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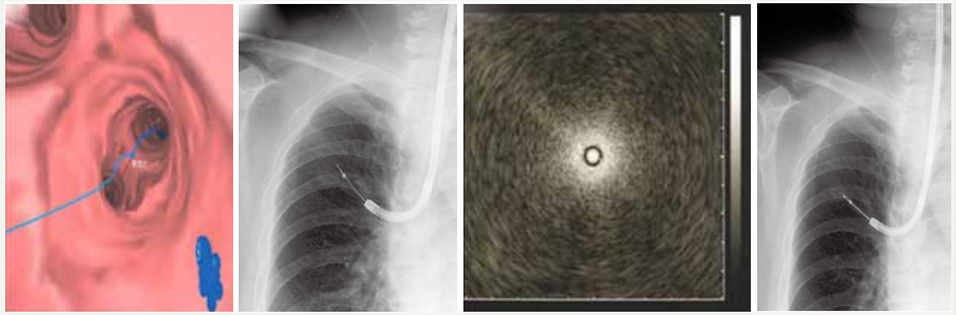

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The bronchoscopy procedure. (See PE82 in this issue).

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Tecemotide (L-BLP25) versus placebo after chemoradiotherapy for stage III non-small cell lung cancer (START): a randomized, double-blind, phase III trial

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Incorporating an effective and tolerable immunotherapeutic as part of maintenance therapy for unresectable stage III non-small-cell lung cancer (NSCLC) is a potential method of improving overall treatment outcomes (1). Many investigators in the lung cancer community have the research goal of establishing a maintenance therapy that prolongs overall survival by stabilizing disease without significantly decreasing quality of life. Immunotherapy capable of inducing an immune response against a tumor-specific antigen is one such approach anticipated to achieve this goal. The recent publication by Butts *et al*. of the Stimulating Targeted Antigenic Response To NSCLC (START) trial showed that the primary endpoint of a significant difference in overall survival in the treatment group was not met; however, the predefined subgroup of patients receiving concurrent chemoradiotherapy followed by maintenance therapy with tecemotide, an antigen-specific immunotherapy, received a notable survival benefit (n=806; HR 0.78, 95% CI: 0.64-0.95, P=0.016) (2).

The START trial was a randomized, double-blind, placebo-controlled phase III trial investigating tecemotide (L-BLP25), an active immunotherapeutic agent, following chemoradiotherapy for inoperable stage III NSCLC (2). The study was performed to evaluate the effectiveness of tecemotide as a maintenance therapy following either concurrent or sequential chemoradiotherapy. Tecemotide is designed to mount an immune response to the cell-surface glycoprotein, Mucin-1 (MUC1), which is aberrantly glycosylated in various epithelial cell cancers, including NSCLC. When MUC1 in these types of cancers is aberrantly glycosylated, it is more efficiently processed into peptides and loaded onto human lymphocyte antigen (HLA) molecules (3-5). This could yield a tumor-specific epitope repertoire bound to HLA molecules and presented on the surface of neoplastic cells that can be recognized by MUC1-specific cytotoxic T-lymphocytes (CTLs). Upon administration, tecemotide is assumed to be taken up by antigen presenting cells. Its peptide compound is subsequently presented to HLA class I and class II molecules, thus eliciting a T_H1 immune response which produces MUC1-specific CTLs.

In this international study, the investigators faced many challenges, including a study suspension, which complicated the conduct of the study and made the results difficult to interpret. All histology subtypes of stage III NSCLC were included. A total of 1,513 patients from 33 countries were enrolled and randomized 2:1 using double-blind methods, stratified by stage (IIIA *vs.* IIIB), response to chemoradiotherapy (stable disease *vs.* objective response), delivery of chemoradiotherapy (concurrent *vs.* sequential) and region (North America and Australia, Western Europe, Rest of World). There was no standardization for chemoradiotherapy except that it required only two cycles of platinum-based chemotherapy, along with a minimum of 50 Gy radiation between four and 12 weeks before randomization. There was no standardization of the chemotherapy schedule, dose intensity, or radiation therapy quality or technique. Patients that participated

from North America and Australia almost exclusively received concurrent chemoradiotherapy while most patients from Eastern European sites received sequential chemoradiotherapy. Overall survival was the primary endpoint studied, while secondary endpoints included time to disease progression, time to symptom progression, 1-3 years survival, and safety. The primary endpoint analysis was adjusted for the randomization strata.

Although the results showed no significant survival benefit between tecemotide and placebo treatment groups when analyzing the outcome independently of chemoradiotherapy dose schedule (HR 0.88, 95% CI: 0.75-1.03; P=0.123), a sub-group analysis of patients receiving concurrent chemoradiotherapy followed by tecemotide showed a notable overall survival benefit. Median overall survival in this group was 30.8 months compared to 20.6 months in those patients receiving concurrent chemoradiotherapy followed by placebo (HR=0.78, 95% CI: 0.64-0.95; P=0.016). The investigators speculated on a number of possible reasons for the difference in tecemotide activity following sequential versus concurrent chemoradiotherapy including initial tumor burden, type of chemotherapy (e.g., taxanes *vs.* etoposide) and their effect on immunogenic *vs.* tolerogenic cell death (6), and poorer performance status at the beginning of the trial. However, to confirm the START trial findings and attribute the survival increase to tecemotide maintenance therapy following concurrent chemoradiotherapy, these investigators have initiated a second Phase III study of tecemotide maintenance therapy after concurrent chemoradiotherapy. Additionally, the results of the START trial have led to the modification of an ongoing Phase III study in Asia to exclude sequential therapies and focus solely on concurrent therapy options.

In addition to the new and modified clinical pathways, post-clinical studies using an immune intact human MUC1-expressing lung cancer mouse model (hMUC1. Tg C57BL/6 mice) are also underway with the goal of identifying effective dosing schedules of combination therapy. Using this model, we have previously shown that tecemotide can induce a specific antigen response and produce modest antitumor effects as a single agent. In addition, cisplatin/tecemotide combination therapy results in additive antitumor effects, while therapeutic doses of cisplatin or localized radiation did not interfere with the immune response to tecemotide (7-9). Additional questions we plan to address include the role of cyclophosphamide in enhancing the immune response to tecemotide, and the potential for inducing acquired drug/immune resistance. Perhaps even more important will be the determination of the factors and timing that result in the development of immune exhaustion following prolonged antigen challenge and methods of reversing immune resistance such as anti-PDL-1 therapy (10,11). In this context, it is essential to monitor the immune response of cancer patients receiving immunotherapy over time and identify parameters that correlate with survival. For instance, it may be worthwhile to investigate an indicator of antigen-specific immune responses to ensure that a given patient is at least exhibiting an immunological response throughout the treatment period.

If the results of the START trial are confirmed, and perhaps further refined with a better understanding of the methods of administering combination therapies while avoiding immune exhaustion and acquired immune resistance, an effective maintenance therapy for patients with unresectable stage III NSCLC is on the horizon.

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References

1. Lwin Z, Riess JW, Gandara D. The continuing role of chemotherapy for advanced non-small cell lung cancer in the targeted therapy era. J Thorac Dis 2013;5:S556-S564.
2. Butts C, Socinski MA, Mitchell PL, et al. Tecemotide (L-BLP25) versus placebo after chemoradiotherapy for stage III non-small-cell lung cancer (START): a randomised, double-blind, phase 3 trial. Lancet Oncol 2014;15:59-68.
3. Hanisch FG, Schwientek T, Von Bergwelt-Baildon MS, et al. O-Linked glycans control glycoprotein processing by antigen-presenting cells: a biochemical approach to the molecular aspects of MUC1 processing by dendritic cells. Eur J Immunol 2003;33:3242-54.
4. Hiltbold EM, Alter MD, Ciborowski P, et al. Presentation of MUC1 tumor antigen by class I MHC and CTL function correlate with the glycosylation state of the protein taken Up by dendritic cells. Cellular Immunology 1999;194:143-9.
5. Purcell AW, van Driel IR, Gleeson PA. Impact of glycans on T-cell tolerance to glycosylated self-antigens. Immunology

and Cell Biology 2008;86:574-9.
6. Green DR, Ferguson T, Zitvogel L, et al. Immunogenic and tolerogenic cell death. Nat Rev Immunol 2009;9:353-63.
7. Kao CJ, Wurz GT, Monjazeb AM, et al. Antitumor effects of cisplatin combined with tecemotide immunotherapy in a human MUC1 transgenic lung cancer mouse model. Cancer Immunol Res 2014;2:581-9.
8. Kao CJ, Wurz GT, Schröder A, et al. Clarifying the pharmacodynamics of tecemotide (L-BLP25)-based combination therapy. Oncoimmunology 2013;2:e26285.
9. Wurz GT, Gutierrez AM, Greenberg BE, et al. Antitumor effects of L-BLP25 Antigen-Specific tumor immunotherapy in a novel human MUC1 transgenic lung cancer mouse model. J Transl Med 2013;11:64.
10. Fourcade J, Sun Z, Pagliano O, et al. PD-1 and Tim-3 regulate the expansion of tumor antigen-specific $CD8^+$ T cells induced by melanoma vaccines. Cancer Res 2014;74:1045-55.
11. Ramlogan-Steel CA, Steel JC, Morris JC. Lung cancer vaccines: current status and future prospects. Transl Lung Cancer Res 2014;3:46-52.

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Editorial

Unresectable stage III non-small cell lung cancer: is Tecemotide a new START for our patients?

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Approximately one third of all non-small cell lung cancer (NSCLC) patients present with unresectable locally advanced stage III disease. The treatment of these patients remains one of the major challenges of contemporary oncology. During the past three decades, gradual progress has been made in the curative-intent treatment. In the 80-ies, these patients were treated with radiotherapy as a single modality, resulting in a median overall survival (OS) of about 10 months. In a landmark trial in early 90ies, it became clear that adding cisplatin-based chemotherapy to radiotherapy improved median OS from approximately 10 to 14 months (1). Subsequent studies established that concurrent delivery of both modalities further improved median OS by an additional 4 to 18 months, compared with sequential delivery, which corresponded to an absolute 4.5% gain in 5-year OS to 15.1% (2). Based on these results, the concurrent delivery of 60 Gy of chest radiation and two cycles of cisplatin-based doublet chemotherapy is our current standard of care for fit patients with unresectable stage III NSCLC (3).

Strategies to increase survival further have mainly focused on three aspects: (I) improvements in systemic therapy; (II) improvements in radiation therapy; or (III) consolidation of the initial response by maintenance therapy (4).

Regarding the first point, cisplatin-etoposide remained the doublet of choice for many of us, because of the vast experience with it, and as only this regimen can be delivered in full dose concurrently with radiotherapy. More modern doublets with e.g., vinorelbine, paclitaxel, docetaxel, or gemcitabine need a clear dose reduction in concurrent schedules, so the gain of more modern chemotherapy may be offset by the lower dose. There were high hopes that pemetrexed doublets, which have been shown to be one of the most effective regimens in advanced non-squamous NSCLC and which can be safely delivered in full dose concurrent with radiotherapy (5), would be a step forward. This question was addressed in the PROCLAIM trial (ClinicalTrials.gov identifier NCT00686959), the phase III trial comparing cisplatin-etoposide with cisplatin-pemetrexed in this setting. However, hope was in vain. The safety monitoring committee had to stop the inclusion prematurely, because the goal of achieving the primary end point (significantly improved OS with cisplatin-pemetrexed) was deemed impossible. The addition of targeted agents to chemoradiotherapy is also very attractive. However, almost none of the agents that were successful in advanced NSCLC, such as gefitinib, erlotinib, bevacizumab, have made it to phase III trials yet. The best hopes were for the EGFR antibody cetuximab, which was studied in the phase III intergroup trial RTOG 0617 (ClinicalTrials.gov identifier NCT00533949). Hope was in vain, addition of cetuximab to contemporary chemoradiotherapy did not deliver significant benefit in OS (6).

Concerning the radiation therapy, the conviction was that delivery of higher doses in a setting with good quality control would improve OS without worrisome impact on toxicity. This aspect was studied as well in the phase 3 RTOG 0617 trial, comparing standard (60 Gy) and high-dose (74 Gy) radiotherapy. Hope was in vain, and quite unexpectedly, in the setting of chemoradiation for stage III NSCLC, higher dose (74 Gy) proved to be inferior, both in terms of OS and locoregional control (7).

Thirdly, as systemic maintenance therapy was shown to improve OS in advanced NSCLC, several attempts to similarly consolidate the results of the concurrent treatment phase in stage III NSCLC by a maintenance strategy were made. Consolidation with for instance docetaxel or gefitinib has been assessed. Hope was in vain, these strategies did not improve OS rates and tended to result in increased toxicity (8,9). Overall, these data clearly indicate the urgent need for novel therapeutic options in the challenging setting of stage III NSCLC.

New insights in the interaction between tumors and the immune system of the host have led to the development of promising immunotherapies for NSCLC treatment. The term cancer immunotherapy covers any interaction with the immune system to treat cancer, and two quite different approaches can be distinguished (10). The first approach is non-antigen-specific modulation of the immune system, for instance by inhibition of immune checkpoints on T-cells, one of the most promising developments in advanced NSCLC (11). The second approach is antigen-specific immunotherapy or therapeutic cancer vaccination. Tumor vaccines prime the immune system to produce antibodies and effector T-cells specifically directed against tumor-associated antigens.

In a recent issue of *Lancet Oncology*, the results of the phase III, placebo-controlled, randomized *START* trial with the tumor vaccine Tecemotide in the setting after chemoradiotherapy for stage III NSCLC were reported [*S*timulating *T*argeted *A*ntigenic *R*esponse *T*o NSCLC, (12)]. Tecemotide is one of the modern vaccines with a relevant antigenic target and a strong immuno-adjuvant and delivery system. The antigenic target is a tandem repeat of 25 amino acids of the core of the MUC1 protein providing antigenic epitopes for T cells (hence its previous name BLP-25). The adjuvant is based on monophosphoryl lipid A, supporting the T-cell response by inducing pro-inflammatory cytokines (via toll-like receptor stimulation). Both components are presented in a liposomal formulation to further enhance the antigen uptake by antigen-presenting cells, thereby stimulating the resulting immune reaction (13). In a previous phase II randomized trial, a signal of prolonged OS in a subgroup analysis of stage IIIB NSCLC patients treated with Tecemotide versus patients with best supportive care alone had been noted (median OS of 30.6 versus 13.3 months, respectively; HR 0.55, 95% CI: 0.30-1.00), together with excellent tolerability (14). In START, patients who had stable or responsive disease after chemoradiotherapy were randomly assigned in a double-blinded fashion (2:1 ratio) to receive Tecemotide vaccine (N=829) or placebo (N=410) weekly for 8 weeks, followed by once every 6 weeks until progression. The primary endpoint was OS, the authors hypothesized to observe a median OS of 20 months in the placebo arm versus an improved median OS of 26 months in the Tecemotide arm. The START investigators did not find a significant difference in median OS between patients that received Tecemotide and those that received placebo (25.6 versus 22.3 months, adjusted HR 0.88, 95% CI: 0.75-1.03; P=0.123). Interestingly, they did identify a favorable effect of the vaccine in the predefined large (N=806) subgroup of patients initially treated with concurrent chemoradiotherapy, with a remarkable 10.2 months improvement in median OS (30.8 versus 20.8 months in the placebo group, adjusted HR 0.78, 95% CI: 0.64-0.95; P=0.016). In contrast, patients that had previously been treated with sequential chemoradiotherapy obtained no clinical benefit from Tecemotide. Moreover, Tecemotide was very well tolerated, most reported reactions were grade 1 or 2 local or flu-like reactions. Importantly, there was no increase in severe immune-related adverse events and no increase in (symptoms of) radiation pneumonitis.

Overall, the study hypothesis of the START trial was well designed. The median OS estimates in the study hypothesis (20 and 26 months) were somewhat higher in comparison with observations made in contemporary trials in the setting of stage III NSCLC, but the START trial only included patients showing at least stable disease after completion of chemoradiotherapy, which supports the higher OS estimates in the START study design.

The START trial, however, leaves some important questions unanswered. Above all, the reason why Tecemotide was associated with improved OS in patients initially treated with concurrent and not with sequential chemoradiotherapy. As also hypothesized by the authors, differences in the patient's performance status and tumor characteristics between both subgroups may have influenced the study result. Treatment with concurrent chemoradiotherapy requires a good performance status and usually smaller tumor sizes. Since both of these factors often coincide with better function of the immune system, differences in these variables may indeed have led to the OS benefit with Tecemotide in the concurrently treated subgroup. Variation in the time window between the delivery of radiation and vaccine therapy may also have influenced the effect of Tecemotide across both subgroups. In colon cancer cells, it has e.g., been shown that MUC1 expression is upregulated in the first days after radiotherapy

and that its expression is higher in a pro-inflammatory microenvironment (15).

When looking at the median survival of patients that were randomized to the placebo arm, there are also some remarkable findings. Firstly, the median OS in the sequential chemoradiotherapy plus placebo arm was higher than in the concurrent chemoradiotherapy plus placebo arm (25 versus 21 months, respectively). This finding is surprising since, as stated before, concurrent delivery of chemo- and radiotherapy has been established as superior to sequential delivery in terms of disease control, which also implies that disease progression is more frequent after sequential chemoradiotherapy. As the START trial only included patients with at least stable disease after initial therapy, the patients in the sequential arm of the START trial likely form a unique subgroup that bares tumors with another (better) biology compared with tumors in the general sequentially treated stage III NSCLC population. Secondly, there is also no clear explanation for the observed difference in OS between the concurrent chemoradiotherapy plus placebo arm of the START study and placebo groups in other contemporary studies in stage III NSCLC [e.g., 28.7 months in the RTOG 0617 trial (7) versus 21 months in the START concurrent chemoradiotherapy plus placebo arm]. However, this may relate to differences in the staging work-up, e.g., the lesser use of PET-CT in the START study (16), leading to more frequent inclusion of patients with subclinical distant metastases. Moreover, some patients in START received what is now considered suboptimal radiotherapy, as the protocol mandated a dose of at least 50 Gy.

As with many oncological treatments, the separation of patients that do benefit from therapy from those who do not by the use of predictive factors is of paramount importance. In that respect, START did not perform very well, mainly due to difficulty of obtaining good tissue samples in stage III NSCLC in general, and certainly after chemoradiotherapy. Plasma samples were available, and in an exploratory analysis antinuclear antibody and soluble MUC1 protein emerged as of potential interest (17).

In summary, the START trial did not meet its overall primary endpoint, but the difference in median OS of about 10 months in the preplanned subanalysis of more than 800 patients with concurrent chemoradiotherapy is remarkable, certainly in the challenging setting of stage III NSCLC, where almost no progress in systemic therapy has been made over the last decade. To confirm the benefit of Tecemotide in patients treated with concurrent chemoradiotherapy, studies now restrict recruitment to this specific patient subgroup. The ongoing phase III INSPIRE trial assesses Tecemotide in Asian stage III NSCLC patients after concurrent chemoradiotherapy [ClinicalTrials.gov identifier NCT01015443 (18)]. Moreover, Tecemotide as maintenance therapy after initial concurrent chemoradiotherapy in stage III NSCLC will be studied in a global confirmatory trial (START 2, ClinicalTrials.gov identifier NCT02049151), which has just started recruitment. The latter trial will be more homogeneous than the previous START trial, as all patients will have concurrent therapy, and as radiotherapy is more standardized according to contemporary guidelines.

Of course, in parallel with confirmatory clinical trials, more fundamental studies assessing the importance of MUC1 in NSCLC, the mechanism of action of Tecemotide, and the interaction between chemoradiotherapy and immunotherapy also need to be performed. Analysis of tissue, preferably before chemoradiotherapy, before the start of Tecemotide and at the time of progression, will be important in this respect. Smaller exploratory trials in dedicated centers may be of additional benefit for this purpose.

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References

1. Dillman RO, Seagren SL, Propert KJ, et al. A randomized trial of induction chemotherapy plus high-dose radiation versus radiation alone in stage III non-small cell lung cancer. N Engl J Med 1990;323:940-5.
2. Aupérin A, Le Péchoux C, Rolland E, et al. Meta-analysis of concomitant versus sequential radiochemotherapy in locally advanced non-small cell lung cancer. J Clin Oncol 2010;28:2181-90.
3. Vansteenkiste J, De Ruysscher D, Eberhardt WE, et al. Early and locally advanced non-small cell lung cancer (NSCLC): ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2013;24 Suppl 6:vi89-98.
4. Vansteenkiste J. Vaccine therapy for NSCLC: does promise meet reality? J Clin Oncol 2013;31 Suppl:abstr 7500.
5. Choy H, Schwartzberg LS, Dakhil SR, et al. Phase 2

study of pemetrexed plus carboplatin, or pemetrexed plus cisplatin with concurrent radiation therapy followed by pemetrexed consolidation in patients with favorable-prognosis inoperable stage IIIA/B non-small cell lung cancer. J Thorac Oncol 2013;8:1308-16.
6. Bradley J, Masters G, Hu C, et al. An intergroup randomized phase III comparison of standard-dose (60 Gy) versus high-dose (74 Gy) chemoradiotherapy (CRT) +/– cetuximab (cetux) for stage III non-small cell lung cancer (NSCLC): Results on cetux from RTOG 0617. J Thorac Oncol 2013;8 Suppl 2:3S.
7. Bradley JD, Paulus R, Komaki R, et al. A randomized phase III comparison of standard-dose (60 Gy) versus high-dose (74 Gy) conformal chemoradiotherapy with or without cetuximab for stage III non-small cell lung cancer: Results on radiation dose in RTOG 0617. J Clin Oncol 2013;31 Suppl:abstr 7501.
8. Hanna N, Neubauer M, Yiannoutsos C, et al. Phase III study of cisplatin, etoposide, and concurrent chest radiation with or without consolidation docetaxel in patients with inoperable stage III non-small-cell lung cancer: the Hoosier Oncology Group and U.S. Oncology. J Clin Oncol 2008;26:5755-60.
9. Kelly K, Chansky K, Gaspar LE, et al. Phase III trial of maintenance gefitinib or placebo after concurrent chemoradiotherapy and docetaxel consolidation in inoperable stage III non-small cell lung cancer: SWOG S0023. J Clin Oncol 2008;26:2450-6.
10. Decoster L, Wauters I, Vansteenkiste JF. Vaccination therapy for non-small-cell lung cancer: review of agents in phase III development. Ann Oncol 2012;23:1387-93.
11. Brahmer JR, Tykodi SS, Chow LQ, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med 2012;366:2455-65.
12. Butts C, Socinski MA, Mitchell PL, et al. Tecemotide (L-BLP25) versus placebo after chemoradiotherapy for stage III non-small-cell lung cancer (START): a randomised, double-blind, phase 3 trial. Lancet Oncol 2014;15:59-68.
13. Sangha R, Butts C. L-BLP25: a peptide vaccine strategy in non small cell lung cancer. Clin Cancer Res 2007;13:s4652-4.
14. Butts C, Murray N, Maksymiuk A, et al. Randomized phase IIB trial of BLP25 liposome vaccine in stage IIIB and IV non-small cell lung cancer. J Clin Oncol 2005;23:6674-81.
15. Kang Y, Hirano K, Suzuki N, et al. Increased expression after X-irradiation of MUC1 in cultured human colon carcinoma HT-29 cells. Jpn J Cancer Res 2000;91:324-30.
16. Thatcher N, Shepherd FA, Mitchell P, et al. Geographic differences in the combined-modality treatment of stage III unresectable non-small cell lung cancer: Results from a global phase III trial of tecemotide (LBLP25). J Thorac Oncol 2013;8 Suppl 2:135S.
17. Mitchell PL, Butts CA, Socinski MA, et al. Tecemotide (L-BLP25) in unresectable stage III non-small cell lung cancer in the phase III START study: Further endpoint and exploratory biomarker results. J Thorac Oncol 2013;8 Suppl 2:135S-136S.
18. Wu YL, Park K, Soo RA, et al. INSPIRE: A phase III study of the BLP25 liposome vaccine (L-BLP25) in Asian patients with unresectable stage III non-small cell lung cancer. BMC Cancer 2011;11:430.

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Editorial

Cetuximab in advanced non-small cell lung cancer (NSCLC): the showdown?

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Nowadays, epidermal growth factor receptor (EGFR) is an important therapeutic target in non-small-cell lung cancer (NSCLC). Monoclonal antibodies (mAbs) targeting the extra-cellular domain of EGFR together with small molecule tyrosine kinase inhibitors (TKIs) have been exploited pharmacologically to block EGFR activation. While the EGFR-TKIs erlotinib and gefitinib are established treatment options for patients with advanced NSCLC, above all in patients with activating EGFR mutations (exon 19 deletion and mutation L858R in exon 21), the role of cetuximab (mAb) was recently clarified. Cetuximab (marketed as Erbitux) is a chimeric human/murine monoclonal immunoglobulin G1 antibody, that inhibits the receptor function, mediates antibody-dependent cell-mediated cytotoxicity and receptor downregulation, leading to a mitigation of EGFR activity. Several phase II trials have evaluated if cetuximab in combination with different first-line chemotherapy regimens could enhance synergic effect. First, the promising efficacy results of the addiction of cetuximab to cisplatin plus vinorelbine as first-line treatment in the phase II Lung Cancer Cetuximab Study [LUCAS; overall response rate (ORR): 35% *vs.* 28%] (1) led to the FLEX (First-Line Erbitux in Lung Cancer) phase III trial (2,3). In this landmark phase III trial, the combination with cetuximab significantly improved overall survival (OS, primary endpoint) compared with chemotherapy alone (cisplatin plus vinorelbine) in 1,125 chemo-naïve patients with advanced EGFR-positive NSCLC (median OS: 11.3 versus 10.1 months, respectively; HR: 0.871, P=0.044). This small but significant survival benefit was seen in all histological subgroups. Progression-free survival (PFS) time was similar, showing a median 4.8 months in both groups (HR: 0.943, P=0.39) (2). As expected with an anti-EGFR antibody, acne-like skin rash, diarrhoea, and infusion-related reactions were more common in patients given cetuximab plus chemotherapy. Interestingly, early-onset acne-like rash of any grade was associated with better outcome: median survival of 15.0 *vs.* 8.8 months (HR: 0.63, P<0.001) (3).

Another phase II trial, SWOG S0342, evaluated concurrent and sequential administration of cetuximab with a standard chemotherapy (carboplatin plus paclitaxel) regimen in untreated patients with advanced NSCLC (4). Both arms meet the predefined efficacy end point of median OS time of ≥10 months; RR and PFS were similar, as well as grade 3 rash, whereas sensory neuropathy was higher in the concurrent arm. The concurrent regimen was chosen in subsequent phase III trial BMS-099 (Bristol-Myers Squibb 099), testing the addition of cetuximab to carboplatin plus paclitaxel in 676 chemo-naïve patients with advanced NSCLC, without restrictions based on histology or EGFR expression (5). Although BMS-099 did not meet its primary end point (PFS, 4.4 *vs.* 4.24 months; HR: 0.902, P=0.24), there were some similarities with the FLEX trial. Both studies reported a statistically significant benefit in ORR with the addition of cetuximab to platinum-based chemotherapy (36% *vs.* 29% in FLEX; 25.7% *vs.* 17.2% in BMS), and failed to show any improvement in PFS. However, the difference in OS was similar in both studies (approximately 1.3-month increase in median OS and 11% to 13% reduction in the death risk), although BMS099 lacked power to detect a difference of this magnitude with statistical significance (5).

Cetuximab added to a platinum agent (mostly carboplatin)

plus gemcitabine in chemo-naïve patients with advanced NSCLC, regardless of EGFR expression, resulted in a higher RR (27.7% *vs.* 18.2%) and longer PFS (median 5.09 *vs.* 4.21 months) compared to chemotherapy alone, in another phase II trial (6). To better understand the real impact of cetuximab-based treatment in first-line setting, a metanalysis including 2018 patients from four randomized trials, was performed. The survival benefit of chemotherapy plus cetuximab compared to chemotherapy alone [regardless of the chemotherapy protocol used: cisplatin plus vinorelbine (1-3), platin plus paclitaxel (5), and platin plus gemcitabine (6)] was confirmed in chemo-naïve patients with advanced NSCLC (7). Despite these positive results—in biomarker unselected population—both the FDA and the EMEA rejected the licensing of cetuximab in combination with chemotherapy for first-line therapy of advanced NSCLC in consideration of the small OS benefit of the addition of cetuximab to chemotherapy, which should be weighed against its side effects, the weekly administration, and costs.

The identification of a biomarker predictive of a treatment benefit associated with the addition of cetuximab to first-line chemotherapy for NSCLC would enable a personalised approach to care. To pursue this possibility, retrospective analyses of FLEX and BMS-099 investigated a panel of candidate pretreatment molecular markers (KRAS mutational status, EGFR mutational status, and EGFR copy number) in tumours, but none of these have a predictive role in clinical benefit (8,9). Interestingly, tumour EGFR expression levels seemed to be associated with clinical outcome in FLEX study patients (10). In a further prospective analysis of this study, Pirker *et al.* collected tumour EGFR expression data to generate an immunohistochemistry score (H score), to provide a more detailed assessment of EGFR protein expression, and to evaluate its role as predictive biomarker of survival benefit. The H score takes into account the percentage of cells (0-100%) in each intensity category (0-3+) and computes a final score, on a continuous scale between 0 and 300.

High EGFR expression according to a tumour IHC score of 200 or more seems to be the only effective pre-treatment biomarker so far identified for the prediction of clinical benefit from chemotherapy plus cetuximab in the first-line treatment of advanced NSCLC (10).

Although the predictive role of EGFR expression levels seems to emerge from this analysis, FDA and EMA rejected the approval of cetuximab in high score EGFR expression NSCLC due the fact that the data come from a subgroup analysis. They required a confirmatory prospective trial that the pharmaceutical company has decided not to run.

Most patients receiving front-line cytotoxic therapy for advanced NSCLC experience progressive disease. Several single agents are approved for use in advanced, second-line NSCLC, including pemetrexed, docetaxel, and erlotinib. However, in patients who become refractory to front-line chemotherapy, no new treatment has shown significant survival benefit in unselected patient populations for the past decade outside of single-agent therapy. Based on promising safety and efficacy results of combined regimen (cetuximab plus docetaxel) in a phase II trial (11), the SELECT study evaluated if the addition of cetuximab to standard chemotherapy might improve outcome in patients with pretreated advanced NSCLC (12). In this open-label phase III trial, Kim and colleagues randomized 938 patients with metastatic, unresectable, or locally advanced NSCLC to four arms of treatment: 605 patients received pemetrexed (301 patients with cetuximab and 304 alone) and 333 received docetaxel (167 in combination with cetuximab and 166 alone). The initial primary analysis was a comparison of the ORR between chemotherapy alone or combined with weekly cetuximab. However during the trial, the primary endpoint was changed to compare PFS with cetuximab plus pemetrexed versus pemetrexed alone, on an intention-to-treat basis, after data publication of phase III trial, in which pemetrexed showed a clinically equivalent efficacy outcomes to docetaxel, with fewer side-effects (13). The addition of cetuximab to pemetrexed did not improve PFS (2.9 *vs.* 2.8 months, respectively; HR: 1.03, P=0.76), nor there were improvements in any of the other assessed efficacy or quality-of-life measures, including OS (6.9 *vs.* 7.8 months, respectively; HR 1.01, P=0.86). Data from pre-specified efficacy subgroup analyses by EGFR status and histology (squamous *vs.* non-squamous) confirmed any improvement in outcome. There were no significant differences between the two treatment groups in median PFS or in OS, when assessed by EGFR staining intensity (positive: EGFR 1+, 2+, 3+/negative: EGFR undetectable) and H-score (low H-score: <200/high H-score: ≥200). More and worse adverse events (AEs) were recorded with cetuximab plus pemetrexed, mainly due to skin-related toxic effects (grade 3-4 acneiform rash: 11% *vs.* 0%), gastrointestinal symptoms (grade 1-2 diarrhoea: 27% *vs.* 13%; grade 1-2 mucositis oral: 18% *vs.* 7%), and hypomagnesaemia (grade 1-2: 19% *vs.* 6%).

These disappointing results confirmed the ineffectiveness of the combination of cetuximab and pemetrexed, already reported in a single arm phase II study (14), suggesting that the addition of cetuximab in an unselected patient

population in this setting is unlikely to result in significantly superior outcomes to single-agent therapy alone.

Nowadays, cetuximab failed to demonstrate a great and clinically significantly survival benefit when combined with chemotherapy regimens (mono- or poly-chemotherapy), regardless line setting (first- or second line). Furthermore, the data reported by Kim and colleagues (12) highlighted that the use of cetuximab in unselected patients not only did not improve outcomes, but also worsened toxic effects. So the identification of NSCLC patients that might potentially benefit from treatment with this monoclonal antibody is needful, but not yet clarified. The use of EGFR staining intensity and H-score, for selection of patients need to be confirmed in prospective trials but pharmaceutical company decided to stop the cetuximab clinical development in NSCLC.

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References

1. Rosell R, Robinet G, Szczesna A, et al. Randomized phase II study of cetuximab plus cisplatin/vinorelbine compared with cisplatin/vinorelbine alone as first-line therapy in EGFR-expressing advanced non-small-cell lung cancer. Ann Oncol 2008;19:362-9.
2. Pirker R, Pereira JR, Szczesna A, et al. Cetuximab plus chemotherapy in patients with advanced non-small-cell lung cancer (FLEX): an open-label randomised phase III trial. Lancet 2009;373:1525-31.
3. Gatzemeier U, von Pawel J, Vynnychenko I, et al. First-cycle rash and survival in patients with advanced non-small-cell lung cancer receiving cetuximab in combination with first-line chemotherapy: a subgroup analysis of data from the FLEX phase 3 study. Lancet Oncol 2011;12:30-7.
4. Herbst RS, Kelly K, Chansky K, et al. Phase II selection design trial of concurrent chemotherapy and cetuximab versus chemotherapy followed by cetuximab in advanced-stage non-small-cell lung cancer: Southwest Oncology Group study S0342. J Clin Oncol 2010;28:4747-54.
5. Lynch TJ, Patel T, Dreisbach L, et al. Cetuximab and first-line taxane/carboplatin chemotherapy in advanced non-small-cell lung cancer: results of the randomized multicenter phase III trial BMS099. J Clin Oncol 2010;28:911-7.
6. Butts CA, Bodkin D, Middleman EL, et al. Randomized phase II study of gemcitabine plus cisplatin or carboplatin [corrected], with or without cetuximab, as first-line therapy for patients with advanced or metastatic non small-cell lung cancer. J Clin Oncol 2007;25:5777-84.
7. Thatcher N, Linchy TJ. Cetuximab plus platinum-based chemotherapy as 1st-line treatment in patients with nonsmall cell lung cancer (NSCLC): A meta-analysis of randomized phase II/III trials. WCLC 2009;4:S5-968; [abstr A3.7].
8. O'Byrne KJ, Gatzemeier U, Bondarenko I, et al. Molecular biomarkers in non-small-cell lung cancer: a retrospective analysis of data from the phase 3 FLEX study. Lancet Oncol 2011;12:795-805.
9. Khambata-Ford S, Harbison CT, Hart LL, et al. Analysis of potential predictive markers of cetuximab benefit in BMS099, a phase III study of cetuximab and first-line taxane/carboplatin in advanced non-small-cell lung cancer. J Clin Oncol 2010;28:918-27.
10. Pirker R, Pereira JR, von Pawel J, et al. EGFR expression as a predictor of survival for first-line chemotherapy plus cetuximab in patients with advanced non-small-cell lung cancer: analysis of data from the phase 3 FLEX study. Lancet Oncol 2012;13:33-42.
11. Kim ES, Mauer AM, William WN Jr, et al. A phase 2 study of cetuximab in combination with docetaxel in chemotherapy-refractory/resistant patients with advanced nonsmall cell lung cancer. Cancer 2009;115:1713-22.
12. Kim ES, Neubauer M, Cohn A, et al. Docetaxel or pemetrexed with or without cetuximab in recurrent or progressive non-small-cell lung cancer after platinum-based therapy: a phase 3, open-label, randomised trial. Lancet Oncol 2013;14:1326-36.
13. Hanna N, Shepherd FA, Fossella FV, et al. Randomized phase III trial of pemetrexed versus docetaxel in patients with non-small-cell lung cancer previously treated with chemotherapy. J Clin Oncol 2004;22:1589-97.
14. Jalal S, Waterhouse D, Edelman MJ, et al. Pemetrexed plus cetuximab in patients with recurrent non-small cell lung cancer (NSCLC): a phase I/II study from the Hoosier Oncology Group. J Thorac Oncol 2009;4:1420-4.

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Editorial

Fertility concerns and preservation strategies in young women with breast cancer

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Prospective study of fertility concerns and preservation strategies in young women with breast cancer was a prospective survey of attitudes regarding fertility among younger women (age 40 and under) with breast cancer in the US (1). The study examined whether or not fertility was discussed when the patient was diagnosed, whether or not the patient had fertility concerns when treatment began, what steps could be taken to preserve fertility, and what effect fertility concerns actually had on treatment decisions.

The concept of survivorship, or helping individuals diagnosed cancer to lead full lives, has gained widespread acceptance. In Europe and the US, guidelines have been issued predicated on the recognition that fertility preservation (FP) should be considered for all patients with cancer who are of reproductive age and who will be undergoing anti-cancer drug therapy (2-4). Other reports, however, have suggested that breast cancer specialists' knowledge and their attitudes towards actively FP were related to their efforts to provide information to patients (5). These studies also surmised that there are differences in the information and options offered to patients depending on the physician. This study by Ruddy *et al.* reported that 68% patients discussed fertility issues with their physicians before starting therapy. Previous studies from Europe and the US have found that patients are not adequately informed of the effects of drug therapy on reproductive function, including early menopause, or of the FP (6-11). Those previous studies noted differences in whether or not FP was explained depending on aspects such as the patient's age at diagnosis, whether or not the patient had children, the patient's educational level, the type of drug therapy, and the stage of cancer. Several problems for oncologists are not known a reproductive specialist to confer with and time constraints upon clinical practice (5). Issues that need to be dealt with are creation of an information network for oncologists and reproductive specialists and establishment of a system for collaboration.

That said, there were very few prospective studies on the current state of patients' attitudes. This study by Ruddy *et al.* found that half of all patients were concerned about becoming infertile after treatment and that greater concern about fertility was associated with younger age, nonwhite race, not having children, and receipt of chemotherapy. According to the study, concerns about fertility were significantly affected by social background. These findings do not necessarily apply everywhere around the world. Aspects such as the average marrying age, the role of women in society, and childrearing conditions differ considerably in depending on the country and region. However, the incontrovertible fact is that numerous patients need to be fully informed about a treatment's effects on their fertility before that treatment begins.

A more significant finding reported by Ruddy *et al.* is that there were instances where the strategy for treatment of breast cancer was changed because of its potential effects on fertility. In many of those instances, standard postoperative drug therapy was originally intended to limit recurrence as much as possible but this regimen was modified or its duration was shortened. Such a decision must be made prudently since it has the potential to worsen a patient's prognosis. A patient must be fully informed of a treatment's effects on her fertility and other options, including preservation of fertility, in order to choose a treatment. However, there appears to be little available evidence of

this at the current point in time. Effects on fertility differ depending on the patient's age, ovarian reserve, and type of medication, so many uncertainties remain with regard to whether a patient will be able to have children after undergoing drug therapy. Thus, research is beginning to look at indices to predict ovarian reserve prior to treatment and after drug therapy. Anti-Mullerian hormone (AMH) is one such index. Patients with chemotherapy-related amenorrhea are reported to have low levels of AMH prior to treatment (12,13). If research into these factors progresses, then the subsequent findings will help with patient discussions of fertility prior to treatment and allow planning of a treatment strategy in light of survivorship after treatment.

Medical reproductive technology is being used to grant the wishes of younger patients with cancer who are undergoing anti-cancer drug therapy but who want to have children. Embryos or oocytes are being preserved before the start of drug therapy, and the cryopreservation of ovarian tissue has recently been attempted (14). This technique could preserve reproductive function if guidelines on the standard treatment for breast cancer are followed. This technique would prove extremely useful in terms of both treating breast cancer and preserving reproductive function to the maximum extent. However, this technique still has a number of issues, such as the fact that its effects on breast cancer prognosis are not known, it results in a low rate of successful conception, and it is highly expensive. The study on ovarian tissue cryopreservation by Fabbri *et al.* used a small subject sample. A major issue with ovarian tissue cryopreservation is that no studies have reported the prognosis when patients with breast cancer use reproductive medicine. FP often involves inducing oocyte stimulation in order to harvest numerous eggs from the patient and it increases levels of estrogen in the blood compared to those during a normal menstrual cycle, so a hormone-sensitive tumor may recur or become more aggressive. Over the past few years, studies have begun to attempt to limit the rise in estrogen levels to the extent possible by inducing oocyte stimulation using aromatase inhibitors (15,16).

The dearth of evidence has hampered the informing of patients with breast cancer about fertility and the selection of a suitable treatment in accordance with the patient's wishes. However, a study on the decision to undergo counseling with regard to early menopause when treatment starts and treatment's effects on reproductive function has reported that counseling should not be limited to patients who actually have their reproductive function preserved (17). Instead, the study reported that all women of childbearing age with breast cancer who underwent counseling had a higher QOL after treatment. Moreover, another study indicated that an explanation needs to be provided by an oncologist as well as a reproductive specialist so that a patient can select a treatment without regrets (7). Patients lack adequate knowledge of issues with their reproductive function at the start of treatment, but providing information at the appropriate time both allows the patient to select a treatment without regrets (18) and it significantly alleviates subsequent distress and symptoms due to menopause (19). Prospective studies like that by Ruddy *et al.* need to be assembled and current conditions and needs must be prospectively studied, as the study by Ruddy *et al.* did, in accordance with social backgrounds in different places. Based on these findings, fertility counseling systems and FP strategies tailored to those places must be crafted by medical personnel in multiple disciplines such as oncologists and reproductive specialists.

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References

1. Ruddy KJ, Gelber SI, Tamimi RM, et al. Prospective study of fertility concerns and preservation strategies in young women with breast cancer. J Clin Oncol 2014;32:1151-6.
2. Loren AW, Mangu PB, Beck LN, et al. Fertility preservation for patients with cancer: American Society of Clinical Oncology clinical practice guideline update. J Clin Oncol 2013;31:2500-10.
3. Peccatori FA, Azim HA Jr, Orecchia R, et al. Cancer, pregnancy and fertility: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2013;24 Suppl 6:vi160-70.
4. ISFP Practice Committee, Kim SS, Donnez J, et al. Recommendations for fertility preservation in patients with lymphoma, leukemia, and breast cancer. J Assist Reprod Genet 2012;29:465-8.
5. Shimizu C, Bando H, Kato T, et al. Physicians' knowledge, attitude, and behavior regarding fertility issues for young breast cancer patients: a national survey for breast care specialists. Breast Cancer 2013;20:230-40.
6. Niemasik EE, Letourneau J, Dohan D, et al. Patient perceptions of reproductive health counseling at the time of cancer diagnosis: a qualitative study of female California

cancer survivors. J Cancer Surviv 2012;6:324-32.
7. Scanlon M, Blaes A, Geller M, et al. Patient Satisfaction with Physician Discussions of Treatment Impact on Fertility, Menopause and Sexual Health among Pre-menopausal Women with Cancer. J Cancer 2012;3:217-25.
8. Letourneau JM, Smith JF, Ebbel EE, et al. Racial, socioeconomic, and demographic disparities in access to fertility preservation in young women diagnosed with cancer. Cancer 2012;118:4579-88.
9. Karaöz B, Aksu H, Küçük M. A qualitative study of the information needs of premenopausal women with breast cancer in terms of contraception, sexuality, early menopause, and fertility. Int J Gynaecol Obstet 2010;109:118-20.
10. Rippy EE, Karat IF, Kissin MW. Pregnancy after breast cancer: the importance of active counselling and planning. Breast 2009;18:345-50.
11. Duffy CM, Allen SM, Clark MA. Discussions regarding reproductive health for young women with breast cancer undergoing chemotherapy. J Clin Oncol 2005;23:766-73.
12. Partridge AH, Ruddy KJ, Gelber S, et al. Ovarian reserve in women who remain premenopausal after chemotherapy for early stage breast cancer. Fertil Steril 2010;94:638-44.
13. Su HI, Sammel MD, Green J, et al. Antimullerian hormone and inhibin B are hormone measures of ovarian function in late reproductive-aged breast cancer survivors. Cancer 2010;116:592-9.
14. Fabbri R, Vicenti R, Magnani V, et al. Cryopreservation of ovarian tissue in breast cancer patients: 10 years of experience. Future Oncol 2012;8:1613-9.
15. Oktay K, Hourvitz A, Sahin G, et al. Letrozole reduces estrogen and gonadotropin exposure in women with breast cancer undergoing ovarian stimulation before chemotherapy. J Clin Endocrinol Metab 2006;91:3885-90.
16. Azim AA, Costantini-Ferrando M, Oktay K. Safety of fertility preservation by ovarian stimulation with letrozole and gonadotropins in patients with breast cancer: a prospective controlled study. J Clin Oncol 2008;26:2630-5.
17. Letourneau JM, Ebbel EE, Katz PP, et al. Pretreatment fertility counseling and fertility preservation improve quality of life in reproductive age women with cancer. Cancer 2012;118:1710-7.
18. Peate M, Meiser B, Friedlander M, et al. It's now or never: fertility-related knowledge, decision-making preferences, and treatment intentions in young women with breast cancer--an Australian fertility decision aid collaborative group study. J Clin Oncol 2011;29:1670-7.
19. Schover LR, Jenkins R, Sui D, et al. Randomized trial of peer counseling on reproductive health in African American breast cancer survivors. J Clin Oncol 2006;24:1620-6.

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Editorial

Fertility issues in young breast cancer patients: what women want

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Breast cancer is the most common tumor type in young women of reproductive age: approximately 7% of breast cancer cases are diagnosed in women ≤40 years and this corresponds to more than 40% of all malignancies diagnosed in this age group (1).

The available anticancer treatments (surgery, radiotherapy, chemotherapy, endocrine therapy and biologic therapy) have improved both disease-free survival (DFS) and overall survival (OS) in young early breast cancer patients but they can cause acute and chronic side effects, such as a negative impact on gonadal function that may lead to impaired fertility (2). The fertility issues in these patients have acquired a growing importance in the last few years not only because of the improved prognosis of cancer patients but also due to the tendency of delaying child-bearing in western countries, so that many women can be childless or may want to enlarge their family at the time of breast cancer diagnosis (3). A new medical discipline, named "oncofertility" (a product of the crosstalk between oncology and reproductive medicine) has emerged in recent years: it is a new concept that describes an exciting integrated network of clinical resources with the goal to develop methods to spare or restore reproductive function in young cancer patients (4).

There are four main available strategies, standard and experimental, for fertility preservation in young early breast cancer patients: embryo cryopreservation, cryopreservation of oocytes, cryopreservation of ovarian tissue and temporary ovarian suppression with luteinizing hormone-releasing hormone analogues (LHRHa).

To date, cryopreservation of embryos and of mature oocytes are the only techniques that are considered standard by the American Society of Clinical Oncology (ASCO) (5) and the European Society for Medical Oncology (ESMO) (6); on the contrary, cryopreservation of ovarian tissue or cryopreservation of immature oocyte or of oocytes matured *in vitro* and temporary ovarian suppression with LHRHa are still considered experimental. The main factors that should be considered for the choice between the available fertility preservation techniques for young women candidates for anticancer therapies are: patient's age and ovarian reserve, type of anticancer therapy planned, availability of a partner at the time of diagnosis, the time available before the initiation of anticancer treatments, and the possibility that cancer has metastasized to the ovaries (5).

Irrespective of the pro and contra of the different strategies, every young breast cancer patient who is candidate to anticancer therapies (particularly chemotherapy and endocrine therapy) should have access to fertility counseling to receive information about ovarian damage due to such treatments (5). Health care providers should be knowledgeable about guidelines on fertility preservation in patients with cancer diagnosed at young age: they have the responsibility to raise awareness on potential fertility impairment due to anti-cancer therapies and should be able to deal with these issues. Fertility counseling is the key moment to discuss the fertility issues before the time of treatment initiation: it should include a detailed description of all the available strategies to preserve fertility which are appropriate for that particular patient including techniques, timing, possible complications, success rates, costs and ethical implications (7). A major objective is to elucidate the patient about what is well-known and considered standard and what is still experimental about these techniques: more

than one strategy can sometimes be applied to the same patient to increase the chances of maintaining fertility and future pregnancies (7).

The available evidence suggests that infertility resulting from cancer treatment may be associated with psychosocial distress and negative impacts on global health of young breast cancer survivors (2,8). However, while the majority of data regarding fertility concerns in young women with breast cancer has focused on long-term survivors, little is known on the burden of fertility concerns at the time of breast cancer diagnosis before the initiation of anticancer treatments. Particularly, few data are available on the possible impact of fertility concerns on treatment decisions and about patients' preferences among the available strategies of fertility preservation.

The paper recently published by Ruddy and colleagues in the *Journal of Clinical Oncology* has a great importance in the understanding of the burden of fertility issues in young breast cancer patients at the time of diagnosis (9). The "Helping Ourselves, Helping Others: The Young Women's Breast Cancer Study" is a large prospective multicentre cohort study of young women with newly diagnosed stage I-III breast cancer and age ≤40 years. The primary endpoint of the study was the assessment of fertility concerns; other important objectives of the trial were the understanding of how fertility concerns affect treatment decisions and the preferences of women regarding the available fertility preservation strategies. Of the 1,511 women invited to participate between November 2006 and December 2012, only 620 were included in the final analysis. At the time of breast cancer diagnosis, the median age of the participants was 37 years (range, 17-40 years), 76% were married and 34% were childless. Regarding tumor characteristics, 58% were grade III, 29% were estrogen receptor-negative, 36% were progesterone receptor-negative and 30% were HER2-positive. A total of 424 women (68%) discussed fertility issues with their physicians before starting therapy: no increasing or decreasing trends in the likelihood of these discussions were shown over time between 2006 and 2012. At the time of decision making about treatment, 319 women (51%) had concerns about becoming infertile after treatment: again for fertility concerns, no change over time between 2006 and 2012 was shown. At the multivariable logistic regression model, the factors associated with greater statistically significant likelihood of fertility concerns were: age [age ≥35 *vs.* <35: odds ratio (OR) 0.26, 95% confidence interval (CI): 0.18-0.40, P<0.001], race (white *vs.* not: OR 0.38, 95% CI: 0.20-0.72, P=0.003), receipt of chemotherapy (received chemotherapy *vs.* none: OR 1.61, 95% CI: 1.04-2.50, P=0.03) and currently childless (has children *vs.* does not: OR 0.17, 95% CI: 0.11-0.26, P<0.001). It is noteworthy that 112 patients (18%) reported that concerns about fertility affected their decisions regarding the medical treatment they would undergo: 4 women (1%) chose not to receive chemotherapy, 12 (2%) chose one chemotherapy over another, 6 (1%) considered not receiving endocrine therapy, 19 (3%) decided not to receive endocrine therapy, and 71 (11%) considered receiving endocrine therapy for <5 years. Despite the great proportion of women concerned about fertility, few patients (65 women, 10%) took special steps to lessen their chance of infertility: 46 women (7%) underwent embryo cryopreservation, 7 (1%) oocyte cryopreservation and 19 (3%) accepted the administration of LHRHa during chemotherapy. The proportion of patients who pursued fertility preservation techniques seemed to increase over time (from 5% in 2006 to 15% in 2012) (9). In conclusion, the main findings of the study are that 32% of women did not discuss fertility issues with their physicians, 18% of women decided not to receive the proposed treatments because of fear of infertility and, despite the high proportion of women concerned about fertility, only 10% underwent one of the available strategies to preserve fertility.

It is well know that the fertility issues are not always dealt with appropriately by physicians thus depriving patients of the opportunity to access effective fertility preservation techniques (10). Fortunately, the data seems to be improving: a recent German study showed that the proportion of patients who could not remember proper counselling about the risk of fertility impairment due to anticancer treatments decreased significantly over time (11). In the years 1980-1984 the proportion of patients who reported no memories of counselling was 67% while in the years 2000-2004 decreased to 50% (P<0.001) (11). Another recent Swedish study reported similar findings with less than half of women (48%) reporting to have received information about treatment impact on fertility and 14% who reported having received information about the available fertility preservation strategies (12). In the study by Ruddy *et al.*, fewer women, nearly one third (32%), did not discuss fertility issues with their physicians: however, as discussed by authors themselves, many women were enrolled at Institutions that focus particularly on care of young women, and so fertility discussions might be rarer and less substantive elsewhere (9). Several factors may hinder the discussion between the medical oncologists and

the patients: the fact that oncologists might not be aware of the clinical recommendations related to the issue of fertility preservation or not being update on the subject, the lack of ad hoc multi-disciplinary teams, and factors related to the patient (level of education, prognosis, sex, parental status, marital status, having children at diagnosis, age and pubertal status, economic opportunities) (13). Despite a trend towards a reduction in the number of women who do not receive fertility counseling at the time of diagnosis, more efforts should be made to continue to improve communication about fertility risks and options to preserve fertility.

Furthermore, an accurate communication has a great importance to avoid the possibility that concerns about the risk of infertility would have an impact on treatment decisions. In 2004, Partridge and colleagues reported that out of 657 young early breast cancer patients interviewed, 193 (29%) indicated that concerns about fertility impacted on their treatment decisions (14). Particularly, authors found that women who reported greater concern about fertility required greater risk reduction from chemotherapy and were much less likely to accept a higher risk of infertility from adjuvant chemotherapy than women who were less concerned about fertility (14). In the study by Ruddy *et al.* the proportion of patients who decided not to receive the initially prescribed treatments due to infertility concerns was lower but still significant (112/620 women, 18%) (9). However, although evidence suggests that some patients prefer to receive less effective treatments in order to prevent long-term complications such as infertility, many of them prefer not to deal with these concerns with their treating physicians. Then, it is up to the clinician to deliver limited information and delegating the task to other members of the multidisciplinary team, such as specialised nurses, whose role in this area is widely recognized (8).

Finally, despite a growing amount of evidence suggesting that fertility issues are of great importance for young women diagnosed with cancer, limited data exist about the proportion of patients who do make use of fertility preservation techniques. The percentage of patients who choose to undergo fertility preservation strategies (oocytes/embryos cryopreservation or ovarian tissue cryopreservation) after fertility counseling reported in the literature varies from 2% to over 50% (12,15). Ruddy and colleagues reported that out of 620 women, 65 (10%) took special steps to lessen chance of infertility (7% underwent embryo cryopreservation, 1% oocyte cryopreservation and 3% accepted the administration of LHRHa) but with an increasing trend over time in the proportion of patients who pursued fertility preservation techniques (from 5% in 2006 to 15% in 2012) (9). Similar findings come from our experience: approximately 22% of breast cancer patients accepted to undergo fertility counseling performed by the reproductive physician and 8% underwent surgical fertility preservation techniques (oocytes cryopreservation or ovarian tissue cryopreservation) (7); however, a significant greater proportion of patients (85%) compared to the study by Ruddy *et al.* accepted to undergo the administration of LHRHa during chemotherapy, since this strategy is recommended by the Italian guidelines for fertility preservation in breast cancer patients (7). Despite a high proportion of young women with breast cancer reporting to be concerned about fertility, only a small proportion of them decide to undergo one of the available fertility preservation strategies. Possible explanations are: a lack of discussion of fertility issues between patients and physicians (as discussed above), concerns about the safety of these techniques, fear of a negative impact of pregnancy after breast cancer, and inadequate access to these strategies. Regarding the safety of the techniques, particularly for hormone responsive tumors, there are still some concerns about a possible negative impact of the ovarian stimulation required for oocyte and embryo cryopreservation. To try to reduce the potential risk of short-term exposure to high estrogen levels, alternative approaches for ovarian stimulation with letrozole or tamoxifen have been developed (16). As reported by Azim and colleagues in the largest experience with the use of cryopreservation strategies in breast cancer patients, at a median follow up of 23.4 months after chemotherapy, the hazard ratio for recurrence after *in vitro* fertilization was 0.56 (95% CI: 0.17-1.9) and the survival of patients that underwent cryopreservation strategies was not compromised compared with controls (17). However, further research, including longer-term follow-up for both cryopreservation strategies and for LHRHa administration, is needed to confirm the safety of these procedures. Another important point to keep in mind during fertility counseling is the fear of some patients (but also of some physicians) about the occurrence of congenital abnormalities and the potential obstetric and birth complications due to previous cancer treatments, and the theoretic risk that pregnancy might have negative consequences on patients' prognosis. The available evidence suggests that the occurrence of congenital abnormalities of infants born to women with a history of breast cancer is similar to that of the general population (6). On the other side, a relatively higher

incidence of birth complications (caesarean section, preterm birth, babies with low birth weight), in women previously treated for breast cancer as compared to the general population has been reported (18): therefore, a close monitoring of pregnancy in women previously treated for cancer is recommended. So far, it is well established that women who become pregnant after breast cancer do not have a worse prognosis: neither in young women with estrogen receptor-positive breast cancer any difference in DFS and OS has been observed between women who became pregnant after breast cancer and non-pregnant patients (19). Finally, it is mandatory for oncologists to cooperate with one or more Reproductive Units to give their patients the opportunity to undergo a well-timed and complete reproductive counseling. According to the results of a recent survey on post-treatment quality of life (QoL) in young women with cancer who were counseled either by the oncology team or by fertility specialists, the specialized counseling about reproductive loss and pursuing fertility preservation is associated with less regret and greater QoL for survivors (20). Therefore, a well-organized interaction between oncology and reproductive units is the first step to be accomplished to face the management of fertility issues in young cancer patients (7).

In summary, despite many young breast cancer patients have concerns about fertility at the time of diagnosis, only a minority undergo one of the available fertility preservation strategies and little over one-sixth change their therapeutic strategy. More efforts are needed to ameliorate the communication on the fertility issues in all women of reproductive age diagnosed with cancer to improve their opportunities to participate in informed decisions regarding their treatment and future reproductive ability. Future researches are needed to better understand the factors that influence patients' choice: these would help physicians to improve the quality of their fertility counseling.

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References

1. Anders CK, Johnson R, Litton J, et al. Breast cancer before age 40 years. Semin Oncol 2009;36:237-49.
2. Lee SJ, Schover LR, Partridge AH, et al. American Society of Clinical Oncology recommendations on fertility preservation in cancer patients. J Clin Oncol 2006;24:2917-31.
3. Johnson JA, Tough S; Society of Obstetricians and Gynaecologists of Canada. Delayed child-bearing. J Obstet Gynaecol Can 2012;34:80-93.
4. Woodruff TK. The Oncofertility Consortium--addressing fertility in young people with cancer. Nat Rev Clin Oncol 2010;7:466-75.
5. Loren AW, Mangu PB, Beck LN, et al. Fertility preservation for patients with cancer: American Society of Clinical Oncology clinical practice guideline update. J Clin Oncol 2013;31:2500-10.
6. Peccatori FA, Azim HA Jr, Orecchia R, et al. Cancer, pregnancy and fertility: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2013;24 Suppl 6:vi160-70.
7. Lambertini M, Anserini P, Levaggi A, et al. Fertility counseling of young breast cancer patients. J Thorac Dis 2013;5:S68-80.
8. Rosen A, Rodriguez-Wallberg KA, Rosenzweig L. Psychosocial distress in young cancer survivors. Semin Oncol Nurs 2009;25:268-77.
9. Ruddy KJ, Gelber SI, Tamimi RM, et al. Prospective study of fertility concerns and preservation strategies in young women with breast cancer. J Clin Oncol 2014;32:1151-6.
10. Duffy CM, Allen SM, Clark MA. Discussions regarding reproductive health for young women with breast cancer undergoing chemotherapy. J Clin Oncol 2005;23:766-73.
11. Hohmann C, Borgmann-Staudt A, Rendtorff R, et al. Patient counselling on the risk of infertility and its impact on childhood cancer survivors: results from a national survey. J Psychosoc Oncol 2011;29:274-85.
12. Armuand GM, Rodriguez-Wallberg KA, Wettergren L, et al. Sex differences in fertility-related information received by young adult cancer survivors. J Clin Oncol 2012;30:2147-53.
13. King JW, Davies MC, Roche N, et al. Fertility preservation in women undergoing treatment for breast cancer in the UK: a questionnaire study. Oncologist 2012;17:910-6.
14. Partridge AH, Gelber S, Peppercorn J, et al. Web-based survey of fertility issues in young women with breast cancer. J Clin Oncol 2004;22:4174-83.
15. Lawrenz B, Jauckus J, Kupka MS, et al. Fertility preservation in >1,000 patients: patient's characteristics, spectrum, efficacy and risks of applied preservation techniques. Arch Gynecol Obstet 2011;283:651-6.
16. Oktay K, Buyuk E, Libertella N, et al. Fertility preservation in breast cancer patients: a prospective controlled comparison of ovarian stimulation with

tamoxifen and letrozole for embryo cryopreservation. J Clin Oncol 2005;23:4347-53.
17. Azim AA, Costantini-Ferrando M, Oktay K. Safety of fertility preservation by ovarian stimulation with letrozole and gonadotropins in patients with breast cancer: a prospective controlled study. J Clin Oncol 2008;26:2630-5.
18. Dalberg K, Eriksson J, Holmberg L. Birth outcome in women with previously treated breast cancer--a population-based cohort study from Sweden. PLoS Med 2006;3:e336.
19. Azim HA Jr, Kroman N, Paesmans M, et al. Prognostic impact of pregnancy after breast cancer according to estrogen receptor status: a multicenter retrospective study. J Clin Oncol 2013;31:73-9.
20. Letourneau JM, Ebbel EE, Katz PP, et al. Pretreatment fertility counseling and fertility preservation improve quality of life in reproductive age women with cancer. Cancer 2012;118:1710-7.

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Editorial

Female breast cancer in Europe: statistics, diagnosis and treatment modalities

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In this issue of the Journal of thoracic diseases, Zeng *et al*. (1) present an extremely interesting synthesis of 145 population-based cancer registries submitting qualified cancer incidence and mortality data to National Cancer Registration Center of China. The authors provide interesting age-standardized incidence and mortality rates, highlighting higher incidence rates in urban areas as well as meaningful geographical disparities.

Based on the most recent data, China exhibits considerably lower incidence and mortality rates for female breast cancer than Europe; more specifically, the age-standardized rate (ASR) per 100,000 was equal to 25.9 in China (1), whereas the respective rates according to GLOBOCAN 2012 were 71.1 for Europe with Greece presenting with more favorable rates (43.9) (2) (*Figure 1A*). Regarding the lower rates in China, the underlying explanation remains elusive as a host of factors including genetic, environmental, lifestyle and somatometric differences have been acknowledged (3,4). As far as the favorable profile of Greece compared to the rest of Europe is concerned, once again the specific explanations remain to be uncovered; nevertheless, genetic differences, adherence to Mediterranean diet, consumption of olive oil, prolonged sun exposure (5-7) may have contributed.

In accordance with incidence rates, China exhibited lower breast cancer mortality rates [6.6 per 100,000 (1)], whereas the respective mortality rate in Europe was equal to 16.1 (2); of note, the discrepancies in mortality rates across Europe were relatively milder (*Figure 1B*). Especially

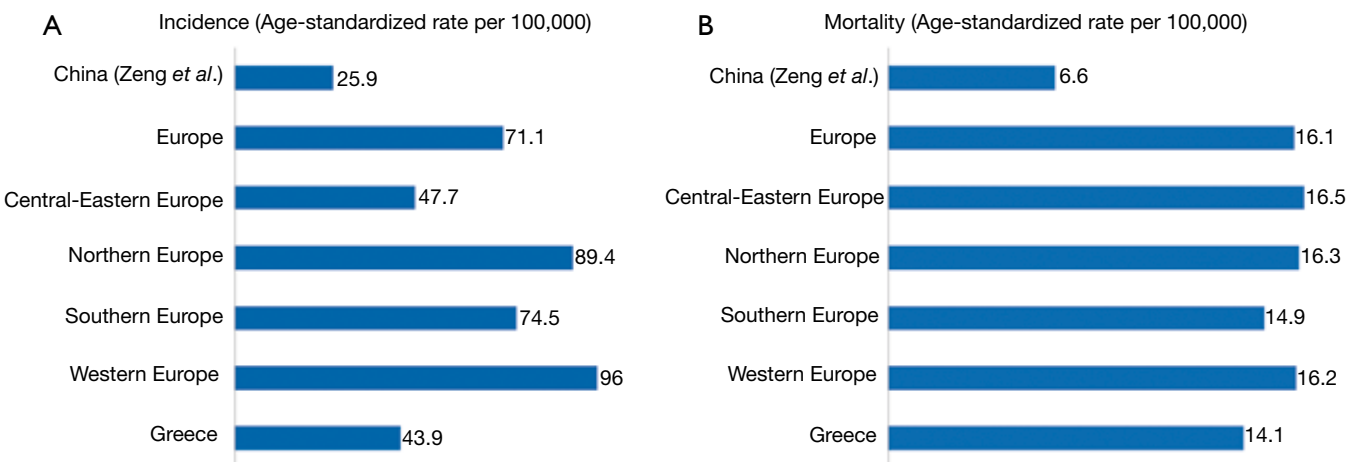

Figure 1 Incidence and mortality age-standardized rate in Europe, according to GLOBOCAN 2012.

regarding Greece, the bridging of the gap with the rest of Europe may be attributed to the lack of State implemented national screening program potentially leading to diagnosis at a more advanced stage, to the existence of geographically isolated, remote regions (small islands and mountainous regions) in the country, etc. Indeed, a recent study published by the Hellenic Cooperative Oncology Group pointed to worst outcomes from breast cancer among women residing in distant Greek regions (8). Nevertheless, these questions remain to be addressed by future studies as detailed relevant nationwide data are not currently available. Of note, previous studies issued by our research team in tertiary Breast Units have highlighted the suboptimal adherence of women to the worldwide breast cancer screening recommendations (9,10).

On the other hand, treatment modalities among Europe do not seem to exhibit significant differences given the common regulatory agency—European Medicines Agency (EMEA)—for the evaluation of approved agents, the adherence to European Society for Medical Oncology (ESMO) guidelines, etc. (11-13). However, national guidelines in each European country exist, exhibiting slight discrepancies in screening, treatment modalities and surveillance of female breast cancer patients.

In conclusion, it seems that the article by Zeng *et al.* (1) provides interesting insight into significant questions regarding female breast cancer epidemiology surpassing the boundaries of China. It seems important to develop careful public health plans, conduct screening strategies and adopt cancer prevention measures in order to guide scientific research applicable and treatment applicable to each country and consequently reduce breast cancer burden.

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References

1. Zeng H, Zheng R, Zhang S, et al. Female breast cancer statistics of 2010 in China: estimates based on data from 145 population-based cancer registries. J Thorac Dis 2014;6:466-70.
2. Ferlay J, Soerjomataram I, Ervik M, et al. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer; 2013. Available online: http://globocan.iarc.fr, accessed on May 20, 2013.
3. Ziegler RG, Hoover RN, Pike MC, et al. Migration patterns and breast cancer risk in Asian-American women. J Natl Cancer Inst 1993;85:1819-27.
4. Adami HO, Hunter D, Trichopoulos D. Textbook of Cancer Epidemiology. NY: Oxford University Press, USA, 2008.
5. Schwingshackl L, Hoffmann G. Adherence to Mediterranean diet and risk of cancer: A systematic review and meta-analysis of observational studies. Int J Cancer 2014. [Epub ahead of print].
6. Engel LS, Satagopan J, Sima CS, et al. Sun exposure, vitamin D receptor genetic variants, and risk of breast cancer in the Agricultural Health Study. Environ Health Perspect 2014;122:165-71.
7. Psaltopoulou T, Kosti RI, Haidopoulos D, et al. Olive oil intake is inversely related to cancer prevalence: a systematic review and a meta-analysis of 13,800 patients and 23,340 controls in 19 observational studies. Lipids Health Dis 2011;10:127.
8. Panagopoulou P, Gogas H, Dessypris N, et al. Survival from breast cancer in relation to access to tertiary healthcare, body mass index, tumor characteristics and treatment: a Hellenic Cooperative Oncology Group (HeCOG) study. Eur J Epidemiol 2012;27:857-66.
9. Domeyer PJ, Sergentanis TN, Katsari V, et al. Screening in the era of economic crisis: misperceptions and misuse from a longitudinal study on Greek women undergoing benign vacuum-assisted breast biopsy. Asian Pac J Cancer Prev 2013;14:5023-9.
10. Zografos GC, Sergentanis TN, Zagouri F, et al. Breast self-examination and adherence to mammographic follow-up: an intriguing diptych after benign breast biopsy. Eur J Cancer Prev 2010;19:71-2.
11. Senkus E, Kyriakides S, Penault-Llorca F, et al. Primary breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2013;24 Suppl 6:vi7-23.
12. Cardoso F, Harbeck N, Fallowfield L, et al. Locally recurrent or metastatic breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2012;23 Suppl 7:vii11-9.
13. Azim HA Jr, Michiels S, Zagouri F, et al. Utility of prognostic genomic tests in breast cancer practice: The IMPAKT 2012 Working Group Consensus Statement. Ann Oncol 2013;24:647-54.

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Editorial

The burden of breast cancer from China to Italy

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Breast cancer is the second most common cancer in the world and the most frequent cancer among women, with an estimated 1.67 million new cancer cases diagnosed in 2012 (25% of all cancers) (1). In Europe the estimated age-adjusted annual incidence of breast cancer in 2012 was 94.2/100 thousand and the mortality 23.1/100 thousand (2). In USA, according to the SEER database [2006-2010], the age-adjusted annual incidence of breast cancer was 123.8/100 thousand and the mortality 22.6/100 thousand (3). The median age at breast cancer diagnosis is 61 years: about 10% of breast cancers occur among women aged younger than 50 years, while 65% occur among women aged 65 years or older (3). Overall, 60% of breast cancers are diagnosed at a localized stage, 32% at a regional stage and 5% at an advanced stage. The 5-year relative survival rate for women diagnosed with localized breast cancer is 98.6%; survival declines to 84.4% for regional stage and 24.3% for distant stage (3). In western countries, due to both early detection through screening programs and the improvement in the available treatment strategies, the percentage of women surviving at least 5 years after diagnosis and treatment has shifted from 74.8% in the early 1970s to 90.3% in the late 1990s (3).

In Italy, it has been estimated that approximately 48,000 new cases of breast cancer has been diagnosed in 2013 (4). Excluding skin cancers, breast cancer is the most common cancer diagnosed in women: a total of 41% are diagnosed in the age group 0-49 years, 36% in patients aged 50-69 years and 21% in women older than 70 years (2,4,5). Breast cancer incidence and prevalence present a marked north-to-south gradient: the incidence rates are respectively 124.9, 100.3 and 95.6 per 100 thousand in the northern, central and southern areas (5). Regarding breast cancer prevalence, the proportion of prevalent cases in the northern area is remarkably higher (2,055-2,331 per 100 thousand) than in the central area (1,795 per 100 thousand) and about twice than in the southern area (1,151 per 100 thousand). In Italy, breast cancer mortality increased until the late 1980s reaching its maximum value at approximately 27 per 100 thousand, and started to decline thereafter (approximately –1.6%/year) (2). The mortality rate started to decline from the late 1980s in the northern central regions and from the mid-1990s in the southern regions. The 5-year relative survival increased from 78% in 1990-1992 to 87% in 2005-2007 (6,7); age standardized mortality rates are lower in the central area (20.6 per 100 thousand) than in the northern (24.7 per 100 thousand) and southern (25.2 per 100 thousand) areas (4).

Breast cancer is a major burden also for Chinese women: Zeng and colleagues recently described the epidemiology of breast cancer in China in 2010, reporting breast cancer statistics by age and geographical area (8). Authors estimated the status of female breast cancer based on existing population-based cancer registries' data available in 2010; these registries covered approximately 12.96% of the overall female population in China. The estimated number of female breast cancer cases was about 208 thousand; the overall crude incidence rate was 32.43 per 100 thousand, accounting for 16.2% of all cancer cases in Chinese women (first cause of cancer diagnosis). The rates standardized by World population and by China population were 24.20 per 100 thousand and 25.89 per 100 thousand respectively. The estimated number of female breast cancer death was about 55.5 thousand with an overall crude mortality rate of 8.65 per 100 thousand, accounting for 7.90% of all cancer deaths (fifth cause of cancer deaths in Chinese women). After age standardization by China population and World population, the standardized rates were 6.56 per 100 thousand and

6.36 per 100 thousand respectively. After stratification by area, the incidence of breast cancer was higher in urban area than in rural area. The age-specific incidence rate resulted relatively low before 25 years old, but dramatically increased after then; the trend of age-specific incidence in urban and rural area were similar as the overall incidence. The mortality rates by geographical area had a similar pattern as the incidence rates, increasing with age. In conclusion, breast cancer is still a major health burden in China, especially for women living in urban area; authors suggested that prevention strategies (for example weight control and breastfeeding promotion), high quality screening and diagnosis might help to reduce breast cancer mortality and to control the disease (8).

In the interpretation of the epidemiology of breast cancer, multiple risk factors, the implementation of breast cancer screening and the improvement in cancer therapy should be taken into account. In recent years, the substantial progresses in the management of stage I-III breast cancer led to a better prognosis for breast cancer patients. However, many women every year in the world die for disease; strategies for primary and secondary prevention should be implemented to reduce the burden of breast cancer.

Several risk factors are associated with breast cancer risk; they are divided into two categories: the non-modifiable and the potentially modifiable risk factors (9). The purpose of primary prevention is to reduce the incidence of cancer, keeping under control the modifiable risk factors, such as: life-style (obesity, alcohol consumption, diet rich in carbohydrates and saturated fats, physical inactivity), some reproductive factors (nulliparity, old age at first pregnancy, no breastfeeding) and use of hormone replacement therapy.

Petracci and colleagues identified three modifiable risk factors in Italian women (physical activity, alcohol consumption and body mass index) on which to base prevention strategies (9). Authors showed that a regular daily physical activity combined with a balanced mediterranean diet can reduce the risk of postmenopausal breast cancer women of approximately 1.6% in 20 years, rising to 3.2% in women with a positive family history and 4.1% in women at high risk (9).

Not only the improvements in lifestyle but also a pharmacological prophylactic treatment (chemoprevention) seems to have an important role in reducing the incidence of breast cancer, especially for women at high risk (defined as a 10-year risk of breast cancer of 5% or more). Recently, Cuzick and colleagues performed an individual participant data meta-analysis to better clarify the role of selective estrogen receptor modulators (SERMs) in the primary prevention of breast cancer (10). Authors showed an overall 38% reduction in breast cancer incidence with the use of tamoxifen or other SERMs [hazard ratio (HR) 0.62, 95% confidence interval (CI) 0.56-0.69]; the benefit was shown only for estrogen receptor positive breast cancer (HR 0.49, 95% CI: 0.42-0.57) and not for estrogen receptor negative tumours (HR 1.14, 95% CI: 0.90-1.45) (10). Two recently published randomized trials assessed the role of aromatase inhibitors (AIs) for breast cancer prevention in high risk postmenopausal women. In the MAP.3 trial, exemestane significantly reduced the incidence of all breast cancer by 53% (HR 0.47, 95% CI: 0.27-0.79) and invasive breast cancer by 65% (HR 0.35, 95% CI: 0.18-0.70) after a median follow-up of 3 years (11). The IBIS-II trial, after a median follow-up of 5.0 years, showed a reduction in the risk of breast cancer and ductal carcinoma in situ by more than 50% with the use of anastrozole (HR 0.47, 95% CI: 0.32-0.68) (12). Both trials recorded no significant differences between groups for cardiovascular events; many side-effects associated with estrogen deprivation were poorly more frequent in the treatment group than in the placebo group, indicating that most of these symptoms are not drug related. Based on this data, the American Society of Clinical Oncology (ASCO) guidelines recommend the use of chemoprevention in women at increased risk of breast cancer, defined as individuals with a 5-year projected absolute risk of breast cancer ≥1.66% (based on the National Cancer Institute Breast Cancer Risk Assessment Tool or an equivalent measure) or women diagnosed with lobular carcinoma in situ. Specifically, in women at increased risk of breast cancer older than 35 years, tamoxifen (20 mg per day for 5 years) should be discussed as an option to reduce the risk of estrogen receptor-positive breast cancer; in postmenopausal women, raloxifene (60 mg per day for 5 years) and exemestane (25 mg per day for 5 years) should also be discussed as options (13). In Italy the use of SERMs or AIs for breast cancer prevention is only possible so far in clinical trials or as "off-label" drugs.

The goal of secondary prevention is to detect cancer at an early stage; among the current available screening methods, mammography seems to be the most sensitive and specific. Mammography screening has been shown to have the greatest effect on breast cancer mortality reduction in the age group of 50-69 years and it is recommended by the European Society for Medical Oncology (ESMO) guidelines in this age group (14). In contrast, the role of

mammography screening in women aged 40-49 years is debated and seems to be associated with lower efficacy and cost-effectiveness (14).

The increase in survival among breast cancer patients observed in Italy since 1990, is consistent with the improvement in local and systemic treatment and, above all, with the diffusion of screening programs. Organized screening programs started at the beginning of the 1990s and contributed to an increase in breast cancer detected at an early stage and with a better prognosis (15). They were first restricted to certain northern areas, including less than 5% of the target population (16). From the second half of the 1990s different screening programs were activated in central Italy coming to involve 15% of the women between 50 and 69 years (16). In Italy, breast cancer screening is based on bilateral mammography and is recommended every two years in women aged 50-69 years. In women aged 40-49 years, mammography should be performed according to individual risk factors (for example family history and the density of breast tissue); recently, according to the National Plan for Prevention 2005-2007 mammographic screening should be extended in women with 45-49 years of age, performed every 12-18 months. For women older than 70 years, there is no evidence of the efficacy of mammography screening and therefore is not recommended. Since 2007, the organized screening activity is present in all Italian regions but with great differences in the adherence among the different geographical areas. Data analysis from 2000-2010 shows that crude attendance rate reached an acceptable value of 50%, but with a higher level of participation in northern and central Italy as compared to southern Italy where attendance rates are still inadequate and do not reach the acceptable standard (17). The rise in breast cancer incidence showed in Italy during the 1990s might be explained by the effect of screening, especially in the northern-central area, while the stabilization of incidence rates estimated for many northern-central regions from the late 1990s could be explained by the exhaustion of the initial screening effect (18). Such a situation cannot yet be hypothesized for southern regions because of the late initiation and the low adherence to the screening program.

The improvements in breast cancer care, including the more widespread use of adjuvant systemic therapies, and the progress in radiotherapy and surgery, are likely to be the major determinants of the mortality reduction in Italy in the late 1980s and early 1990s, before the screening program could have produced its effect. The decrease observed in mortality for the northern-central regions from the late 1980s and for Italy as a whole from the early 1990s may be related to both improved treatment and early detection diffusion. The delay in the mortality decline in the southern regions may be explained with the limited coverage areas of screening programs, the lesser attendance in screening programs with more late stage diagnosis of breast cancer and the disparities in cancer care with respect to northern-central regions (18,19). The current gap in the breast cancer prevalence between northern-central and southern regions is mainly attributable to the pronounced differences in past levels, which were much higher in the northern and central areas than in the south (20).

In conclusion, breast cancer is still a major global health problem. The geographical variation in female breast cancer burden can be partially explained by differences in the risk factors and in the distribution of screening among different countries and among different regions within the same country. Lifestyle interventions such as weight reduction, low-fat diet, reduced alcohol intake and exercise should be included in programs to prevent breast cancer, and for women at high risk of breast cancer, where available, chemoprevention strategies could also be proposed. Moreover, a more widespread screening activity would help to close the gap between countries and regions within the same country. A continuous monitoring of regional epidemiological indicators for breast cancer is very important to evaluate the effect of different health measures taken to control breast cancer.

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References

1. Available online: Globocan 2012: http://globocan.iarc.fr/Default.aspx
2. Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, et al. Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. Eur J Cancer 2013;49:1374-403.
3. Surveillance, Epidemiology, and End Results Program. Available online: http://seer.cancer.gov/
4. AIRTUM Working Group. Italian cancer figures, report 2013: Multiple tumours. Epidemiol Prev 2013;37:1-152.
5. Rossi S, Crocetti E, Capocaccia R, et al. Estimates of cancer burden in Italy. Tumori 2013;99:416-24.
6. Sant M, Allemani C, Santaquilani M, et al.

EUROCARE-4. Survival of cancer patients diagnosed in 1995-1999. Results and commentary. Eur J Cancer 2009;45:931-91.
7. De Angelis R, Sant M, Coleman MP, et al. Cancer survival in Europe 1999-2007 by country and age: results of EUROCARE--5-a population-based study. Lancet Oncol 2014;15:23-34.
8. Zeng H, Zheng R, Zhang S, et al. Female breast cancer statistics of 2010 in China: estimates based on data from 145 population-based cancer registries. J Thorac Dis 2014;6:466-70.
9. Petracci E, Decarli A, Schairer C, et al. Risk factor modification and projections of absolute breast cancer risk. J Natl Cancer Inst 2011;103:1037-48.
10. Cuzick J, Sestak I, Bonanni B, et al. Selective oestrogen receptor modulators in prevention of breast cancer: an updated meta-analysis of individual participant data. Lancet 2013;381:1827-34.
11. Goss PE, Ingle JN, Alonanni B, et al. Selective oestrogen receptor modulators in prevention of breast cancer: an updated meta-analysis of i
12. Cuzick J, Sestak I, Forbes JF, et al. Anastrozole for prevention of breast cancer in high-risk postmenopausal women (IBIS-II): an international, double-blind, randomised placebo-controlled trial. Lancet 2014;383:1041-8.
13. Visvanathan K, Hurley P, Bantug E, et al. Use of pharmacologic interventions for breast cancer risk reduction: American Society of Clinical Oncology clinical practice guideline. J Clin Oncol 2013;31:2942-62.
14. Senkus E, Kyriakides S, Penault-Llorca F, et al. Primary breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2013;24 Suppl 6:vi7-23.
15. Foca F, Mancini S, Bucchi L, et al. Decreasing incidence of late-stage breast cancer after the introduction of organized mammography screening in Italy. Cancer 2013;119:2022-8.
16. Giordano L, Giorgi D, Piccini P, et al. Time trends of process and impact indicators in Italian breast screening programmes--1996-2005. Epidemiol Prev 2008;32:23-36.
17. Giordano L, Giorgi D, Ventura L, et al. Time trends of process and impact indicators in Italian breast screening programmes (2000-2010). Epidemiol Prev 2012;36:28-38.
18. Grande E, Inghelmann R, Francisci S, et al. Regional estimates of breast cancer burden in Italy. Tumori 2007;93:374-9.
19. Sant M, Minicozzi P, Allemani C, et al. Regional inequalities in cancer care persist in Italy and can influence survival. Cancer Epidemiol 2012;36:541-7.
20. Guzzinati S, Buzzoni C, De Angelis R, et al. Cancer prevalence in Italy: an analysis of geographic variability. Cancer Causes Control 2012;23:1497-510.

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Perspective

Tyrosine kinase inhibitors re-treatment beyond progression: choice and challenge

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Abstract: Tyrosine kinase inhibitors (TKIs) are highly effective agents for the treatment of lung cancer which harbors activated gene mutation. However, for patients with failure of TKI, TKI re-treatment beyond progression (TRBP) is still a potential option that has been proven by many literatures. In this review, we summarize the clinical evidence of TRBP and discuss the potential mechanisms to overcome TKI-acquired resistance

Keywords: Non-small cell lung cancer (NSCLC); tyrosine kinase inhibitor (TKI); re-treatment; EGFR; acquired resistance

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In the past decade, tyrosine kinase inhibitors (TKIs) including gefitinib and erlotinib have been proven effective in the treatment of patients with non-small cell lung cancer (NSCLC) (1,2). Moreover, it's reported that TKI re-treatment beyond progression (TRBP) also contributes to achieve a long-term survival (3,4). However, who should receive TRBP? This issue is still unclear.

Predictors

Generally, the best evidence for TRBP predictors should be derived from large-scale randomized control trails or high-level Meta-analysis. Unfortunately, it's lack now. Two on-going studies including ASPIRATION and IMPRESS may address this issue soon. Recently *EGFR* gene mutation is recognized as the best predictor of TKI response. Some studies suggest it a potential predictor of TRBP for NSCLC patients (4). However, as we know, *EGFR* gene tests are only launched in very few hospitals of China. A large number of patients can not do such tests in primary hospitals without test equipment. Thus, those patients have to be switched to chemotherapy without opportunity to receive TRBP. Moreover, regardless of re-biopsy risk, expensive cost of health care is another question. A convenient and effective predictor is required urgently. In fact, gene tests are not irreplaceable. Even for EGFR mutation test, the best predictor to TKI response, the predictive accuracy is 70% approximately. It may be taken place by the other approaches such as clinically identified models.

In the previous reports (5-7), TRBP has been proven effective for TKI responders. A Japanese report showed that a 39-year-old male patient received initial crizotinib treatment and achieved a significant response persisted for 4 months, after disease progression, crizotinib was discontinued. However, five months after that, crizotinib was re-administrated and still achieved a significant response persisted for 2.5 months (8). Another study also suggests that TKI re-treatment is better option after failure of TKI treatment for NSCLC patients once responded from the prior TKI treatment (9). Taken together, initial response to TKI may be a potential predictor of TRBP. Based on these promising findings, a series of clinically TKI-failure models were established and explored using Bayesian discriminant analysis, named as dramatic progression group, gradual progression group and local progression group. It is a novel method derived from clinical experiences and mathematical calculation. Novel criteria for TKI failure models in NSCLC were addressed (10). Conclusively, TRBP should

be given in those with slow disease progression or less new lesions rather in those with massive disease progression. It's meaningful in the developing countries.

Mechanisms

TKI-acquired resistance has many sorts of mechanisms. The main mechanisms are summarized as follows (11): (I) EGFR signaling pathway is abnormal such as EGFR amplification, T790M mutation and other components disorders; (II) regardless of whether EGFR signaling pathway is normal or abnormal, the other signaling bypasses take its place, such as c-MET amplification and PIK3CA mutation; (III) histopathological features have been changed, such as small cell lung cancer (SCLC) transformation and epithelial to mesenchymal transition (EMT). To overcome these, the relative therapies are various. EGFR-TKI re-treatment is one of them that may be due to the complicated molecular reactions. Until now, it is unclear. To our knowledge, we speculate that despite of disease progression, EGFR signaling crosstalk remains stationary so that it plays a role on cancer cell potentially. The EGFR pathway factors could be re-activated by TKI re-treatment or other regimens. Thus, TKI re-treatment with or without time interval could be still at work. However, for patients with EGFR pathway dysfunction, such as rapid progression with SCLC transformation, TKI re-treatment might be out of work.

Hypothesis

Until now, TKI-retreatment is an extremely controversial topic. Who and when should receive TKI-retreatment after failure of TKI? Although it is unclear, we proposed our hypothesis as follows: three time-points are supposed in a TKI-using patient's history including time-point before initial TKI treatment (named as "A"), time-point after initial TKI treatment but before TRBP (named as "B"), and time-point after TRBP (named as "C"). In our hypothesis, two groups ("a" and "b") are defined as a set of potential predictors in their corresponding time-points ("A" and "B"), including initial responses, gene tests by biopsy/re-biopsy, best change of baseline, biomarker expression and other criteria.

Generally, a time interval is shorter, a prediction is more accurate; however, applicable value of prediction should be decreased due to a narrow time window. Thus, EGFR mutation has been proven to be the best predictor in group "a" for prediction of TKI initial response (B), but may be not the best in group "b" for TRBP (C). Some clinical feathers such as initial response in group "b" should be better in terms of inexpensive cost and available definition for TRBP indications. In the previous report, three mainly clinical parameters as duration of disease control (DDC), the volume doubling time (VDT) of target lesions and scores for progression in non-target lesions. Additionally, scores for clinical symptom were analyzed as well (10). In another report in ESMO 2012 (Abstract 1253p), NSCLC patients initially responded to TKI (CR, PR and SD) should be suitable to receive TRBP. The benefit rate (SD and PR) is approximate 30-33% (5). Therefore, initial response which defined as best TKI response lasting for four weeks represents time duration to TKI response before VDT in time-axis. It is an important parameter so that should be analyzed. However, clinical symptoms which represents patient's basic diseases and performance status (PS) are complicated and non-specific. In previous reports, patients with old age or poor PS scores have been advised to receive TRBP (3,4). Thus, it should be recommended as a candidate parameter with a considerable weight.

Conclusions

Definitely, TRBP based on some intrinsic properties deserves further investigations. To date, more and more studies focused on clinical parameters rather than molecular features for prediction of TKI treatment such as first cycle rash (12). Herein, clinical features such as initial response may imply a good response to TRBP. It will be approved by two prospective studies (IMPRESS and ASPIRATION), and able to select the candidates for TRBP efficiently and inexpensively.

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References

1. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 2009;361:947-57.
2. Shaw AT, Yeap BY, Solomon BJ, et al. Effect of crizotinib on overall survival in patients with advanced non-small-cell lung cancer harbouring ALK gene rearrangement: a retrospective analysis. Lancet Oncol 2011;12:1004-12.
3. Camidge DR, Bang YJ, Kwak EL, et al. Activity and safety of crizotinib in patients with ALK-positive non-small-cell

lung cancer: updated results from a phase 1 study. Lancet Oncol 2012;13:1011-9.
4. Nishie K, Kawaguchi T, Tamiya A, et al. Epidermal growth factor receptor tyrosine kinase inhibitors beyond progressive disease: a retrospective analysis for Japanese patients with activating EGFR mutations. J Thorac Oncol 2012;7:1722-7.
5. Tu L, Sun L. Re-challenge treatment of small-molecular inhibitors in NSCLC patients beyond progression. J Thorac Dis 2012;4:647-9.
6. Kim YH, Fukuhara A, Mishima M. Should Epidermal Growth Factor Receptor-Tyrosine Kinase Inhibitor Be Continued beyond Progressive Disease? Case Rep Oncol 2011;4:470-4.
7. Watanabe S, Tanaka J, Ota T, et al. Clinical responses to EGFR-tyrosine kinase inhibitor retreatment in non-small cell lung cancer patients who benefited from prior effective gefitinib therapy: a retrospective analysis. BMC Cancer 2011;11:1.
8. Matsuoka H, Kurata T, Okamoto I, et al. Clinical response to crizotinib retreatment after acquisition of drug resistance. J Clin Oncol 2013;31:e322-3.
9. Song Z, Yu X, He C, et al. Re-administration after the failure of gefitinib or erlotinib in patients with advanced non-small cell lung cancer. J Thorac Dis 2013;5:400-5.
10. Yang JJ, Chen HJ, Yan HH, et al. Clinical modes of EGFR tyrosine kinase inhibitor failure and subsequent management in advanced non-small cell lung cancer. Lung Cancer 2013;79:33-9.
11. Sequist LV, Waltman BA, Dias-Santagata D, et al. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. Sci Transl Med 2011;3:75ra26.
12. Lee SM, Khan I, Upadhyay S, et al. First-line erlotinib in patients with advanced non-small-cell lung cancer unsuitable for chemotherapy (TOPICAL): a double-blind, placebo-controlled, phase 3 trial. Lancet Oncol 2012;13:1161-70.

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Original Article

Nocturnal pulse rate and symptomatic response in patients with obstructive sleep apnoea treated with continuous positive airway pressure for one year

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Background: Obstructive sleep apnoea (OSA) is the most common form of sleep-disordered breathing and a known risk factor for cardiovascular disease. We hypothesised that in patients with OSA the characteristics of nocturnal pulse rate (PR) are associated with changes in blood pressure and daytime sleepiness, following commencement of continuous positive airway pressure (CPAP) therapy.

Methods: Pulse oximetry data, demographics, daytime sleepiness and blood pressure were recorded at baseline and at one year follow up. Patients with OSA were grouped according to positive and negative changes in the PR (ΔPR) response during the first night of pulse oximetry before commencement of CPAP.

Results: A total of 115 patients (58 with OSA and 57 matched subjects without OSA) were identified and included in the analysis. The scale of improvement in daytime sleepiness could be predicted by a negative or positive ΔPR, as recorded in the initial screening pulse oximetry [ΔESS –5.8 (5.1) *vs.* –0.8 (7.2) points, P<0.05]. A negative correlation was observed between mean nocturnal PR and changes in systolic blood pressure (SBP) after one year of CPAP treatment (r=–0.42, P<0.05).

Conclusions: Mean nocturnal PR prior to CPAP initiation was associated with changes in SBP at one year follow up. A descending nocturnal PR in patients with OSA, prior to CPAP initiation, might help to identify a symptomatic response from long term CPAP treatment.

Keywords: Blood pressure; oximetry; continuous positive airway pressure (CPAP); Epworth sleepiness scale (ESS)

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Background

Obstructive sleep apnoea (OSA) is the most common form of sleep-disordered breathing (1), it contributes to increased mortality rates secondary to associated cardiovascular (2) and metabolic risks (3), with cognitive deficits impairing work efficiency (4). It is characterised by repetitive episodes of nocturnal apnoeas or hypopnoeas with arousal from sleep leading to an increased sympathetic tone (5).

Continuous positive airway pressure (CPAP) is the most effective available treatment for moderate to severe OSA (6), reducing apnoeas, hypopnoeas and, in symptomatic responders, daytime symptoms like sleepiness (7). However, not every patients benefits symptomatically (8) and the long-term impact of CPAP on arterial blood pressure and cardiovascular risks is not entirely understood: while the acute effects of CPAP on blood pressure have been clearly demonstrated, long term outcomes are still equivocal (9). Key mechanisms linking sleep-disordered breathing and

cardiovascular problems are likely to be multi-factorial and potentially involve sympathetic activity (10), intermittent hypoxia (11), inflammation (12), hyper-coagulation (13) and endothelial dysfunction (14).

Heart rate is a predictor of hypertension and a major risk factor for cardiovascular mortality in non-OSA populations, despite considerable differences between the genders (heart rate-to-blood pressure correlation) (15). Increased heart rate in OSA may derive from a high sympathetic tone and previous studies have shown that an increased sympathetic activation is associated with OSA in men but the evidence is lacking in women. Sympathetic activation leads to dysregulation of the circadian heart rate rhythm, potentially affecting systemic arterial blood pressure, heart rate and cardiac arrhythmias (16). Non-invasive monitoring and recording of nocturnal pulse rate (PR), a surrogate of heart rate (17), is safe, widely available and could be used to better understand the sympathetic activity in OSA (18,19).

It is important to predict long-term symptomatic outcomes and associated cardiovascular risks in patients with OSA when commencing them on CPAP therapy. We hypothesised that nocturnal PR in patients with OSA, measured during the diagnostic night as a marker for sympathetic tone, can help to predict long term changes in daytime sleepiness and blood pressure that are associated with CPAP therapy.

Patients and methods

The study was approved by the local institution's review board (registration number: 2012-2773). Patients referred to the Sleep Disorders Centre at Guy's & St. Thomas' NHS Foundation Trust, London, UK for suspected OSA were screened using nocturnal pulse oximetry (Pulsox 300i, Konica Minolta sensing Inc, Hachioji, Tokyo, Japan) for two consecutive nights at home. We analysed the pulse and oxygen trace with PULSOX DS-5 software (Anandic Medical Systems, Feuerthalen, Zurich/Switzerland). The device gives the moving average of the last eight PRs at one-second intervals. We selected the night with the better quality and longer recording time for further analysis.

Inclusion criteria for the OSA group were clinical presentation suspicious of OSA plus a 4% oxygen desaturation index (4% ODI) ≥5/h. We also included a matched control group of patients without OSA. The inclusion criteria were an ODI <5/h and a pulse rise index (PRI) lower than 20/h. The PRI is defined as an increase in the PR by more than six beats per minute and these events are counted over a one hour period. Patients without significant OSA can exhibit an increased PRI caused by periodic limb movements or other underlying conditions leading to autonomic arousals and an increase sympathetic tone. We therefore decided to exclude subjects from the control group if they had an abnormal high PRI. On the other hand, patients with OSA have commonly an elevated PRI and, therefore, we did not use this criterion for the OSA group. We selected the threshold of five events/hour for the 4% ODI in line with recommendations for the apnoea-hypopnoea-index and the National Institute of Clinical Excellence (NICE) guidance (7).

Exclusion criteria for both groups were ongoing use of heart rate-affecting medications (digoxin, anti-arrhythmic agents, beta-blockers or calcium antagonists, including verapamil and diltiazem), chronic atrial fibrillation and other paroxysmal or permanent arrhythmias, second or third degree atrio-ventricular block, permanent pacemaker, diagnosis of periodic limb movement disease (PLMD), age under 18 and over 70 years old, pregnancy, acute/critical illness (decompensated heart failure or end stage COPD and renal failure), patients admitted to a hospital ward.

Patients who discontinued CPAP over the study period were excluded, as were those who were lost to follow up and those with corrupted pulse oximetry data. The pulse oximetry data of the patients with a clear history of snoring, witnessed apnoeas and excessive daytime sleepiness [EDS, as assessed by the Epworth sleepiness scale (ESS) >10 points] (20), were subsequently reviewed by a physician experienced in sleep medicine and the diagnosis of OSA was confirmed (4% ODI of ≥15/h, or ≥5/h plus elevated ESS) and CPAP titration was initiated. CPAP treatment was started after 2-weeks of pressure titration using an AutoSet device (APAP, S8/S9, ResMed Ltd, Sydney, Australia). Optimal CPAP pressure was identified as the 95th percentile of APAP pressure. Upon initiation of CPAP therapy, patients attended an induction by specialised sleep technicians, blood pressure and weight were recorded. Patients with treated OSA were followed to assess weight, blood pressure, ESS and adherence to CPAP therapy at one year. To study the PR trend throughout the night, we calculated the difference between mean PR of the last and the first hour of the nocturnal recording. In order to be consistent throughout the study we decided that the first and last hour of pulse oximetry was likely not to represent sleep. The patients were further analysed in a subgroup according to ascending (positive ΔPR) or descending (negative ΔPR) PR.

Blood pressure was measured with an automated

Table 1 Demographics, clinical characteristics, oximetry results in OSA and non-OSA patients

Demographics and baseline measurements	OSA group (n=58)	Non OSA group (n=57)	P value
Age (years)	49.4 (1.2)	46.4 (1.7)	0.161
Weight (kg)	109.7 (3.6)	95.0 (7.0)	0.105
ESS (points)	12.4 (0.6)	8 (0.8)	<0.0001
Sex (males/females)	36/22	34/23	0.850
ODI (h^{-1}), at baseline	28.9 (3.3)	2.0 (0.1)	<0.0001
Nocturnal pulse rise index (>6 bpm, h^{-1})	46.0 (2.5)	13.1 (0.6)	<0.0001
Mean pulse rate (bpm)	71.7 (1.2)	62.3 (1.4)	<0.0001
Mean SpO_2 (%)	92.7 (0.4)	95.4 (0.2)	<0.0001
Mean pulse rate (1st hour of sleep, bpm)	76.1 (1.5)	65.5 (1.6)	<0.0001
Mean pulse rate (last hour of sleep, bpm)	69.5 (1.2)	60.5 (1.4)	<0.0001
ΔNocturnal pulse rate during a single night (bpm)	–6.6 (0.9)	–4.9 (0.7)	0.148

ODI, oxygen desaturation index; Δ, difference between mean pulse rates of the first versus last hour of nocturnal recording; bpm, beats per minute; ESS, Epworth sleepiness scale.

device (Mindray VS-800, Medical International Limited, Shenzhen, China) according to the American Heart Association reccomendations (21). Subjective daytime sleepiness was assessed using the ESS (20). The primary end point was to compare mean PR and ΔPR of the first screening night of pulse oximetry with the change of blood pressure at one year in patients with OSA treated with CPAP. Our secondary end point was to determine whether changes in daytime sleepiness (ΔESS) differed between patients with different PR responses (ΔPR) over one year of CPAP treatment. Adherence to treatment and compliance were measured at follow up as the number of hours per night and the overall percentage of CPAP usage.

Statistical analysis

Statistical analysis of the data was performed using GraphPad Prism (Version 5.02, GraphPad Software Inc, San Diego, California/USA). Data are reported as mean (standard deviation, SD), if not otherwise indicated, correlations were stated including the 95% confidence interval (CI). Following testing for normality, similarity of two means was compared using student's *t*-test and Chi-square or Fisher's exact tests statistics in case of normal distribution; otherwise Wilcoxon signed-rank test was used. Within group variables were compared using student *t*-test for paired data. Spearman's rank correlation coefficient was calculated for non-normally distributed variables. For all tests, a value of P<0.05 was considered significant (22).

Results

In the final analysis, we included 58 patients with OSA [36 males, age 49.4 (1.2) years, weight 109.7 (3.6) kg] and 57 without OSA [34 males, age 46.4 (1.7) years, weight 95.0 (7.0) kg].

The two groups were matched regarding gender (P=0.850), age (P=0.161) and weight (P=0.105). Patients with OSA were more sleepy (P<0.0001). The ODI, PRI, mean PR, mean saturation and ΔPR were significantly different between the groups (P<0.0001, respectively; *Table 1*).

Mean PR was higher in male subjects with OSA compared to men without OSA [71.3 (1.6) *vs.* 58.5 (1.7) min^{-1}, P<0.0001]; in the control group female subjects had a higher mean PR than men [68.1 (2.1) *vs.* 58.4 (1.7) min^{-1}, P<0.001].

In the OSA group, ΔPR correlated negatively with mean oxygen saturation (r=–0.39, 95% CI, –0.59 to –0.13, P<0.01), as did the mean PR (r=–0.4, 95% CI, –0.6 to –0.14, P<0.01). The mean PR and the change in systolic blood pressure (ΔSBP) at one year follow up correlated negatively (r=–0.42, 95% CI, –0.66 to –0.1, P<0.05; *Figure 1*). There was no correlation between ΔPR and systolic (r=–0.049, P=0.77) or diastolic blood pressure (DBP) (r=–0.146, P=0.38).

Following diagnosis of OSA and commencing on CPAP treatment, patients in the OSA group at one year follow up had used the device for 5.0 (1.9) hours per night with an average total percentage of nights used of 63.0% (36.7%).

In a subgroup analysis of patients with OSA who were grouped depending on the change in nocturnal PR (positive

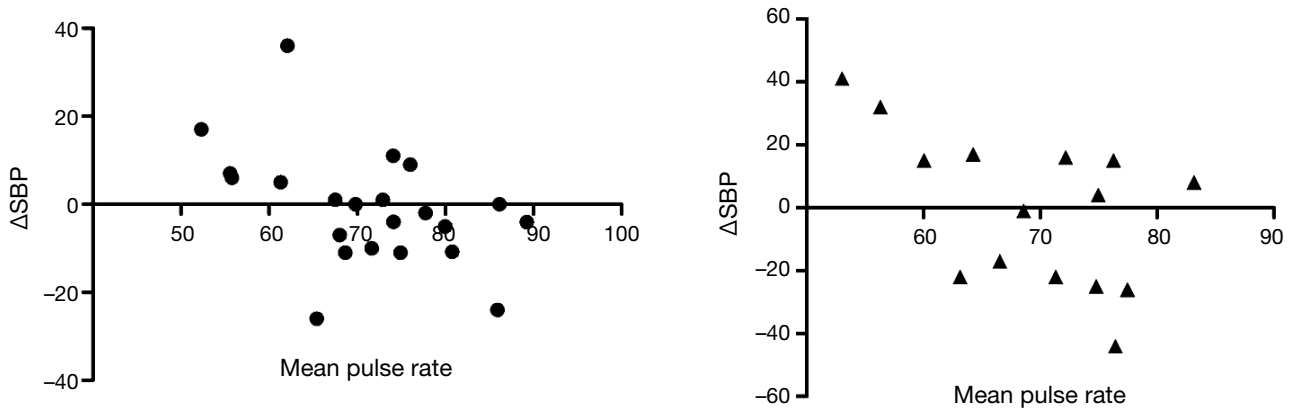

Figure 1 Negative association between changes in systolic blood pressure over one year and mean nocturnal heart rate at baseline in patients with obstructive sleep apnoea. Men (n=36/58, circles, r=–0.44, 95% CI, –0.73 to –0.01, P<0.05) and women (n=22/58, triangles, r=–0.54, 95% CI, –0.83 to –0.034, P<0.05). SBP, systolic blood pressure; Δ, delta.

Table 2 Change in weight, blood pressure and sleepiness between baseline and follow up in OSA patients

	OSA group		
	Baseline	One year follow up	P value
Weight	109.7 (3.6)	108.1 (24.6)	0.115
SBP	134.6 (14.2)	135.9 (17.4)	0.745
DBP	85.1 (9.2)	85.1 (9.1)	0.960
ESS	12.4 (0.6)	7.5 (5.3)	<0.001

SBP, systolic blood pressure; DBP, diastolic blood pressure; ESS, Epworth sleepiness scale; N/A, not applicable.

vs. negative ΔPR) we found that, at baseline, the weight of the patients with a positive ΔPR (n=11) was higher [124.4 (26.6) *vs.* 104.8 (23.1) kg, P<0.05] and the patients were more sleepy compared to the patients with a negative ΔPR (n=47) [10.5 (5.8) *vs.* 6.9 (4.9) points in the ESS, P<0.05]. At one year follow up, there was no significant difference between the groups with respect to SBP or DBP), but the level of daytime sleepiness had significantly reduced in those with a negative ΔPR compared to those with a nocturnal rise in the PR [ΔESS –5.8 (5.1) *vs.* –0.8 (7.2) points, P<0.05, *Tables 2* and *3*].

Discussion

The mean nocturnal PR of patients with OSA is elevated when compared to those who do not experience nocturnal sleep disruption and the nocturnal change in PR (ΔPR) is associated with a better symptomatic response to CPAP treatment at one year. In OSA patients, mean nocturnal PR correlated inversely with ΔSBP in the one year follow up. While most of the OSA patients demonstrated a reduction in nocturnal PR (negative ΔPR), those with a rise in nocturnal PR (positive ΔPR) revealed lower mean oxygen saturations at baseline and had symptomatically responded less to CPAP treatment at one year.

Clinical significance

Various factors may influence nocturnal PR. Non-rapid eye movement (REM) sleep is associated with reduced sympathetic nerve activity, reduction in blood pressure and lower heart rate, whilst REM sleep is associated with more irregular ventilation and increases in blood pressure and heart rate (5). Our data support the hypothesis that an increased PR in patients with OSA reflects an enhanced sympathetic activation (23) and therefore contributes to the development of hypertension in OSA patients, representing a major risk factor for cardiovascular and non-cardiovascular morbidity and death (15).

Understanding nocturnal PR and its trend during the night appears to be important for patients with sleep apnoea, but has not been considered yet as a diagnostic or prognostic tool. Nocturnal pulse oximetry is frequently used for the diagnosis of sleep apnoea in European-based sleep centres (24), but more recently its use has also been assessed elsewhere (25). Complementing the analysis of the recorded oxygen trace by considering the trend of the nocturnal PR might help to early stratify sleep apnoea patients who are

Table 3 Demographics, clinical characteristics, oxymetry results and treatment data in the OSA group, stratified into ascending (+ΔPR) and descending (–ΔPR) nocturnal pulse rate

	OSA group (n=58)		P value
	Negative ΔPR (n=47)	Positive ΔPR (n=11)	
Demographics			
Age (years)	48.6±9.9	52.7±6.7	0.200
Sex (males/females)	36/11	5/6	0.964
Weight (kg)	107.1±26.0	122.7±26.2	0.105
Measurements at baseline			
ESS (points)	12.7±5.0	11.4±4.3	0.429
SBP (mmHg)	133.8±15.1	138.3±8.1	0.457
DBP (mmHg)	85.4±9.3	84.0±9.1	0.729
ODI (h^{-1})	27.7±19.9	35.9±42.4	0.317
Nocturnal pulse rise index (h^{-1}, >6 bpm)	45.6±17.7	47.8±26.6	0.737
Mean pulse rate (bpm)	71.4±8.8	73.07±11.9	0.591
Mean SpO_2 (%)	93.2±2.2	90.96±3.9	0.012
Measurements at one year follow up			
Weight (kg)	104.8±23.1	124.4±26.6	0.028
ESS (points)	6.9±4.9	10.6±5.8	0.038
SBP (mmHg)	133.9±15.4	145.6±24.1	0.107
DBP (mmHg)	84.1±9.1	89.9±8.3	0.129
Average daily use of CPAP (hours/night)	5.18±1.9	4.5±2.2	0.303
CPAP/nights used (%)	65.5±36.3	50.8±38.4	0.276
Delta between baseline and one year follow up			
ΔWeight (kg)	–1.5±4.9	1.6±7.3	0.115
ΔESS (points)	–5.8±5.1	–0.8±7.2	0.010
ΔSBP (mmHg)	–0.2±21.6	7.3±23.6	0.418
ΔDBP (mmHg)	–1.5±13.0	5.9±11.3	0.180

ODI, oxygen desaturation index; Δ, delta; bpm, beats per minute; ESS, Epworth sleepiness scale.

likely to symptomatically respond to treatment.

Kawano *et al.* (26) have recently shown that mean heart rate over 24 hrs correlates well with the apnoea-hypopnea-index (AHI), nocturnal SpO_2 and arousal index in patients with OSA. In our study, the change in nocturnal PR (ΔPR) and mean PR correlated inversely with mean oxygen saturation. This observation links the tonic chemoreflex activation to increased sympathetic activity in patients with OSA.

We found a difference between the mean nocturnal PR in male OSA patients and matched male subjects in the control group. However, there was no difference in female subjects in the two groups, presumably because heart rate in the control group was higher in females, as had been described in previous studies (27). Moreover, the association between SBP and PR in male and female subjects suggests a possible gender-specific difference in the OSA population (28) (*Figure 1*). A significant association between sleep-related breathing disorders and hypertension had previously been reported only in males (29), while a questionnaire-based, case-controlled study found that the association of sleep-related breathing disorders and hypertension was independent of BMI in women, but not in men (30). Nevertheless, gender-specific differences have been poorly evaluated and reliable data in large cohorts of women are needed.

Our findings are consistent with results by Sanner and colleagues who showed that elevated heart rate predicts a better CPAP effect on blood pressure (31) and may indicate the higher sympathetic impact on heart rate and blood

pressure (32). However, in their study no consideration was given to exclude patients treated with heart rate affecting drugs.

In our study, patients with a fall in nocturnal PR (negative ΔPR) demonstrated a significant decrease in weight at follow up. This finding might help to identify patients that benefit more from CPAP in terms of reducing their overall cardiovascular risk. In order to confirm this hypothesis the change in nocturnal PR needs to be validated prospectively in a larger cohort of patients with OSA.

The benefit of CPAP on blood pressure and body weight is still under debate: in unselected OSA patients, CPAP has modest effects on blood pressure. In contrast, patients with more severe OSA and difficult-to-control hypertension seem to benefit significantly (33,34). Similarly, an impact of CPAP therapy on body weight has not been clearly demonstrated: a recent randomised sham-controlled study concluded that reducing visceral obesity in men with OSA cannot be achieved by CPAP alone (35).

Limitations

Our study was an observational cohort in which we were limited to pre-recorded data; we cannot rule out that our final analysed sample of patients was not representative for OSA patients. Both groups of patients included in the analysis were obese and this could have affected heart rate because of an increased sympathetic and reduced vagal activity (36). In this study we investigated for possible correlations between nocturnal PR in patients with OSA and weight change, following initiation of CPAP. We acknowledge that in our study we only measured weight and not BMI. However, it has to be considered that height in the adult population does not regularly change over time. The measurement of weight at baseline and at the follow up appointments will therefore indicate the same amount of change in the parameter that would have been found if BMI had been measured. A prospective study with height and BMI measurements could help to better match groups. Moreover, ΔPR may not accurately reflect PR when asleep. However, the availability of nocturnal pulse oximetry enhances the clinical usefulness of this method. Blood pressure was only recorded during the initial CPAP titration and at one year follow up; it could therefore be argued that the CPAP titration period may have affected the baseline blood pressure. Compliance to CPAP treatment in the OSA patients was limited and this could have further affected the outcome parameters. However, our study highlights the fact that there are sparse data on the change in blood pressure of OSA patients after CPAP treatment has continued for more than six months (9).

Conclusions

This study reveals that nocturnal PR is increased in OSA patients reflecting an elevated sympathetic tone. A nocturnal reduction in PR during the diagnostic night is associated with an improved symptomatic response to CPAP therapy at one year.

Further prospective studies are needed to better assess the long term effect of CPAP on blood pressure and body weight and to confirm the possible predictive role of mean PR and ΔPR in OSA patients. However, recording nocturnal PR and ΔPR is a widely available method and may help to determine OSA patients with a symptomatic response to CPAP and those with an increased cardiovascular risk.

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References

1. Young T, Palta M, Dempsey J, et al. The occurrence of sleep-disordered breathing among middle-aged adults. N Engl J Med 1993;328:1230-5.
2. He J, Kryger MH, Zorick FJ, et al. Mortality and apnea index in obstructive sleep apnea. Experience in 385 male patients. Chest 1988;94:9-14.
3. Martinez D, Klein C, Rahmeier L, et al. Sleep apnea is a stronger predictor for coronary heart disease than traditional risk factors. Sleep Breath 2012;16:695-701.
4. Philip P, Sagaspe P, Lagarde E, et al. Sleep disorders and accidental risk in a large group of regular registered highway drivers. Sleep Med 2010;11:973-9.
5. Somers VK, Dyken ME, Mark AL, et al. Sympathetic-nerve activity during sleep in normal subjects. N Engl J Med 1993;328:303-7.
6. Sullivan CE, Issa FG, Berthon-Jones M, et al. Reversal of obstructive sleep apnoea by continuous positive airway pressure applied through the nares. Lancet 1981;1:862-5.
7. National Institute for Health and Clinical Excellence. Continuous positive airway pressure for the treatment of

obstructive sleep apnoea/hypopnoea syndrome. No. of guidance 139, 2008; London: National Institute for Health and Clinical Excellence.
8. Weaver TE, Maislin G, Dinges DF, et al. Relationship between hours of CPAP use and achieving normal levels of sleepiness and daily functioning. Sleep 2007;30:711-9.
9. Parati G, Lombardi C, Hedner J, et al. Position paper on the management of patients with obstructive sleep apnea and hypertension: joint recommendations by the European Society of Hypertension, by the European Respiratory Society and by the members of European COST (COoperation in Scientific and Technological research) ACTION B26 on obstructive sleep apnea. J Hypertens 2012;30:633-46.
10. Fletcher EC. Effect of episodic hypoxia on sympathetic activity and blood pressure. Respir Physiol 2000;119:189-97.
11. Prabhakar NR, Kumar GK. Mechanisms of sympathetic activation and blood pressure elevation by intermittent hypoxia. Respir Physiol Neurobiol 2010;174:156-61.
12. Del Rio R, Moya EA, Parga MJ, et al. Carotid body inflammation and cardiorespiratory alterations in intermittent hypoxia. Eur Respir J 2012;39:1492-500.
13. Terada S, Koyama T, Watanabe H, et al. Abnormal coagulation and platelet profile in patients with obstructive sleep apnea syndrome. Int J Cardiol 2011;146:423-5.
14. Bruno RM, Rossi L, Fabbrini M, et al. Renal vasodilating capacity and endothelial function are impaired in patients with obstructive sleep apnea syndrome and no traditional cardiovascular risk factors. J Hypertens 2013;31:1456-64.
15. Palatini P, Julius S. Heart rate and the cardiovascular risk. J Hypertens 1997;15:3-17.
16. Somers VK, Dyken ME, Clary MP, et al. Sympathetic neural mechanisms in obstructive sleep apnea. J Clin Invest 1995;96:1897-904.
17. Khandoker AH, Karmakar CK, Palaniswami M. Comparison of pulse rate variability with heart rate variability during obstructive sleep apnea. Med Eng Phys 2011;33:204-9.
18. Pepperell JC, Ramdassingh-Dow S, Crosthwaite N, et al. Ambulatory blood pressure after therapeutic and subtherapeutic nasal continuous positive airway pressure for obstructive sleep apnoea: a randomised parallel trial. Lancet 2002;359:204-10.
19. Gil E, Orini M, Bailón R, et al. Photoplethysmography pulse rate variability as a surrogate measurement of heart rate variability during non-stationary conditions. Physiol Meas 2010;31:1271-90.
20. Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep 1991;14:540-5.
21. Pickering TG, Hall JE, Appel LJ, et al. Recommendations for blood pressure measurement in humans and experimental animals: part 1: blood pressure measurement in humans: a statement for professionals from the Subcommittee of Professional and Public Education of the American Heart Association Council on High Blood Pressure Research. Circulation 2005;111:697-716.
22. Fisher RA. eds. Statistical Methods and Scientific Inference. New York: Hafner, 1956.
23. Narkiewicz K, Somers VK. Interactive effect of heart rate and muscle sympathetic nerve activity on blood pressure. Circulation 1999;100:2514-8.
24. Rosen CL, Auckley D, Benca R, et al. A multisite randomized trial of portable sleep studies and positive airway pressure autotitration versus laboratory-based polysomnography for the diagnosis and treatment of obstructive sleep apnea: the HomePAP study. Sleep 2012;35:757-67.
25. Vázquez JC, Tsai WH, Flemons WW, et al. Automated analysis of digital oximetry in the diagnosis of obstructive sleep apnoea. Thorax 2000;55:302-7.
26. Kawano Y, Tamura A, Watanabe T, et al. Influence of the severity of obstructive sleep apnea on heart rate. J Cardiol 2010;56:27-34.
27. Lavie-Nevo K, Pillar G. Evening-morning differences in blood pressure in sleep apnea syndrome: effect of gender. Am J Hypertens 2006;19:1064-9.
28. Alajmi M, Mulgrew AT, Fox J, et al. Impact of continuous positive airway pressure therapy on blood pressure in patients with obstructive sleep apnea hypopnea: a meta-analysis of randomized controlled trials. Lung 2007;185:67-72.
29. Hedner J, Bengtsson-Boström K, Peker Y, et al. Hypertension prevalence in obstructive sleep apnoea and sex: a population-based case-control study. Eur Respir J 2006;27:564-70.
30. Marrone O, Bonsignore MR, Fricano L, et al. Gender and the systemic hypertension-snoring association: a questionnaire-based case-control study. Blood Press 1998;7:11-7.
31. Sanner BM, Tepel M, Markmann A, et al. Effect of continuous positive airway pressure therapy on 24-hour blood pressure in patients with obstructive sleep apnea syndrome. Am J Hypertens 2002;15:251-7.
32. Kufoy E, Palma JA, Lopez J, et al. Changes in the heart

rate variability in patients with obstructive sleep apnea and its response to acute CPAP treatment. PLoS One 2012;7:e33769.
33. Obstructive Sleep Apnoea Syndrome. Report of a joint Nordic project. Finnish Office for Health Care Technology Assessment. Obstructive Sleep Apnoea Syndrome. Report of a joint Nordic project. Helsinki: Finnish Office for Health Care Technology Assessment (FinOHTA) 2007.
34. Giles TL, Lasserson TJ, Smith BH, et al. Continuous positive airways pressure for obstructive sleep apnoea in adults. Cochrane Database Syst Rev 2006;(3):CD001106.
35. Hoyos CM, Killick R, Yee BJ, et al. Cardiometabolic changes after continuous positive airway pressure for obstructive sleep apnoea: a randomised sham-controlled study. Thorax 2012;67:1081-9.
36. Grassi G, Vailati S, Bertinieri G, et al. Heart rate as marker of sympathetic activity. J Hypertens 1998;16:1635-9.

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Original Article

Pulmonary hypertension in end-stage renal disease and post renal transplantation patients

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Background: Information regarding lung function parameters and functional capacity in renal failure and post renal transplantation patients with pulmonary hypertension (PH) is limited. The purpose of this study was to examine the clinical characteristics of patients with PH who were receiving hemodialysis (HD) or peritoneal dialysis (PD) or who had undergone renal transplantation.

Methods: A prospective study was performed on 116 patients (HD =55, PD =17, and post renal transplantation =44) who underwent Doppler echocardiography. PH was defined as systolic pulmonary artery pressure (SPAP) ≥40 mmHg. Demographic information, clinical characteristics, pulmonary function tests (PFTs) and the six-minute walk test (6MWT) were collected and compared between the patients with and without PH.

Results: Twelve (21.8%) patients receiving HD, four (23.5%) patients receiving PD, and eight (18.2%) post renal transplantation patients had PH. In the HD group, the physiological indicators (including pulmonary function test parameters, the final Borg score, and walking distance during the 6MWT) were all significantly lower in the patients with PH compared with those without PH (all P<0.0001). However, in the PD and post renal transplantation groups, no significant differences were noted in the demographic characteristics or in the physiological parameters when the PH patients were compared with those without PH (all P>0.05).

Conclusions: Among HD patients, marked aberrations in PFT results or walking distance may identify a subset of patients suffering from PH.

Keywords: Pulmonary hypertension (PH); renal failure; hemodialysis (HD); functional capacity; lung function

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Introduction

Pulmonary hypertension (PH) has emerged as a major complication of several systemic disorders. The estimated prevalence rates of PH range from 16-58% for patients receiving hemodialysis (HD), 12-42% for patients receiving peritoneal dialysis (PD), and 5-14% among patients who have undergone renal transplantation (1-11). Importantly, it has been found that patients with PH who experience renal failure show significantly higher rates of morbidity and mortality (1,2). The pathogenesis of renal failure-associated PH is complex, and it may include metabolic and hormonal derangements, high cardiac output due to arterio-venous fistula (AVF), impaired endothelial function, anemia, fluid overload, and other factors (3,12,13). Because of the plethora of possible mechanisms by which it exerts its effects, PH related to renal failure falls into group 5 (14).

Impaired lung function and exercise capacity are commonly observed in patients with chronic renal failure. Such limitations are due to several factors, including inflammation, myopathy, neuropathy, metabolic acidosis,

and others (15-18). However, there is little information available regarding pulmonary function parameters and functional capacity among patients with renal failure-associated PH.

In this study, we sought to determine the incidence of PH in three groups: patients receiving HD, patients receiving PD, and patients who had undergone renal transplantation. Additionally, we sought to compare the results of the pulmonary function tests (PFTs) and the six-minute walk test (6MWT) in the PH patients versus those without PH in the three groups.

Materials and methods

Study population

Patient selection

This was a prospective, descriptive clinical study conducted between November 2008 and June 2010. Patients were included in the study if they were aged 18 years or above, were receiving HD or PD or were post-renal transplantation patients, and for whom the cause of PH was unknown. Patients were excluded they were known to have a chest wall deformity, parenchymal lung disease, a history of pulmonary embolism, collagen vascular disease, congestive heart failure, significant valvular heart disease, or chronic liver disease. Additionally, six patients were not included in the study because they were unable to perform the PFTs and 6MWT. In addition, 12 patients refused to participate in the study.

Patient assessment

A standard form was used to collect information regarding demographics, clinical factors, duration of dialysis, etiology of renal failure, and vascular access location. Echocardiography, PFTs, arterial blood gas (ABG) sampling, 6MWT, and blood testing for hemoglobin, creatinine, albumin, serum calcium, phosphorus, and parathyroid hormone levels were performed in all participants. For patients with PH, chest radiography and computed tomography were included in the systemic diagnostic evaluation.

Echocardiograms and estimation of systolic pulmonary artery pressure (SPAP)

Two-dimensional, M-mode, and Doppler echocardiography exams were performed on all of the participants by two experienced echo technicians dedicated to this study. The Phillips Sonos 5,500 imaging system (Phillips Co., Andover, MA, USA), equipped with a 3.2 MHz transducer, was used. Multiple views using different acoustic windows were obtained to measure the most optimal tricuspid regurgitation (TR) jet signal using continuous wave (CW) Doppler at a sweep speed of 100 to 200 mm/s. Only CW signals that demonstrated the peak velocity of the TR jet were used for this analysis. SPAP was estimated based on the modified Bernoulli equation as follows (19) : 4 V^2 (V = peak velocity of TR in meters per second, obtained using the CW Doppler) was added to the estimated right atrial pressure (RAP). The RAP was estimated based on the dimensions of the inferior vena cava (IVC) during inspiration. The RAP was estimated to be 5 mmHg if the IVC size was less than 2.0 cm and collapsed by 50% during inspiration, 10 mmHg if the IVC was less than 2.0 cm and did not collapse by 50%, 15 mmHg if the IVC was greater than or equal to 2.0 cm and collapsed more than 50%, and 20 mmHg if the IVC was greater than or equal to 2.0 cm and did not collapse by 50%. A patient was considered to have PH if the SPAP was greater than or equal to 40 mmHg (20). All of the studies were evaluated off line by an experienced echocardiographer who was blinded to the patients' clinical data.

Physiological measurements

Immediately after echocardiography, the patients performed PFTs (PFT Masterscreen; Jaeger, Hoechberg, Germany) using standard methodologies. These tests included spirometry, plethysmography, and measurement of the diffusion capacity of the lung for carbon monoxide (DLco) (21-23). ABG values (Rapid Lab 865; Bayer, Plymouth, UK) were obtained for the partial pressure of oxygen (PaO_2), the partial pressure of carbon dioxide ($PaCO_2$), and the extent of oxygen saturation (SaO_2). After the PFTs and ABG sampling, the patients were asked to perform the 6MWT in accordance with ATS guidelines (24). SpO_2 and the Borg dyspnea index (25) were recorded at the beginning and end of a six-minute walk. At the end of the test, the total distance walked in meters was documented.

In the HD group, echocardiograms, PFTs, and the 6MWT were performed within one hour after the completion of HD to assure that the patients were at the optimal dry weight. In the PD group, the dialysis fluid was drained before the echocardiograms and physiological tests were performed.

Table 1 Etiology of renal failure in the different groups

Etiology	HD (n=55) (%)	Post-transplant (n=44) (%)	PD (n=17) (%)	P value
Diabetes mellitus	24 (43.6)	8 (18.2)	7 (41.2)	0.022
Hypertension	12 (21.8)	9 (20.4)	2 (11.8)	0.656
Glomerulonephritis	6 (10.9)	7 (15.9)	1 (5.9)	0.524
Chronic pyelonephritis	3 (5.4)	3 (6.8)	0	0.555
Nephrolithiasis	1 (1.8)	1 (2.3)	0	0.827
Unknown	24 (43.6)	19 (43.2)	7 (41.2)	0.093

Patients could have multiple etiology of renal failure. HD, hemodialysis; PD, peritoneal dialysis.

Blood samples

Blood tests for hemoglobin, creatinine, albumin, serum calcium, phosphorus, and parathyroid hormone level were performed in all participants within one week of the physiological studies.

Statistical analysis

Descriptive statistics (the mean values, standard deviations, and percentages) were used to describe the quantitative and categorical study variables. Chi-square statistics were used to assess the differences between proportions. Student's *t*-test for independent samples was applied to compare the mean values of continuous variables. A two-sided P value <0.05 was considered statistically significant. All of the analyses were performed using Statistical Package for the Social Sciences software (SPSS, version 18.0; SPSS Inc., Chicago, IL, USA).

Results

A total of 116 patients (HD =55, post renal transplantation =44, and PD =17) were eligible for the study. Chronic renal failure secondary to diabetes mellitus was more frequently noted in the HD and PD groups than in the post renal transplantation group (*Table 1*) (P=0.022). However, the distributions of other causes of chronic renal failure were not significantly different among the three groups (*Table 1*) (all P>0.05).

In the HD group, PH was detected in 12 (21.8%) patients. The comparisons of the demographic and clinical characteristics and the laboratory data for the HD patients with PH and those without PH are shown in *Table 2*. The HD patients with PH were significantly older than those without PH (P=0.003). No differences in gender or the duration of dialysis were noted between the patients with and without PH. However, the PH group had significantly lower forced vital capacity (FVC) [(55.2±9.7)% predicted *vs.* (82.0±22.0)% predicted, P<0.0001], forced expiratory volume in one second (FEV_1) [(58.6±10.7)% predicted *vs.* (83.6±21.9)% predicted, P<0.0001], total lung capacity (TLC) [(63.4±21.7)% predicted *vs.* (80.4±17.1)% predicted, P=0.007], and DL_{CO} [(44.0±23.2)% predicted *vs.* (68.2±19.9)% predicted, P=0.002] compared with the patients without PH. In addition, the HD patients with PH had a significantly shorter walking distance [(192.5±120.0) *vs.* (358.4±97.9) meters, P<0.0001] and a higher dyspnea score at the end of the 6MWT [(3.8±2.4) *vs.* (1.5±1.2), P<0.0001] compared with the patients without PH. There was no significant difference between those with and without PH with regard to hemoglobin, creatinine, albumin, phosphorus, calcium, parathyroid hormone levels, HD access, and shunt location.

In the post renal transplantation group, PH was noted in eight (18.2%) patients. The clinical characteristics and laboratory data for the patients with and without PH are shown in *Table 3*. No between-group differences were observed with regard to age, gender, disease duration, PFTs, ABG, or 6MWT. With regard to the laboratory results, the hemoglobin level was the only parameter that was significantly lower in the PH group [(11.7±2.3) *vs.* (13.3±1.6) g/dL, P=0.026].

Among the patients receiving PD, PH was noted in four (23.5%) patients. However, in this group, the clinical characteristics, physiological parameters, and laboratory data did not differ between patients with and without PH (*Table 4*).

The clinical characteristics, physiological parameters, and laboratory data for the patients with PH in all three groups (HD, post renal transplantation, and PD) are summarized in *Table 5*.

Table 2 Comparison between hemodialysis patients with and without pulmonary hypertension

	With PH (n=12)	Without PH (n=43)	P value
Age (years)	58.0±15.6	43.9±13.5	0.003
Male/female	5/7	16/27	0.681
Dialysis duration (years)	5.0±5.3	3.4±4.1	0.289
SPAP (mmHg)	53.2±8.3	32.1±4.2	<0.0001
Pulmonary function test			
FVC (% predicted)	55.2±9.7	82.0±22.0	<0.0001
FEV_1 (% predicted)	58.6±10.7	83.6±21.9	<0.0001
FEV_1/FVC (ratio)	87.3±10.0	86.9±7.8	0.881
TLC (% predicted)	63.4±21.7	80.4±17.1	0.007
DL_{CO} (% predicted)	44.0±23.2	68.2±19.9	0.002
ABG			
pH	7.4±0.1	7.4±0.0	0.266
PaO_2 (mmHg)	83.8±15.4	89.2±10.7	0.239
SaO_2 (%)	96.3±2.1	97.0±1.1	0.190
6MWT			
Distance (meters)	192.5±120.0	358.4±97.9	<0.0001
Initial Borg score	0.6±1.1	0.1±0.5	0.085
Final Borg score	3.8±2.4	1.5±1.2	<0.0001
Initial SpO_2 (%)	95.4±7.0	98.0±1.4	0.032
Final SpO_2 (%)	93.1±7.4	93.7±6.1	0.794
Laboratory data			
Hemoglobin (g/dL)	11.1±1.7	11.6±1.7	0.386
Creatinine (μmol/L)	752.4±288.6	727.4±377.5	0.832
Albumin (g/L)	32.8±8.2	33.6±6.4	0.719
Parathyroid hormone (pmol/L)	40.6±28.0	46.2±48.6	0.702
Phosphorus (mmol/L)	1.6±0.6	1.5±0.5	0.669
Calcium (mmol/L)	2.2±0.2	2.1±0.2	0.616
Hemodialysis access [%]			
Graft/fistula	7 [58]	22 [51]	0.746
Catheter	5 [42]	22 [51]*	0.751
Shunt location [%]			
Brachial artery	6 [86]	21 [95]	0.753
Radial artery	1 [14]	1 [5]	0.326

The data are presented as the mean ± SD and a number (%). PH, pulmonary hypertension; SPAP, systolic pulmonary artery pressure; FVC, forced vital capacity; FEV_1, forced expiratory volume in one second; TLC, total lung capacity; DL_{CO}, diffusion capacity of lung for carbon monoxide; PaO_2, partial pressure of oxygen in the blood; SaO_2, arterial oxygen saturation; SpO_2, oxygen saturation by pulse oximetry; ABG, arterial blood gas; 6MWT, six-minute walk test; *, one patient had both a venous catheter and an arterio-venous fistula.

Table 3 Comparison between the renal transplantation patients with and without pulmonary hypertension

	With PH (n=8)	Without PH (n=36)	P value
Age (years)	44.9±13.2	42.9±12.8	0.695
Male/female	5/3	26/10	0.586
Duration (years)	5.8±3.9	4.6±4.2	0.445
SPAP (mmHg)	48.9±8.3	28.3±9.8	<0.0001
Pulmonary function test			
FVC (% predicted)	87.5±15.2	90.3±11.4	0.559
FEV_1 (% predicted)	90.4±13.6	88.2±18.5	0.758
FEV_1/FVC (ratio)	87.2±4.5	82.9±8.2	0.171
TLC (% predicted)	81.4±4.5	78.0±18.1	0.629
DL_{CO} (% predicted)	80.8±18.2	73.5±21.5	0.383
ABG			
pH	7.4±0.0	7.4±0.0	0.586
PaO_2 (mmHg)	91.0±7.4	90.2±9.1	0.818
SaO_2 (%)	97.9±1.0	97.4±1.6	0.482
6MWT			
Distance (meters)	417.9±79.4	441.1±91.0	0.589
Initial Borg score	0	0.1±0.2	0.628
Final Borg score	1.8±2.2	1.1±1.6	0.131
Initial SpO_2 (%)	98.8±1.0	98.4±1.3	0.608
Final SpO_2 (%)	97.4±1.6	96.9±1.3	0.356
Laboratory data			
Hemoglobin (g/dL)	11.7±2.3	13.3±1.6	0.026
Creatinine (μmol/L)	263.9±278.0	159.9±255.6	0.311
Albumin (g/L)	38.1±2.9	36.9±5.9	0.590
Parathyroid hormone (pmol/L)	15.4±10.2	17.1±18.0	0.801
Phosphorus (mmol/L)	1.2±0.2	1.2±0.2	0.509
Calcium (mmol/L)	2.2±0.2	2.2±0.1	0.329

The data are presented as the mean ± SD and a number (%). PH, pulmonary hypertension; SPAP, systolic pulmonary artery pressure; FVC, forced vital capacity; FEV_1, forced expiratory volume in one second; TLC, total lung capacity; DL_{CO}, diffusion capacity of lung for carbon monoxide; PaO_2, partial pressure of oxygen in the blood; SaO_2, arterial oxygen saturation; SpO_2, oxygen saturation by pulse oximetry; ABG, arterial blood gas; 6MWT, six-minute walk test.

During the study period, three patients with PH died (one each in the HD, PD, and post renal transplantation groups) and five patients without PH died (two each in the HD and PD groups and one in the post renal transplantation group). A survival analysis was not performed due to the small sample size and the low number of deaths in each group.

Discussion

The present study demonstrates that PH was relatively common in the patients receiving HD and PD and the post renal transplantation patients. However, significantly impaired lung function and functional capacity were only noted in the patients with PH receiving long-term HD.

The prevalence of PH among the patients receiving long-term HD ranges from 16-58%, depending on the definition of PH, the methodology, the ethnicity of the patients, the institution, and the region of the world (1-7). For example, the majority of the cited studies define PH as an SPAP ≥35 mmHg. However, when the cut-off value of

Table 4 Comparison between the peritoneal dialysis patients with and without pulmonary hypertension

	With PH (n=4)	Without PH (n=13)	P value
Age (years)	45.5±22.8	50.8±18.1	0.638
Male/female	2/2	8/5	0.682
Dialysis duration (years)	3.3±4.0	2.0±2.5	0.461
SPAP (mmHg)	48.2±5.4	32.1±5.1	<0.0001
Pulmonary function test			
FVC (% predicted)	90.0±33.7	90.9±13.3	0.935
FEV_1 (% predicted)	90.1±36.4	94.3±15.6	0.740
FEV_1/FVC (ratio)	81.6±3.6	84.5±7.0	0.453
TLC (% predicted)	83.2±13.9	85.7±16.6	0.792
DL_{CO} (% predicted)	80.7±13.0	72.1±17.6	0.445
ABG			
pH	7.4±0.0	7.4±0.0	0.770
PaO_2 (mmHg)	83.3±12.6	85.7±12.3	0.769
SaO_2 (%)	97.6±0.6	97.1±1.4	0.553
6MWT			
Distance (meters)	435.0±104.0	419.2±97.4	0.807
Initial Borg score	0	0	–
Final Borg score	2.0±1.7	0.8±0.8	0.072
Initial SpO_2 (%)	99.3±0.6	98.2±1.1	0.116
Final SpO_2 (%)	96.0±1.0	96.2±2.8	0.923
Laboratory data			
Hemoglobin (g/dL)	11.6±1.6	11.8±1.5	0.750
Creatinine (μmol/L)	1,016.8±413.1	560.2±364.8	0.050
Albumin (g/L)	30.5±5.4	32.6±4.9	0.475
Parathyroid hormone (pmol/L)	37.3±30.2	35.1±43.1	0.927
Phosphorus (mmol/L)	1.8±0.2	1.4±0.4	0.065
Calcium (mmol/L)	2.2±0.2	2.1±0.2	0.384

Data are presented as the mean ± SD and a number (%). PH, pulmonary hypertension; SPAP, systolic pulmonary artery pressure; FVC, forced vital capacity; FEV_1, forced expiratory volume in one second; TLC, total lung capacity; DL_{CO}, diffusion capacity of lung for carbon monoxide; PaO_2, partial pressure of oxygen in the blood; SaO_2, arterial oxygen saturation; SpO_2, oxygen saturation by pulse oximetry; ABG, arterial blood gas; 6MWT, six-minute walk test.

45 mmHg was applied, the PH estimate was substantially lower (16-20%) (1,2). Furthermore, a higher prevalence of PH was noted when the echocardiograms were performed before dialysis (48-58%) (1,3) compared to when the echocardiograms were performed immediately after dialysis (29-39%) (2,5,6). Many factors have been suggested to contribute to the development of PH in end-stage renal disease. For example, the increase in cardiac output in response to AVF among the patients receiving HD has been implicated in the pathogenesis of PH (5,7,26). However, the lack of a significant difference in cardiac output between the patients with and without PH (27) and the reduction in cardiac output and PAP among the HD patients who underwent kidney transplantation regardless of the status of AVF (whether it remained open or closed) (7) suggest that other mechanisms are involved in the development of PH.

In their study, Ramasubbu *et al.* (1) reported that 63% of HD patients with PH exhibited echocardiographic evidence of elevated pulmonary capillary wedge pressure (PCWP). In addition, they noted a significant correlation between

Table 5 Comparison of patients with pulmonary hypertension among the different renal replacement therapy groups

	HD (n=12)	Post-transplant (n=8)	PD (n=4)	P value
Age (years)	58.0±15.6	44.9±13.2	45.5±22.8	0.171
Male/female	5/7	5/3	2/2	0.659
Duration (years)	5.0±5.3	5.8±3.9	3.3±4.0	0.675
SPAP (mmHg)	53.2±8.3	48.9±8.3	48.2±5.4	0.382
Pulmonary function test				
FVC (% predicted)	55.2±9.7	87.5±15.2	90.0±33.7	<0.0001
FEV_1 (% predicted)	58.6±10.7	90.4±13.6	90.1±36.4	0.001
FEV_1/FVC (ratio)	87.3±10.0	87.2±4.5	81.6±3.6	NS
TLC (% predicted)	63.4±21.7	81.4±4.5	83.2±13.9	NS
DL_{CO} (% predicted)	44.0±23.2	80.8±18.2	80.7±13.0	0.003
ABG				
pH	7.4±0.1	7.4±0.0	7.4±0.0	0.674
PaO_2 (mmHg)	83.8±15.4	91.0±7.4	83.3±12.6	0.441
SaO_2 (%)	96.3±2.1	97.9±1.0	97.6±0.6	0.154
6MWT				
Distance (meters)	192.5±120.0	417.9±79.4	435.0±104.0	<0.0001
Initial Borg score	0.6±1.1	0	0	0.273
Final Borg score	3.8±2.4	1.8±2.2	2.0±1.7	0.183
Initial SpO_2 (%)	95.4±7.0	98.8±1.0	99.3±0.6	0.298
Final SpO_2 (%)	93.1±7.4	97.4±1.6	96.0±1.0	0.256
Laboratory data				
Hemoglobin (g/dL)	11.1±1.7	11.7±2.3	11.6±1.6	0.737
Creatinine (μmol/L)	752.4±288.6	263.9±278.0	1,016.8±413.1	0.001
Albumin (g/L)	32.8±8.2	38.1±2.9	30.5±5.4	0.114
Parathyroid hormone (pmol/L)	40.6±28.0	15.4±10.2	37.3±30.2	0.086
Phosphate (mmol/L)	1.6±0.6	1.2±0.2	1.8±0.2	0.106
Calcium (mmol/L)	2.2±0.2	2.2±0.2	2.2±0.2	0.899

The data are presented as the mean ± SD and a number (%). PH, pulmonary hypertension; SPAP, systolic pulmonary artery pressure; FVC, forced vital capacity; FEV_1, forced expiratory volume in one second; TLC, total lung capacity; DL_{CO}, diffusion capacity of lung for carbon monoxide; PaO_2, partial pressure of oxygen in the blood; SaO_2, arterial oxygen saturation; SpO_2, oxygen saturation by pulse oximetry; ABG, arterial blood gas; 6MWT, six-minute walk test.

PAP and PCWP. In another study, significant increases in the cardiac index, the IVC diameter, and the left atrial diameter, which are all markers of volume overload, were noted in the PH patients receiving long-term HD (2). Collectively, these studies illustrate that chronic volume overload may play a role in the pathogenesis of PH. Other risk factors for PH have also been described, including age, the duration of chronic renal failure, hyperparathyroidism, and increased pulmonary vascular stiffness secondary to endothelial dysfunction (4,7,28,29). Although the purpose of the present study was not to identify variables associated with an increased risk of PH, we found no significant difference between the HD patients with and without PH in terms of age, dialysis duration, hemoglobin, serum calcium, phosphorus, or parathyroid hormone level. Furthermore, neither the type of HD access nor the shunt location was associated with PH. Interestingly, we noted that the PFT parameters and 6MWT were markedly reduced in the PH patients receiving long-term HD compared with those without PH. To the best of our knowledge, this is the first

study to examine the effect of PH on lung function indices and functional capacity among patients with end-stage renal disease.

Because uremic patients often experience dysfunctions in multiple systems, aberrations in PFTs are not uncommon. Previous studies that have examined the effect of dialysis on PFT parameters revealed that a restrictive ventilatory defect was commonly observed among HD patients (16,30,31). In agreement with the cited studies, we noted a similar finding among the HD patients. However, in the present study, marked impairments in lung volume and DLco were also noted in the HD patients with PH, suggesting that this group may represent a distinct entity. Bush and Gabriel (31) and Herrero *et al*. (32) have suggested that the reduction in DLco may be due to chronic pulmonary fibrosis. However, the findings in the present study do not support this hypothesis because our patients with PH demonstrated no evidence of pulmonary fibrosis as assessed by HRCT. In addition, the PFT parameters of the PD and post-transplantation patients were within the normal range, regardless of whether PH was present or not, implying that a separate mechanism was involved in the pathogenesis of PH among HD patients.

In support of this notion, a previous study showed significant impairment in nitric oxide production, a marker of endothelial dysfunction, among PH patients receiving HD (7). Vascular endothelial growth factor (VEGF) is a glycoprotein with potent angiogenic and vascular permeability-enhancing properties, and it is involved in one of the important pathways that has been implicated in the pathogenesis of PH (33,34). Interestingly, hypoxia and acidosis, either alone or in combination, are frequently encountered in dialysis patients, and both conditions are potent inducers of VEGF expression (35-37). Recently, Yuan *et al*. (38) showed that a high serum level of VEGF was an independent predictor of mortality in dialysis patients. However, in the cited study, it was not clear whether the increase in the serum level of VEGF was associated with the presence of PH. As such, future studies are needed to determine the role of endothelial dysfunction and VEGF in the development of PH among dialysis patients.

Several factors, including respiratory status, cardiac involvement, skeletal muscle weakness, malnutrition, metabolic acidosis, corticosteroids, and others, lead to exercise intolerance, which manifests as reduced walking distance among chronic renal failure patients (15,39,40). A striking finding of our study was the significantly shorter walking distance among the PH patients receiving HD compared with those without PH. Moreover, the walking distance was significantly shorter in the HD patients with PH compared with those with PH receiving PD and post renal transplantation patients, substantiating the idea that the presence of PH in HD patients is distinct and has a deleterious effect on the functional capacity of these patients. Because the 6MWT is simple, inexpensive, reproducible, and well-received by patients because it mimics the effort required for daily physical activity, it has become a popular tool for predicting the prognoses of patients with various pulmonary and non-pulmonary diseases and is used as a surrogate marker for responsiveness to therapy in many clinical drug trial studies. Surprisingly, very few studies have attempted to characterize the effect of dialysis on the 6MWT. In addition, there is no information on the best time to perform the 6MWT among dialysis patients. Although in the present study, the walking test was conducted within one hour of the HD to ensure that the patients were at the optimal dry weight, this timing may have had a negative impact on the walking test.

Previous studies (41,42) noted that the patients receiving HD had a significant increase in whole body and muscle protein breakdown, along with a significant increase in inflammatory markers, including interleukin (IL)-6 and fibrinogen, during HD and for two hours afterward. This result implies that HD induces an acute inflammatory response in addition to the persistent chronic inflammatory state that occurs in end-stage renal disease patients. Nonetheless, the significantly shorter walking distance noted in the current study among patients with PH who were receiving HD suggests that PH has a significant negative impact on functional capacity. Thus, future studies are warranted to explore the value of the 6MWT as a screening tool to identify patients with PH and to determine whether this test can be used to predict mortality among PH patients receiving HD.

In the current study, PH was detected in four (23.5%) patients receiving PD. The reported prevalence of PH in PD patients ranges from 12-42%, mostly because of variation in the patient selection criteria (3,8,9). However, because of the small number of patients with PH noted in the present study and the lack of significant differences in the PFT parameters and the 6MWT results between those with and without PH, it is difficult to draw a firm conclusion. Large-scale studies are needed to explore the true impact of PH on the physiological parameters among

patients receiving PD.

Renal transplantation is regarded as the gold standard to restore renal function among end-stage renal disease patients. Simmons *et al*. (43) reported that pro-inflammatory cytokines and oxidative stress markers return to a normal baseline level that is similar to that of healthy controls within two months of renal transplantation. The use of immunosuppressive medications, the restoration of renal function, or perhaps the combination of both may account for the normalization of the markers of oxidative stress and pro-inflammatory cytokines. This may explain the significant reduction in PAP and the normalization of pulmonary function parameters reported in previous studies of patients who underwent renal transplantation (7,12,13,16,44). In the present study, PH was noted in 18% of the patients in the renal transplant group. However, we found no significant difference in the PFT parameters or in the walking distance between those with or without PH. As such, longitudinal follow-up is needed to determine the clinical significance of detecting PH among renal transplant patients.

In conclusion, in this study, we show that PH is commonly observed among patients with end-stage renal disease and post renal transplantation patients. However, the PFT and 6MWT results were only severely compromised in the patients with HD-associated PH. Whether the aberrations in the pulmonary function parameters and functional capacity results that were observed in the HD patients in this study can potentially be used to predict the presence of PH or perhaps as a marker of disease severity to expedite kidney transplantation is unknown and should be explored in future studies.

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References

1. Ramasubbu K, Deswal A, Herdejurgen C, et al. A prospective echocardiographic evaluation of pulmonary hypertension in chronic hemodialysis patients in the United States: prevalence and clinical significance. Int J Gen Med 2010;3:279-86.
2. Agarwal R. Prevalence, determinants and prognosis of pulmonary hypertension among hemodialysis patients. Nephrol Dial Transplant 2012;27:3908-14.
3. Fabbian F, Cantelli S, Molino C, et al. Pulmonary hypertension in dialysis patients: a cross-sectional italian study. Int J Nephrol 2010;2011:283475.
4. Havlucu Y, Kursat S, Ekmekci C, et al. Pulmonary hypertension in patients with chronic renal failure. Respiration 2007;74:503-10.
5. Yigla M, Nakhoul F, Sabag A, et al. Pulmonary hypertension in patients with end-stage renal disease. Chest 2003;123:1577-82.
6. Amin M, Fawzy A, Hamid MA, et al. Pulmonary hypertension in patients with chronic renal failure: role of parathyroid hormone and pulmonary artery calcifications. Chest 2003;124:2093-7.
7. Nakhoul F, Yigla M, Gilman R, et al. The pathogenesis of pulmonary hypertension in haemodialysis patients via arterio-venous access. Nephrol Dial Transplant 2005;20:1686-92.
8. Unal A, Sipahioglu M, Oguz F, et al. Pulmonary hypertension in peritoneal dialysis patients: prevalence and risk factors. Perit Dial Int 2009;29:191-8.
9. Kumbar L, Fein PA, Rafiq MA, et al. Pulmonary hypertension in peritoneal dialysis patients. Adv Perit Dial 2007;23:127-31.
10. Casas-Aparicio G, Castillo-Martínez L, Orea-Tejeda A, et al. The Effect of Successful Kidney Transplantation on Ventricular Dysfunction and Pulmonary Hypertension. Transplant Proc 2010;42:3524-8.
11. Abedini M, Sadeghi M, Naini AE, et al. Pulmonary hypertension among patients on dialysis and kidney transplant recipients. Ren Fail 2013;35:560-5.
12. Abassi Z, Nakhoul F, Khankin E, et al. Pulmonary hypertension in chronic dialysis patients with arteriovenous fistula: pathogenesis and therapeutic prospective. Curr Opin Nephrol Hypertens 2006;15:353-60.
13. Bozbas SS, Akcay S, Altin C, et al. Pulmonary hypertension in patients with end-stage renal disease undergoing renal transplantation. Transplant Proc 2009;41:2753-6.
14. Simonneau G, Robbins IM, Beghetti M, et al. Updated clinical classification of pulmonary hypertension. J Am Coll Cardiol 2009;54:S43-54.
15. Cury JL, Brunetto AF, Aydos RD. Negative effects of

chronic kidney failure on lung function and functional capacity. Rev Bras Fisioter 2010;14:91-8.
16. Karacan O, Tutal E, Colak T, et al. Pulmonary function in renal transplant recipients and end-stage renal disease patients undergoing maintenance dialysis. Transplant Proc 2006;38:396-400.
17. Violan MA, Pomes T, Maldonado S, et al. Exercise capacity in hemodialysis and renal transplant patients. Transplant Proc 2002;34:417-8.
18. McIntyre CW, Selby NM, Sigrist M, et al. Patients receiving maintenance dialysis have more severe functionally significant skeletal muscle wasting than patients with dialysis-independent chronic kidney disease. Nephrol Dial Transplant 2006;21:2210-6.
19. Dabestani A, Mahan G, Gardin JM, et al. Evaluation of pulmonary artery pressure and resistance by pulsed Doppler echocardiography. Am J Cardiol 1987;59:662-8.
20. McLaughlin VV, Archer SL, Badesch DB, et al. ACCF/AHA 2009 expert consensus document on pulmonary hypertension: a report of the American College of Cardiology Foundation Task Force on Expert Consensus Documents and the American Heart Association: developed in collaboration with the American College of Chest Physicians, American Thoracic Society, Inc., and the Pulmonary Hypertension Association. Circulation 2009;119:2250-94.
21. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. Eur Respir J 2005;26:319-38.
22. Wanger J, Clausen JL, Coates A, et al. Standardisation of the measurement of lung volumes. Eur Respir J 2005;26:511-22.
23. Macintyre N, Crapo RO, Viegi G, et al. Standardisation of the single-breath determination of carbon monoxide uptake in the lung. Eur Respir J 2005;26:720-35.
24. ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories. ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med 2002;166:111-7.
25. Borg GA. Psychophysical bases of perceived exertion. Med Sci Sports Exerc 1982;14:377-81.
26. Dagli CE, Sayarlioglu H, Dogan E, et al. Prevalence of and factors affecting pulmonary hypertension in hemodialysis patients. Respiration 2009;78:411-5.
27. Tarrass F, Benjelloun M, Medkouri G, et al. Doppler echocardiograph evaluation of pulmonary hypertension in patients undergoing hemodialysis. Hemodial Int 2006;10:356-9.
28. Harp RJ, Stavropoulos SW, Wasserstein AG, et al. Pulmonary hypertension among end-stage renal failure patients following hemodialysis access thrombectomy. Cardiovasc Intervent Radiol 2005;28:17-22.
29. Akmal M, Barndt RR, Ansari AN, et al. Excess PTH in CRF induces pulmonary calcification, pulmonary hypertension and right ventricular hypertrophy. Kidney Int 1995;47:158-63.
30. Lee HY, Stretton TB, Barnes AM. The lungs in renal failure. Thorax 1975;30:46-53.
31. Bush A, Gabriel R. Pulmonary function in chronic renal failure: effects of dialysis and transplantation. Thorax 1991;46:424-8.
32. Herrero JA, Alvarez-Sala JL, Coronel F, et al. Pulmonary diffusing capacity in chronic dialysis patients. Respir Med 2002;96:487-92.
33. Eddahibi S, Humbert M, Sediame S, et al. Imbalance between platelet vascular endothelial growth factor and platelet-derived growth factor in pulmonary hypertension. Effect of prostacyclin therapy. Am J Respir Crit Care Med 2000;162:1493-9.
34. Mata-Greenwood E, Meyrick B, Soifer SJ, et al. Expression of VEGF and its receptors Flt-1 and Flk-1/KDR is altered in lambs with increased pulmonary blood flow and pulmonary hypertension. Am J Physiol Lung Cell Mol Physiol 2003;285:L222-31.
35. Fukumura D, Xu L, Chen Y, et al. Hypoxia and acidosis independently up-regulate vascular endothelial growth factor transcription in brain tumors in vivo. Cancer Res 2001;61:6020-4.
36. Elias AP, Dias S. Microenvironment changes (in pH) affect VEGF alternative splicing. Cancer Microenviron 2008;1:131-9.
37. Stenmark KR, Fagan KA, Frid MG. Hypoxia-induced pulmonary vascular remodeling: cellular and molecular mechanisms. Circ Res 2006;99:675-91.
38. Yuan J, Guo Q, Qureshi AR, et al. Circulating vascular endothelial growth factor (VEGF) and its soluble receptor 1 (sVEGFR-1) are associated with inflammation and mortality in incident dialysis patients. Nephrol Dial Transplant 2013;28:2356-63.
39. Adams GR, Vaziri ND. Skeletal muscle dysfunction in chronic renal failure: effects of exercise. American journal of physiology. Renal physiology 2006;290:F753-61.
40. Oh-Park M, Fast A, Gopal S, et al. Exercise for the dialyzed: aerobic and strength training during hemodialysis. Am J Phys Med Rehabil 2002;81:814-21.
41. Caglar K, Peng Y, Pupim LB, et al. Inflammatory signals associated with hemodialysis. Kidney Int 2002;62:1408-16.

42. Ikizler TA, Pupim LB, Brouillette JR, et al. Hemodialysis stimulates muscle and whole body protein loss and alters substrate oxidation. Am J Physiol Endocrinol Metab 2002;282:E107-16.
43. Simmons EM, Langone A, Sezer MT, et al. Effect of renal transplantation on biomarkers of inflammation and oxidative stress in end-stage renal disease patients. Transplantation 2005;79:914-9.
44. Guleria S, Agarwal RK, Guleria R, et al. The effect of renal transplantation on pulmonary function and respiratory muscle strength in patients with end-stage renal disease. Transplant Proc 2005;37:664-5.

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Original Article

Pulmonary function assessment in the early phase of patients with smoke inhalation injury from fire

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Objectives: Fire smoke contains toxic gases and numerous chemical compounds produced by incomplete combustion, and may cause injury to the airways. Increased airway reactivity, as well as a decrease in lung function, has been reported as a sequela of smoke inhalation injury. This study was undertaken to assess lung functions in the early phase of patients with smoke inhalation damage from fires.

Methods: A total of 15 patients with fire smoke inhalation (fire smoke group) and 15 subjects with chronic cough but no previous history of lung disease (chronic cough group) were enrolled. For diagnosis of inhalation injury, we performed bronchoscopy, high-resolution computed tomography (HRCT), as well as arterial carboxyhemoglobin (COHb) at admission. Clinical characteristics, pulmonary function tests (PFTs) and mannitol bronchial provocation tests (BPTs) were analyzed and compared between the two groups.

Results: In fire smoke group, initial COHb levels and the PaO_2/FiO_2 ratio were (14.8±18.49)% and 425.7±123.68, respectively. Of seven patients performing HRCT, 4 (57.1%) showed the CT findings compatible with lung involvement of inhalation injury. Post bronchodilator value of the percent of forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV_1) were (76.0±24.27)% and (79.8±27.82)%, respectively. Pre-and post- bronchodilator forced expiratory flow between 25% and 75% of the FVC (FEF_{25-75}) and the percent predicted FEF_{25-75} were 2.41±1.47 *vs*. 2.65±1.45 L (P=0.045), and (68.7±37.29)% *vs*. (76.4±36.70)% (P=0.031), respectively. Two patients (13.3%) had positive bronchodilator response (BDR). In fire smoke and chronic cough group, all the subjects showed mannitol BPTs within normal limits.

Conclusions: Fire smoke inhalation leads to mild obstructive small airway disease pattern of pulmonary function in the early phase of patients with fire smoke damage. Further studies, however, need to be followed to identify the relationship between airway narrowing to inhaled mannitol and smoke inhalation injury.

Keywords: Bronchial provocation tests (BPTs); fires; mannitol; smoke inhalation injury

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Introduction

Fire smoke contains superheated gases, steam, and numerous noxious chemical compounds produced by incomplete combustion. It is generally accepted that the most striking features in the airways of the lungs after smoke inhalation are airway obstruction and bronchoconstriction due to intense inflammatory reaction and inspissated secretions caused by inhalation of particulate debris, irritant materials, fumes, or various toxic vapors (1). The survivors from fire accidents may inhale toxic combustion products generated by fires and present with symptoms that resemble asthma, such as severe cough, sputum, choking sensation, and wheezing (2-4).

Bronchial provocation tests (BPTs) are widely used to identify individuals with bronchial hyperresponsiveness (BHR), which is an important pathophysiological feature of

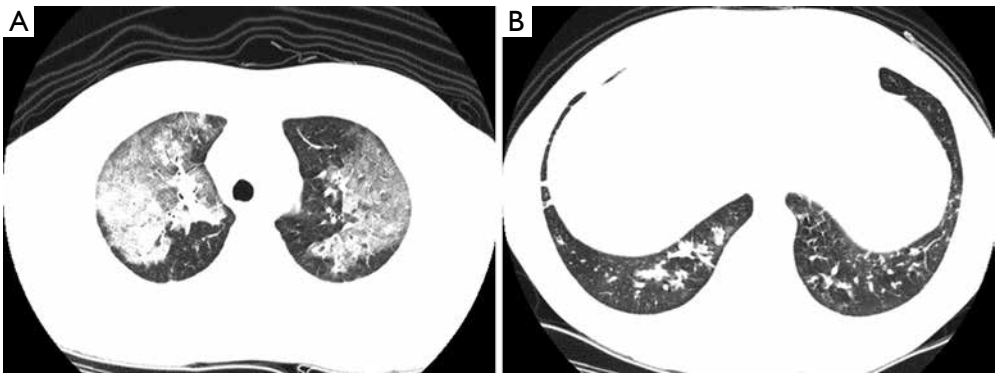

Figure 1 Bronchoscopy showing edema, inflammation, and soot in the carina and both main bronchi in a 22-year-old man with smoke inhalation injury at the first day after admission.

asthma (5). The airways of asthmatic patients are narrowed in response to the inhalation of aerosols of sodium chloride (6). The mechanism causing airway narrowing by hypertonic saline is thought to involve an increase in osmolarity and release of mediators from the mast cells and sensory nerves (6,7). Mannitol with high osmolarity is known to be more potent than sodium chloride in producing mast-cell histamines. An increased airway reactivity in previous healthy individuals may be expected after acute exposure to fire smoke, although the studies have shown that pulmonary function abnormalities in burn patients depend on the type of injury (8,9).

The present study was aimed to assess pulmonary functions by using pulmonary function test (PFT) and mannitol BPT in the early phase of patients who had smoke inhalation injury from fires.

Patients and methods

Study design and subjects

The study was approved by the local institutional review board, and the patients permitted their medical records for research from the hospital database. Between January 2010 and January 2012, we conducted an observational study in consecutive patients hospitalized at the burn center of Hallym University Hangang Sacred Heart Hospital in Seoul, South Korea.

The inclusion criteria were as follows: adult age, minor burns involving a total body surface area of less than 15%, initial arterial carboxyhemoglobin (COHb) levels of more than 5%, or a history of exposure to smoke in closed space and presence of singed nasal hairs, sooty sputum, or auscultatory findings such as wheezing or rhonchi. Diagnosis of inhalation injury was made by fiberoptic bronchoscopy, which was normally performed within 48 h after admission. We characterized bronchoscopic findings for inhalation injury as follows: edema, blistering, carbonaceous material, soot, hemorrhage, inflammation, ulceration, and necrosis of the bronchial mucosa (*Figure 1*). The severity of inhalation injury was graded according to the number of presenting features: near normal, no features present; mild, 1-3 features present; moderate, 4-6 features present; severe, 7-8 features present (10). Patients who had underlying structural lung diseases and past history of tuberculosis, asthma, and atopy were excluded. After admission, patients with smoke inhalation were treated according to the lung care standards of our burn unit, including fluid and nutritional support, maneuvers to prevent pneumonia, endotracheal intubation for airway maintenance, and mechanical ventilation if indicated. We also performed high-resolution computed tomography (HRCT) to identify lung parenchymal involvement of inhalation injury.

During the study periods, the subjects complaining of cough were recruited after matching for age, sex, height and body mass index from our outpatient department after obtaining written informed consent. All participants who had suffered from cough for more than three weeks but had no previous history of respiratory disease or atopy.

High-resolution computed tomography (HRCT)

Computed tomography (CT) scans were acquired using a GE HiSpeed CT/i scanner (GE Medical System, Milwaukee, WI, USA) with no use of contrast media. All CT data were reconstructed by using a high-spatial-frequency (bone)

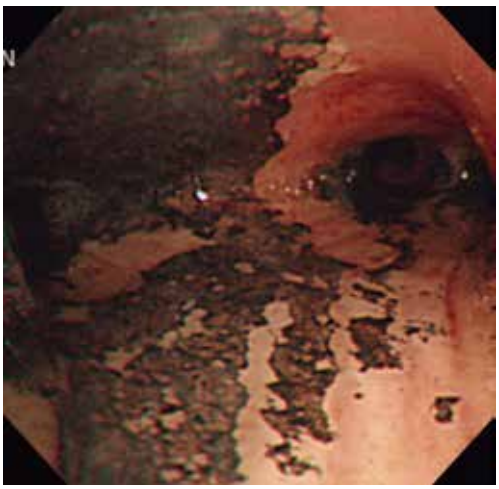

Figure 2 High-resolution computed tomography of a 27-year-old man with 3% of flame burn and inhalation injury at the first day after admission. (A) Extensive ground glass opacities with consolidations were seen in both upper lungs; (B) Peribronchial ground glass attenuations were seen in both lower lungs.

algorithm with a section thickness of 1 mm. Window width and window levels were 400 HU and 20 HU for the mediastinum, and 1,500 HU and –700 HU for the lungs, respectively.

If HRCT findings showed peribronchial ground glass opacity with/or without consolidation, bronchial wall thickenings, branching linear attenuations, atelectasis, or interlobular septal thickening in patients with fire smoke inhalation, we considered inhalation injury to have resulted in lung parenchymal involvement (*Figure 2*).

Each CT finding was interpreted by a radiologist, and defined as; ground glass opacity, a hazy area of air space of increased lung attenuation with preservation of bronchial and vascular markings; consolidation, alveolar space that contains liquid instead of gas; atelectasis, a condition where the alveoli deflated; branching linear attenuations referred to as poorly defined, small centrilobular nodules and branching linear opacity of small airways (2-4 mm diameter) originate from a single stalk; bronchial wall thickening, greater than 0.3 mm or half the width of the accompanying pulmonary artery branch; interlobular septal thickening, greater than 2.0 cm in length and 0.1 mm in thickness (11,12).

PFT and mannitol bronchoprovocation test

Before the PFT and the mannitol BPT, all subjects were asked to refrain from taking short-acting bronchodilators for 6 hours and long-acting bronchodilator for 12 h prior to the study. And the two tests were performed on separate days.

For pulmonary functions test, we used a spirometer (Vmax22; SensorMedics; Yorba Linda, CA, USA), which was calibrated with a 3 L syringe on the morning of each day. In accordance with the American Thoracic Society, forced vital capacity (FVC), forced expiratory volume in 1 second (FEV_1), forced expiratory flow between 25% and 75% of the FVC (FEF_{25-75}), peak expiratory flow (PEF) and diffusing capacity of the lung to transfer carbon monoxide (DLco) were measured. Subsequently, post-bronchodilator values were measured 15-20 min after the administration of an inhaled bronchodilator (e.g., 400 μg of salbutamol) (13). Bronchodilator response (BDR) was assessed by comparing pre- and post- bronchodilator FEV_1. An increase in FEV_1 greater than 200 mL and 12% of the baseline value was accepted as a positive BDR (14).

For mannitol BPT, dry powder mannitol (ARIDOL^{TM}) was prepared in kit form (Pharmaxis Ltd., NSW Australia) containing several capsules with different doses of mannitol. A single capsule contained 0, 5, 10, 20, or 40 mg. The dry powder device used for inhalation was the Osmohaler^{TM} (RS-01, Pastiape^{TM}, Italy). Baseline FEV_1 was measured on presentation at our PFT laboratory. The dose protocol consisted of 0 (empty capsule as a placebo), 5, 10, 20, 40, 80, 160, 160 and 160 mg (15). Doses of more than 80 mg were given in multiples of 40 mg capsules. The FEV_1 was measured twice 60 s after each dose, and the highest FEV_1 value was recorded. The FEV_1 value measured after the 0-mg capsule was taken as the pre-challenge FEV_1 and was used to calculate the percentage decrease in FEV_1 in response to the mannitol challenge. If the FEV_1 fell by more than 10% in response to a single dose, the same dose was repeated. The mannitol challenge was completed when a 15% fall in FEV_1 was documented or a cumulative dose of 635 mg had been administered. After the completion of the test, an inhaled bronchodilator (e.g., 200 μg of salbutamol) was administered.

Statistical analysis

Data were analyzed using dBSTAT for Windows Version 4.0 (DBSTAT Co, Chuncheon, South Korea). Categorical variables were described as frequencies (%) and continuous variables as mean ± standard deviation. We conducted independent two-sample and paired *t*-tests for the comparison of continuous variables and the χ^2 test or Fisher's exact test (if the expected values were below 5)

Table 1 Clinical characteristics of fire smoke group (N=15)

	Value
Burn mode	
Only smoke inhalation	13 (86.7)
Combined flame burn	2 (13.3)
Underlying illness	
Previously healthy	13 (86.7)
Others*	2 (13.3)
ICU care, yes	2 (13.3)
Intubation, yes	2 (13.3)
Initial COHb, %	14.81±18.49
Initial PaO_2/FiO_2, mmHg	425.68±123.68
Initial serum procalcitonin level, ng/mL	0.34±0.50
Bronchoscopy, yes	15 [100]
Date after admission, median days (range)	2 [0-2]
Bronchoscopic grades for inhalation injury	
Near normal	3 [20]
Mild	9 [60]
Moderate	3 [20]
HRCT, yes	7 (46.7)
Date after admission, median days (range)	1 [0-6]
Compatible with inhalation injury	4 (57.1)
PFT date after admission, median days (range)	2 [0-5]
Mannitol BPT date after admission, median days (range)	4 [2-18]

Data are presented as mean ± SD or n (%). *, neurofibromatosis [1], diabetes mellitus with hypertension [1]. Abbreviations: ICU, intensive care unit; COHb, carboxyhemoglobin; HRCT, high-resolution computed tomography; PFT, pulmonary function test; BPT, bronchial provocation test.

for categorical variables. A P value of less than 0.05 was considered statistically significant.

Results

Subjects characteristics

In all, 30 adult participants were enrolled in this study: 15 in fire smoke group (5 men and 10 women) and chronic cough group in 15 (5 men and 10 women).

In fire smoke group, the majority of the patients (86.7%) suffered only from smoke inhalation; only 2 of 15 patients required E-tube insertion for airway maintenance and were admitted to the intensive care units. Initial arterial COHb levels and the PaO_2/FiO_2 ratio were 14.81%±18.40% and 425.68±123.69 mmHg, respectively. Bronchoscopy was performed in all patients, and the grades for inhalation injury were as follows bronchoscopically: near normal, 3 (20%); mild, 9 (60%); moderate, 3 (20%); severe, 0. HRCT was performed in seven patients, and four of those (57.1%) showed the findings compatible with lung parenchymal involvement of inhalation injury (*Table 1*).

Table 2 Patient's characteristics and clinical symptoms between fire smoke and chronic cough group

Parameters	Fire smoke group (N=15)	Chronic cough group (N=15)	P
Age, years	33.93±13.89	33.93±14.51	1.000
Sex, male	5 (33.3)	5 (33.3)	1.000
BMI, kg/m^2	21.19±2.83	20.34±2.18	0.365
Clinical symptoms			
Cough	7 (46.7)	11 (73.3)	0.264
Nocturnal awakening	2 (13.3)	6 (40.0)	0.215
Chest discomfort	0	4 (26.7)	0.100
Chest pain	0	1 (6.7)	1.000
Wheezing	2 (13.3)	0	0.483
Dyspnea	1 (6.7)	0	1.000

Data are presented as mean ± SD or n (%). Abbreviation: BMI, body mass index.

There were no significant differences in demographics and clinical symptoms between the two groups. The most common symptom was cough, 46.7% and 73.3% in fire smoke and chronic cough group, respectively (*Table 2*).

HRCT findings

Of the seven patients in whom HRCT was performed, 4 (57.1%) were found to have lung parenchymal abnormalities on HRCT scans resulted from fire smoke inhalation injury. Peribronchial ground glass opacities were the most common, and followed by segmental or subsegmental consolidations, atelectasis, branching linear attenuations, bronchial wall thickening, and interlobular septal thickening (*Table 3*).

PFT and mannitol bronchoprovocation test

In fire smoke group, FVC and FEF_{25-75} were slightly less than 80% predicted, and other values including FEV_1, PEF,

Table 3 HRCT findings in fire smoke group (N=4)

Findings	Number
Peribronchial ground glass opacities	4 [100]
Segmental or subsegmental consolidations	2 [50]
Segmental or subsegmental atelectasis	2 [50]
Branching linear attenuations	2 [50]
Bronchial wall thickening	1 [25]
Interlobular septal thickening	1 [25]

Data are presented as n [%]. Abbreviation: HRCT, high-resolution computed tomography.

Table 4 Pulmonary function tests in fire smoke group (N=15)

	Before BDR	After BDR	P
FVC, L	2.85±1.15	2.89±1.10	0.448
% predicted	74.3±23.90	76.0±24.27	0.614
FEV_1, L	2.30±1.04	2.41±1.02	0.060
% predicted	76.5±28.49	79.8±27.82	0.078
FEV_1/FVC, %	78.4±11.68	80.9±10.13	0.058
FEF_{25-75}, L	2.41±1.47	2.65±1.45	0.045
% predicted	68.7±37.29	76.4±36.70	0.031
PEF, L	5.23±2.76	5.31±2.60	0.641
% predicted	75.4±34.27	76.9±32.57	0.536
DLco, mL/min/mmHg	18.48±7.52		
% predicted	92.44±22.64		
Positive bronchodilator response	2 (13.3)		

Data are presented as mean ± SD or n (%). Abbreviations: BDR, bronchodilator response; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 second; FEF_{25-75}, forced expiratory flow at 25% to 75% of the FVC; PEF, peak expiratory flow; DLco, diffusing capacity for carbon monoxide.

FEV_1/FVC and DLco were equal to or a little more than 80% predicted. Then, the values of FEF_{25-75} and the percent of FEF_{25-75} before and BDR were 2.4±1.47 L *vs.* 2.65±1.45 L (P=0.045), and 68.7±37.29% *vs.* 76.4±36.70% (P=0.031), respectively. The positive BDR was observed in two patients (13.3%) (*Table 4*).

We compared the results of mannitol BPTs in the two groups, but found no significant differences in baseline FEV_1, maximum decrease in FEV_1, and the total cumulative mannitol dose at the time of the maximum decrease in FEV_1. Moreover, all the subjects showed mannitol BPTs within normal limits (*Table 5*). All study participants were reported to tolerate the tests well without any side effects except complaining of mild cough.

Discussion

The fire victims suffering from smoke inhalation may have a number of complications caused by various pulmonary sequelae. Wheezing and chronic cough may reflect underlying hyperreactive airways. Chronic bronchitis, bronchiolitis obliterans, bronchial stenosis, lung fibrosis, bronchiectasis and atelectasis have been reported to be resulted from exposure to fire smoke and subsequent inflammatory consequences (16).

The understanding of precise pathophysiology in patients who had smoke inhalation damage from fires is of great important for effective airway management. The present study aimed to assess the lung functions in the early phase of patients with smoke inhalation injury. Initially, we taken into account that fire smoke inhalation is a strong stimulus that triggers bronchoconstriction. Unfortunately, we could not detect any significant BHR in mannitol BPTs to explain airway narrowing that commonly occurs after fire smoke inhalation (2,3). Although FEV_1, PEF, FEV_1/FVC and

Table 5 Comparison of mannitol bronchial provocation tests

	Fire smoke group	Chronic cough group	P
A total dose of mannitol used (635 mg)	15 [100]	15 [100]	–
Baseline FEV_1, L	3.05±0.77	2.73±0.82	0.282
Maximum decrease in FEV_1, %	6.53±4.27	6.60±2.95	0.961
FEV_1 at maximum decrease in FEV_1, L	2.9±0.88	2.60±0.82	0.329
Cumulative mannitol dose*, mg	379.67±236.85	355.67±253.22	0.791
Positive results	0	0	

Data are presented as mean ± SD or n (%). *, at maximum decrease in FEV_1. Abbreviations: FEV_1, forced expiratory volume in one1 second; PD15, the provoking dose of mannitol to cause a 15% reduction in FEV_1.

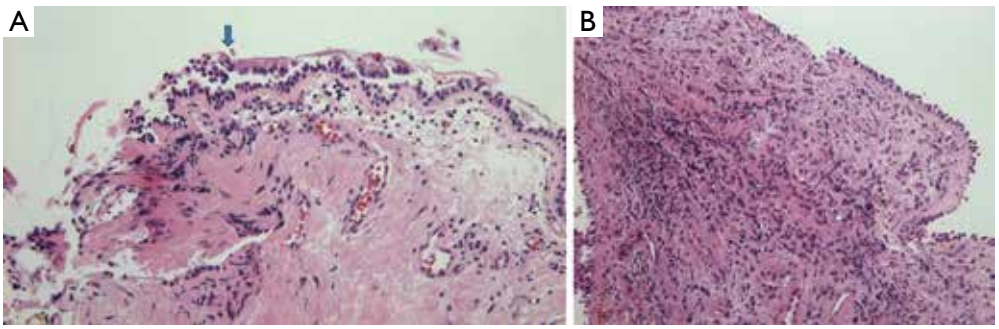

Figure 3 Bronchoscopic biopsy of a 67-year-old woman with smoke inhalation injury. (A) Epithelial exfoliation (arrow), vascular congestion and bronchial dilation (hematoxylin-eosin, ×200); (B) Diffuse aggregates of neutrophils admixed with small numbers of histiocytes, lymphocytes and fibrin subepithelial stroma (hematoxylin-eosin, ×100).

DLco were equal to or a little more than 80% predicted, post-bronchodilator FVC and FEF_{25-75} were less than 80% predicted. Moreover, the percent of FEF_{25-75} was significantly increased after BDR compared to the value before BDR, 68.7% to 76.4% (P=0.031). This finding gives us a relevance to initiate bronchodilators for airway management in patients who had smoke inhalation injury from fires.

Currently, there are two categories of BPTs defined by the mechanisms whereby they stimulate the airways to narrow. The direct stimuli include pharmacologic agents such as histamine or methacholine, which act directly on specific receptors on the bronchial smooth muscle to cause airway contraction. The indirect stimuli refer to physical stimuli such as exercise; hyperventilation; osmotic aerosols including hypertonic saline or mannitol; and adenosine monophosphate, which trigger airway narrowing indirectly by causing the release of various contractile mediators (e.g., leukotrienes, prostaglandins, and histamine) from inflammatory cells within the airways (15). The ultimate goal of precise evaluation of airway narrowing due to an inhalation injury is to provide optimal early management (17-20).

After fire smoke inhalation, a variety of consequences in the respiratory system may be followed by an intense cellular response with neutrophil infiltration, airways edema, bronchoconstriction from aerosolized irritants, small airway occlusion from sloughed endobronchial debris or cast, and alveolar flooding from epithelial disruption (21). These injuries evolve over time and parenchymal lung dysfunction is often minimal for 24-72 h after fire smoke inhalation (22). The airways obstruction and bronchocontriction, which mimic asthma, and usually manifest themselves in the first 24 h (23). In the previous studies, the airways reactivity was assessed by mainly methacholine test, which showed often positive results (2,3). Then, we applied mannitol BPTs on the median four days after admission to a narrow spectrum of patients who mostly had smoke inhalation. Kinsella *et al.* reported that BHR to methacholine usually manifested itself within three days of injury (2). In our study, it remains unclear why BHR to inhaled mannitol was not produced. A delay of the BPT may be a factor not to provoke BHR in fire smoke group. In addition to, one possible explanation is that the airway epithelium after smoke inhalation may be severely injured or even denuded to a degree so that the changes in the airway osmolarity in response to inhaled mannitol could not have occurred. Another reason is that the pathological findings in smoke inhalation injury are quite different from those in asthma, that is, chronic bronchitis with eosinophil infiltration. We have experienced airway inflammatory cells composed mainly of neutrophils, not eosinophils, in the acute stage of inhalation injury (*Figure 3*) (24). Likewise, this is why the study participants did not have BHR in mannitol BPTs, even if they had asthma-like symptoms. Moreover, the increased airway reactivity and prolonged obstructive airway disease have been reported to persist from 3 to 6 months as long-term sequelae of smoke inhalation injury (2,3,25). These findings are the cases when fire victims accompanying inhalation injury suffered from major burn, which probably might have resulted into prolonged BHR, due to intense systemic inflammatory reaction. In our study, the majority of patients had smoke inhalation injury and not fire-related injury. This fact would have contributed to their lack of BHR.

Chest CT scans have been reported to show the superior sensitivity in comparison to chest radiographs for the detection of pulmonary lesion in the early hours of inhalation injury (26). Yamamura *et al.* reported bronchial wall thickening, greater than 2 mm at initial CT scan, was

a predictive marker of prolonged intensive care unit (ICU) stays and the development of pneumonia in patients with inhalation injury (27). In our study, although CT findings such as ground glass opacity with/or without consolidation, atelectasis, branching linear attenuation and interlobular septal thickening, were obtained in patients with smoke inhalation, however, it remains as to which findings affect the pulmonary consequences and how much respiratory function restores since the injury.

The limitations of our study include a small sample size as well as the fact that the participants with chronic cough did not represent individuals with no exposure to fire smoke in whom PFTs were not routinely performed. In addition, we did not have long-term data on late sequelae of inhalation injury because some of the patients refused to undergo BPTs several months after discharge because they did not complain of further symptoms. Despite these limitations, this study demonstrates that FEF_{25-75}, reflecting obstructive small airway disease pattern, was mild decreased before BDR, and significant increased after BDR in fire smoke group, and a small number of patients had positive BDR. These findings may indicate a potential benefit from the use of bronchodilator drugs (28).

In conclusion, the present preliminary study shows that fire smoke inhalation has something with mild obstructive small airway disease pattern of pulmonary function in the early phase in patients with inhalation injury. However, more evidence is necessary to know as to the relationship between airway narrowing to inhaled mannitol and smoke inhalation injury through a large-scaled prospective study.

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References

1. Thorning DR, Howard ML, Hudson LD, et al. Pulmonary responses to smoke inhalation: morphologic changes in rabbits exposed to pine wood smoke. Hum Pathol 1982;13:355-64.
2. Kinsella J, Carter R, Reid WH, et al. Increased airways reactivity after smoke inhalation. Lancet 1991;337:595-7.
3. Park GY, Park JW, Jeong DH, et al. Prolonged airway and systemic inflammatory reactions after smoke inhalation. Chest 2003;123:475-80.
4. Pruitt BA Jr, Flemma RJ, DiVincenti FC, et al. Pulmonary complications in burn patients. A comparative study of 697 patients. J Thorac Cardiovasc Surg 1970;59:7-20.
5. Sterk PJ, Fabbri LM, Quanjer PH, et al. Airway responsiveness. Standardized challenge testing with pharmacological, physical and sensitizing stimuli in adults. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J Suppl 1993;16:53-83.
6. Schoeffel RE, Anderson SD, Altounyan RE. Bronchial hyperreactivity in response to inhalation of ultrasonically nebulised solutions of distilled water and saline. Br Med J (Clin Res Ed) 1981;283:1285-7.
7. Anderson SD, Smith CM. Osmotic challenges in the assessment of bronchial hyperresponsiveness. Am Rev Respir Dis 1991;143:S43-6.
8. Whitener DR, Whitener LM, Robertson KJ, et al. Pulmonary function measurements in patients with thermal injury and smoke inhalation. Am Rev Respir Dis 1980;122:731-9.
9. Demling RH, Crawford G, Lind L, et al. Restrictive pulmonary dysfunction caused by the grafted chest and abdominal burn. Crit Care Med 1988;16:743-7.
10. Khoo AK, Lee ST, Poh WT. Tracheobronchial cytology in inhalation injury. J Trauma 1997;42:81-5.
11. Austin JH, Muller NL, Friedman PJ, et al. Glossary of terms for CT of the lungs: recommendations of the Nomenclature Committee of the Fleischner Society. Radiology 1996;200:327-31.
12. Rossi SE, Franquet T, Volpacchio M, et al. Tree-in-bud pattern at thin-section CT of the lungs: radiologic-pathologic overview. Radiographics 2005;25:789-801.
13. Pellegrino R, Viegi G, Brusasco V, et al. Interpretative strategies for lung function tests. Eur Respir J 2005;26:948-68.
14. Vestbo J, Hurd SS, Agusti AG, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. Am J Respir Crit Care Med 2013;187:347-65.
15. Anderson SD, Brannan J, Spring J, et al. A new method for bronchial-provocation testing in asthmatic subjects using a dry powder of mannitol. Am J Respir Crit Care Med 1997;156:758-65.
16. Lee-Chiong TL Jr. Smoke inhalation injury. Postgrad Med 1999;105:55-62.
17. Brannan JD, Anderson SD, Freed R, et al. Nedocromil sodium inhibits responsiveness to inhaled mannitol

in asthmatic subjects. Am J Respir Crit Care Med 2000;161:2096-9.
18. Brannan JD, Anderson SD, Gomes K, et al. Fexofenadine decreases sensitivity to and montelukast improves recovery from inhaled mannitol. Am J Respir Crit Care Med 2001;163:1420-5.
19. Brannan JD, Gulliksson M, Anderson SD, et al. Inhibition of mast cell PGD2 release protects against mannitol-induced airway narrowing. Eur Respir J 2006;27:944-50.
20. Brannan JD, Koskela H, Anderson SD, et al. Budesonide reduces sensitivity and reactivity to inhaled mannitol in asthmatic subjects. Respirology 2002;7:37-44.
21. Walker HL, McLeod CG Jr, McManus WF. Experimental inhalation injury in the goat. J Trauma 1981;21:962-4.
22. Pruitt BA Jr, Erickson DR, Morris A. Progressive pulmonary insufficiency and other pulmonary complications of thermal injury. J Trauma 1975;15:369-79.
23. Sheridan RL. Airway management and respiratory care of the burn patient. Int Anesthesiol Clin 2000;38:129-45.
24. Clark CJ, Pollock AJ, Reid WH, et al. Role of pulmonary alveolar macrophage activation in acute lung injury after burns and smoke inhalation. Lancet 1988;2:872-4.
25. Moisan TC. Prolonged asthma after smoke inhalation: a report of three cases and a review of previous reports. J Occup Med 1991;33:458-61.
26. Reske A, Bak Z, Samuelsson A, et al. Computed tomography--a possible aid in the diagnosis of smoke inhalation injury? Acta Anaesthesiol Scand 2005;49:257-60.
27. Yamamura H, Kaga S, Kaneda K, et al. Chest computed tomography performed on admission helps predict the severity of smoke-inhalation injury. Crit Care 2013;17:R95.
28. Kim JY, Kim CH, Shin HW, et al. The Findings of Pulmonary Function Test in Patients with Inhalation Injury (Korean). Tuberc Respir Dis 2006;60:653-62.

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Original Article

A comparison between the efficiency of the Xpert MTB/RIF assay and nested PCR in identifying Mycobacterium tuberculosis during routine clinical practice

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Objectives: Polymerase chain reaction (PCR) for the detection of *Mycobacterium tuberculosis* (MTB) is more sensitive, specific, and rapid than the conventional methods of acid-fast bacilli (AFB) smear and culture. The aim of this study was to determine if the Xpert MTB/rifampicin (RIF) assay had additional advantages over nested PCR for the detection of MTB in a geographical area with intermediate tuberculosis (TB) incidence.

Methods: Between February and December 2013, the Xpert MTB/RIF assay and MTB nested PCR, as well as AFB smear and culture, were simultaneously performed on 198 clinical samples (160 pulmonary and 38 non-pulmonary specimens) collected from 171 patients hospitalized at Hallym University Medical Center for possible TB. The accuracy of the diagnosis of MTB culture-positive TB and the turnaround time of reporting laboratory results were calculated and compared. Rifampin resistance by the Xpert MTB/RIF assay was reviewed with that of conventional drug susceptibility testing (DST).

Results: The sensitivity, specificity, and positive and negative predictive values of the Xpert MTB/RIF assay and MTB nested PCR for diagnosis of MTB culture-positive pulmonary TB were 86.1% *vs.* 69.4% (P=0.1563), 97.8% *vs.* 94.1% (P=0.2173), 91.2% *vs.* 75.8% (P=0.1695), and 96.4% *vs.* 92.0% (P=0.2032), respectively. The median turnaround times of the Xpert MTB/RIF assay and MTB nested PCR were 0 [0-4] days and 4 [1-11] days, respectively (P<0.001). Two cases of rifampin resistance, as determined by the Xpert MTB/RIF assay, were found to be multi-drug resistant (MDR) pulmonary TB by DST.

Conclusions: The Xpert MTB/RIF assay seemed to be sensitive, specific, and comparable to nested PCR for identifying MTB among clinically suspected TB patients, and the assay can be valuable in giving a timely identification of resistance to rifampin.

Keywords: *Mycobacterium tuberculosis* (MTB); polymerase chain reaction (PCR); pulmonary tuberculosis

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Introduction

Tuberculosis (TB) remains a serious public health problem because of its high potential for person-to-person transmission. In Korea, where the prevalence of TB is intermediate, the disease is an important public health issue. In 2012 in Korea, the number of reported TB cases was 49,532, and the estimated annual incidence was 108 per 100,000 people (1). Early diagnosis and rapid introduction

of an anti-TB treatment are essential for successful patient outcomes. However, there are shortcomings to the standard diagnostic methods, which include the direct smear for acid-fast bacilli (AFB), which has low sensitivity, and mycobacterial culture, which is slow and usually requires 2-6 weeks to yield a final result (2).

During the last decade, a number of nucleic acid amplification (NAA) methods have been developed for rapid detection and identification of *Mycobacterium tuberculosis* (MTB) in clinical specimens (3,4). These techniques are attractive because they allow for the direct detection of low MTB genomic copy numbers in specimens. Polymerase chain reaction (PCR) is based on NAA methods and is widely used for the rapid diagnosis of TB.

Our institution currently utilizes two commercial standardized PCR procedures, the Xpert MTB/rifampicin (RIF) assay and MTB nested PCR. MTB nested PCR was developed in an effort to identify the members of the MTB complex. The target is the *IS6110* insertion sequence or the *mtp40* gene (5,6). The Xpert MTB/RIF assay, using real-time PCR for the TB-specific *rpoB* gene, is a cartridge-based, automated diagnostic test that can simultaneously identify MTB and resistance to rifampin; it was recently introduced for the rapid diagnosis of TB in Korea. The present study compared the clinical efficiency of the Xpert MTB/RIF assay with that of nested PCR for the detection of MTB among patients with active TB in a newly opened university hospital.

Patients and methods

Study design and specimens

This study was approved by the local institutional review board, with a waiver for obtaining consent from individual patients. We retrospectively examined results from AFB smears and cultures, the Xpert MTB/RIF assay, and MTB nested PCR for 171 patients with suspected TB, in whom those tests were performed at the Hallym University Dongtan Sacred Heart Hospital between February and December 2013. We evaluated the diagnostic accuracy and turnaround time of the Xpert MTB/RIF assay and MTB nested PCR and compared the efficacy of the Xpert MTB/RIF assay with that of the conventional drug susceptibility test (DST) in determining rifampin resistance.

A total of 160 pulmonary and 38 non-pulmonary specimens from 171 suspected TB cases were analyzed in this study. Pulmonary samples included sputum, bronchial washing, and bronchoalveolar lavage fluid, whereas non-pulmonary samples from normally sterile sites consisted of pleural fluid, ascitic fluid, pericardial fluid, joint fluid, cerebrospinal fluid, tissue, or lymph node. If multiple specimens showed positive results from the same patient, only one specimen was used for the analysis.

An initial treatment case of TB was defined as a new patient who had never received treatment for TB or who had taken anti-TB drugs for less than one month. A retreatment case of TB was defined as a patient who was treated after a failure, treated after having previously defaulted, or newly diagnosed with active TB after being previously declared cured or completing treatment (7).

AFB smear and mycobacterial culture

Pulmonary specimens were pretreated with *N*-acetyl-L-cystein-2% NaOH and centrifugation (3,000 ×g for 20 min at 4 ℃). AFB smears were performed using auramine-rhodamine fluorescent staining and confirmed by Ziehl-Neelsen staining. The sediments were inoculated into 3% Ogawa solid media (Asan Pharmaceutical, Seoul, Korea) for eight weeks in 5-10% CO_2 incubators, as well as the BD BACTEC MGIT 960 system (Becton, Dickinson, and Company, Sparks, MD, USA), automated liquid culture system, for 6 weeks. Non-pulmonary specimens were cultured without prior pretreatment. Once cultured, MTB was detected using BD MGIT TB identification test, based-on rapid immunochromatography (Becton, Dickinson, and Company).

Drug susceptibility testing (DST)

All positive cultures were tested for DST. It was performed at the Korean Institute of Tuberculosis by the absolute concentration method, considered the gold standard for detection of isoniazid (INH) and RIF resistance, defined as ≥1% bacterial growth in Löwenstein-Jensen medium at concentrations of 0.2 μg/mL for INH and 40.0 μg/mL for RIF, respectively (8).

MTB nested PCR

For nested PCR, the Seeplex® MTB nested ACE Detection assay (Seegene Inc., Seoul, Korea) was performed according to the manufacturer's instruction. The assay used multi-target (IS6110 and mpb64) PCR, instead of single target PCR for specific detection of MTB. IS6100 is an insertion sequence present in the MTB genome and mpb64 is a conserved sequence present at a single copy in the MTB

genome (5,6). The internal control (520 base pairs) a DNA plasmid, was co-amplified with target DNA to identify processed samples containing substances that might interfere with PCR amplification. Amplified PCR products (190 bp) were electrophoresed and visualized on electrophoresis system.

Xpert MTB/RIF assay

The Xpert MTB/RIF assay (Cepheid Inc., Sunnyvale, CA, USA) was performed according to the manufacturer's instruction. The assay is a fully automated NAA test (by rapid, real-time PCR), was used for the detection of MTB and rifampin resistance. The target was an MTB-specific sequence of the *rpoB* gene, which was labeled with molecular beacons for mutations within the rifampin resistance determining region (9,10). The testing was carried out on the GeneXpert test device platform, which simplifies molecular testing by fully integrating and automating sample preparation, amplification, and detection. A bacterial buffer was added to the clinical specimens before a defined volume was transferred to a cartridge containing all reagents. The plastic cartridge was then introduced to the GeneXpert device, which provided results in less than two hours.

Statistical analysis

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the Xpert MTB/RIF assay and nested TB PCR were calculated, and 95% confidence intervals were estimated. The clinical data of the included patients were described with means, medians, and ranges. Continuous variables were compared by Student's *t*-test or Mann-Whitney U test (if the variables were not normally distributed), whereas the categorical variables were compared using the chi-square test or Fisher's exact test. A P value of <0.05 was considered significant. Statistical analyses were performed using dBSTAT software version 4.0 (dBSTAT Inc., Chuncheon, Korea).

Results

Patient characteristics

The Xpert MTB/RIF assay and MTB nested PCR using pulmonary and non-pulmonary clinical specimens were requested for 171 patients with suspicion of TB. The age of these patients was 58.6±17.58, and 104 (60.8%) patients were male. Hypertension, bronchiectasis, diabetes mellitus, and malignancy were the co-morbidities found in 38 (22.2%), 27 (15.8%), 25 (14.6%), and 15 (8.8%) of the patients, respectively. MTB culture-positive pulmonary TB was finally diagnosed in 36 (21.1%). Seven patients (4.1%) were diagnosed with MTB culture-negative pulmonary TB, based on their clinical symptoms and radiographic findings, even though their AFB smears showed positive results. Extrapulmonary TB was diagnosed in six patients (3.5%), TB pleurisy in three, TB meningitis in two, and TB peritonitis in one (*Table 1*).

Comparison of the Xpert MTB/RIF assay and MTB nested PCR

Of 43 pulmonary TB patients, the Xpert MTB/RIF assay and nested PCR yielded positive results in 32 (94.1%) and 26 (78.8%) patients, respectively (P>0.05). Of the cases of MTB culture-positive pulmonary TB, 31 (91.2%) and 25 (75.8%) were found positive by the Xpert MTB/RIF assay and nested PCR, respectively (P>0.05). None of the extrapulmonary TB patients had positive results in any test. Among the patients with false positive results of the Xpert MTB/RIF assay, non-tuberculous mycobacterial (NTM) disease and bacterial pneumonia were identified in each case (2.9%). Of the patients with false positive MTB nested PCR results, bacterial pneumonia was found in three patients (9.1%) and lung cancer, hemoptysis, sarcoidosis, and empyema in one (3.0%) (*Table 2*).

No differences were observed between the results of the Xpert MTB/RIF assay and MTB nested PCR in patients, according to previous history of treatment for TB (P>0.05). All six patients with extrapulmonary TB corresponded to the initial treatment cases (*Table 3*). Among the 26 patients available data from conventional DST, only two cases (7.7%) showed resistance to RIF by the Xpert MTB/RIF assay and were found to be multi-drug resistant (MDR) MTB by DST on solid culture (*Table 4*).

The median turnaround times from the submission of samples to obtaining results from the laboratory for the Xpert MTB/RIF assay and MTB nested PCR were 0 day (0-4 days) and 4 days (1-11 days), respectively (P<0.001) (*Table 5*).

Diagnostic accuracy of the Xpert MTB/RIF assay and MTB nested PCR for mycobacterial culture positive pulmonary TB

The overall sensitivity, specificity, PPV, and NPV of the

Table 1 Characteristics of the 171 patients in whom the Xpert MTB/RIF assay and MTB nested PCR were performed

Characteristics	
Age, years	58.6±17.58
Male	104 (60.8)
Number of clinical specimens	198
Pulmonary	160 (80.8)
Non pulmonary	38 (19.2)
Co-morbidites	
Hypertension	38 (22.2)
Bronchiectasis	27 (15.8)
Diabetes mellitus	25 (14.6)
Malignancy	15 (8.8)
Cardiovascular disease	13 (7.6)
Stroke	6 (3.5)
Bronchial asthma	5 (2.9)
Dementia	4 (2.3)
Chronic liver disease	3 (1.8)
Chronic kidney disease	3 (1.8)
Taking immunosuppressive drugs	2 (1.2)
HIV/AIDS	1 (0.6)
Final diagnosis	
Pulmonary TB	43 (25.1)
MTB culture positive	36 (21.1)
MTB culture negative	7 (4.1)
Extrapulmonary TB	6 (3.5)
Bacterial pneumonia	45 (26.3)
Lung cancer	13 (7.6)
Nontuberculous mycobacterial lung disease	8 (4.7)
Hemoptysis	8 (4.7)
Lung abscess	7 (4.1)
Transudative pleural effusion	6 (3.5)
Inactive TB sequalae	4 (2.3)
Malignant pleural effusion	4 (2.3)
Empyema	4 (2.3)
Influenza	4 (2.3)
Pulmonary edema	3 (1.8)
Atelectasis	3 (1.8)
Others*	13 (7.6)

*, Fungus ball, 2; interstitial pneumonitis, 2; eosinophilic pneumonia, 2; anthracofibrosis, 2; bronchiectasis, 1; sarcoidosis, 1; pyogenic pericarditis, 1; norcardiosis, 1; chronic respiratory failure, 1. Abbreviations: MTB, *Mycobacterium tuberculosis*; RIF, rifampin; PCR, polymerase chain reaction.

Table 2 Comparison of the results of Xpert MTB/RIF assay and MTB nested PCR

	Number of positive with		P
	Xpert MTB/RIF assay	MTB nested PCR	
Pulmonary TB (n=43)	32 (94.1)	26 (78.8)	0.08
MTB culture positive (n=36)	31 (91.2)	25 (75.8)	0.63
MTB culture negative (n=7)	1 (2.9)	1 (3.0)	–
Extrapulmonary TB (n=6)	0	0	–
Nontuberculous mycobacterial disease	1 (2.9)	0	–
Bacterial pneumonia	1 (2.9)	3 (9.1)	–
Lung cancer	0	1 (3.0)	–
Hemoptysis	0	1 (3.0)	–
Sarcoidosis	0	1 (3.0)	–
Empyema	0	1 (3.0)	–
Total	34 [100]	33 [100]	–

Abbreviations: MTB, *Mycobacterium tuberculosis*; RIF, rifampin; PCR, polymerase chain reaction.

Table 3 Comparison of the results of Xpert MTB/RIF assay and MTB nested PCR in patients with tuberculosis according to previous history of treatment for tuberculosis

	Number of positive with		P
	Xpert MTB/RIF assay	MTB nested PCR	
Pulmonary TB (n=43)			
Initial treatment (n=34)	24	17	0.132
Retreatment (n=9)	8	9	–

Abbreviations: MTB, *Mycobacterium tuberculosis*; RIF, rifampin; PCR, polymerase chain reaction.

Xpert MTB/RIF assay and nested PCR for diagnosis of MTB culture positive pulmonary TB were 86.1% (70.49, 95.28) *vs.* 69.4% (51.89, 83.63), 97.8% (93.63, 99.51) *vs.* 94.1% (88.65, 97.40), 91.2% (76.30, 98.04) *vs.* 75.8% (57.74, 88.88), and 96.4% (91.68, 98.79) *vs.* 92.0% (86.18, 95.95), respectively. No differences were seen among diagnostic

accuracies of the Xpert MTB/RIF assay and nested PCR (*Table 6*).

Discussion

TB remains a major challenge to public health worldwide, especially in endemic areas, despite global efforts to control the disease. Early identification of MTB is very important, as it can help in the initiation of adequate treatment for patients (11-13). PCR is currently the most promising applicative method in the diagnosis of TB. The technique is based on the amplification of a specific genomic sequence of MTB, which is theoretically highly specific and can be useful in giving a rapid diagnosis. However, variable sensitivity (73-80%) and specificity (80-100%) are obtained with different PCR methods, depending on the area of the genome that is amplified and the techniques used for DNA extraction among different laboratories (14-16). Recently, the Xpert MTB/RIF assay, based on real-time PCR, has been developed in an effort to detect MTB, as well as RIF-resistance TB. Its clinical application, however, is limited in Korea, as it has been introduced relatively recently. Because conventional methods, such as AFB smear and culture, can fail due to the paucibacillary nature of TB and presently used PCR methods often show questionable reliability, an evaluation of newer diagnostic methods for TB is important. Therefore, this study evaluated the efficiency of the Xpert MTB/RIF assay compared to preexisting *IS6110*- and *mtp40*-nested PCR to detect MTB in clinical specimens.

In our study, the sensitivity, specificity, PPV, and NPP of the Xpert MTB/RIF assay for diagnosis of MTB culture-positive TB were 86.1%, 97.8%, 91.2%, and 96.4%, respectively, whereas those of nested PCR were 69.4%, 94.1%, 75.8%, and 92.0%, respectively. Out of 43 pulmonary TB patients, no differences were observed between the results of the two tests (P>0.05). Although the sensitivity (86.1%) of the Xpert MTB/RIF assay was higher than that of nested PCR (69.4%), our value was slightly lower than that (90.4%) of a recent study (17). In addition, the turnaround time of the Xpert MTB/RIF assay was shorter than that of the nested PCR, median 0 [0-4] *vs.* 4 [1-11] days, respectively (P<0.001). These findings suggest that the Xpert MTB/RIF assay is comparable to nested PCR for detection of a case of mycobacterial culture-positive TB among clinical TB suspects; in addition, the shorter turnaround time of the assay may help us to initiate anti-TB therapy promptly.

The number of genomic copies in a specimen can affect a

Table 4 Comparison of the Xpert MTB/RIF assay and conventional DST results among mycobacterial culture positive MTB

Conventional DST	Resistance to RIF by Xpert MTB/RIF assay
Susceptible (n=23)	0
INH mono-resistant (n=1)	0
MDR (n=2)	2
Contaminated (n=2)	0
Not done* (n=8)	0

*, Fail to grow on solid culture. Abbreviations: MTB, *Mycobacterium tuberculosis*; RIF, rifampin; INH, isoniazid; PCR, polymerase chain reaction; DST, drug susceptibility test; MDR, multi-drug resistant.

Table 5 Turnaround time of the Xpert MTB/RIF assay and MTB nested PCR (n=171)

From submission of samples to obtaining results from laboratory, days, median [range]		P
Xpert MTB/RIF assay	MTB nested PCR	
0 [0-4]	4 [1-11]	0.000

Abbreviations: MTB, *Mycobacterium tuberculosis*; RIF, rifampin; PCR, polymerase chain reaction.

Table 6 Diagnostic accuracy of the Xpert MTB/RIF assay and MTB nested PCR for the diagnosis of MTB culture positive pulmonary tuberculosis

	Xpert MTB/RIF assay (n=171)	MTB nested PCR (n=171)	P
Sensitivity, % (95% CI)	86.11 (31/36) (70.49, 95.28)	69.44 (25/36) (51.89, 83.63)	0.1563
Specificity, % (95% CI)	97.78 (132/135) (93.63, 99.51)	94.07 (127/135) (88.65, 97.40)	0.2173
PPV, % (95% CI)	91.18 (31/34) (76.3, 98.04)	75.76 (25/33) (57.74, 88.88)	0.1695
NPV, % (95% CI)	96.35 (132/137) (91.68, 98.79)	92.03 (135/138) (86.18, 95.95)	0.2032

Abbreviations: MTB, *Mycobacterium tuberculosis*; RIF, rifampin; PCR, polymerase chain reaction; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

positive PCR result (18). Among 36 pulmonary TB patients with a positive mycobacterial culture, 31 (86.1%) and 25 (69.4%) were found positive with the Xpert MTB/RIF assay and nested PCR, respectively (P>0.05). On the other hand, out of 13 patients, including seven patients with mycobacterial culture-negative pulmonary TB and six patients with extrapulmonary TB, only one patient (7.7%) showed positive results with both tests. These results may come from paucibacillary specimens, such as with TB pleurisy, TB meningitis, and TB peritonitis (19-21). False negative results, however, remain a problem with any test. Among 36 pulmonary MTB culture-positive TB patients, 5 (16.1%) and 11 (30.6%) showed negative results with the Xpert MTB/RIF assay and nested PCR, respectively (P>0.05). A likely reason for the false negative PCR result was the absence of MTB targets (the *rpoB* gene for the Xpert MTB/RIF assay and the *IS6110* or *mtp40* gene for nested PCR) in the requested specimens (22,23). Another possible reason was the number of samples submitted for detection of MTB. More than two sets of sputum samples from patients suspected to have TB are normally requested for AFB cultures. However, the cost and labor of PCR often limits the assay to be performed on one specimen because of sampling difficulties, such as bronchial washing, bronchoalveolar lavage fluid, and body fluid from sterile sites. For these reasons, the sensitivity and PPV of the Xpert MTB/RIF assay and nested PCR in our study (86.1% *vs.* 69.4% and 91.2% *vs.* 75.8%, respectively) were relatively low compared those in other studies (14-16,21,24).

Compared to the Xpert MTB/RIF assay, nested PCR is time consuming and requires manual labor for sample manipulations, which reduce proteins and enzymes that may inhibit the amplification reactions of specimens. This may cause unintentional errors, such as inappropriate specimen dilution and cross-contamination. In our study, among non-TB patients (n=122), excluding 43 pulmonary TB patents and six extrapulmonary TB patients, two (1.6%) and seven patients (5.7%) were positive with the Xpert MTB/RIF assay and nested PCR, respectively (P>0.05). Indeed, nested PCR required multiple user-dependent steps for manipulations, which may give rise to a cross-contamination. In contrast, the Xpert MTB/RIF assay was automatically and simply performed on the GeneXpert device, thus limiting the carryover contamination.

In Korea, MDR-TB strains cause 2.7-3.9% of new TB and 14.0-27.2% of retreatment cases (8,25). In this study, two cases of RIF resistance by the Xpert MTB/RIF assay were found to be MDR-TB patients, one new and one retreatment case of TB. The WHO recommends that the test should be used as the initial diagnostic method in individuals at risk of having MDR-TB or HIV-associated TB and could be used as a follow-up test to sputum microscopy in areas with a low prevalence of MDR-TB or HIV (1). Likewise, the Xpert MTB/RIF assay is likely to play an additional role in detecting MDR-TB strains, regardless of the past history of therapy for TB, after the increase in notification rate of MDR-TB in Korea (1,24). However, we should consider the false negative aspect of the assay, as it may neither detect MTB nor identify RIF resistance in some MDR-TB strains (21,24).

The limitations of our study include a small sample size, as well as the fact that the submitted specimen was not aliquoted into two portions (one for the Xpert MTB/RIF and the other for nested PCR), because of the small amount of volume and sampling difficulties, such as bronchoscopy and body fluid aspiration. Furthermore, both tests were not performed on the same day, in a blind manner by one technician, and independently. This fact may yield some variations in diagnostic accuracy between the Xpert MTB/RIF and nested PCR. We also must consider the cost-effectiveness of PCR techniques, which limits the number times the test can be repeated.

In conclusion, the Xpert MTB/RIF assay appears to have comparable sensitivity and specificity to the nested PCR technique for the routine diagnosis of mycobacterial culture-positive TB. In addition, the assay provides results in a relatively short period, allowing for faster initiation of anti-TB treatment.

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References

1. WHO. Estimates of TB and MDR-TB burden are produced by WHO in consultation with countries. Available online: https://extranet.who.int/sree/Reports?op=Replet&name=%2FWHO_HQ_Reports%2FG2%2FPROD%2FEXT%2FTBCountryProfile&ISO2=KR&LAN=EN&outtype=html
2. Centers for Disease Control and Prevention (CDC). Updated guidelines for the use of nucleic acid amplification tests in the diagnosis of tuberculosis. MMWR Morb Mortal Wkly Rep 2009;58:7-10.
3. Jouveshomme S, Cambau E, Trystram D, et al. Clinical

utility of an amplification test based on ligase chain reaction in pulmonary tuberculosis. Am J Respir Crit Care Med 1998;158:1096-101.
4. Piersimoni C, Scarparo C, Piccoli P, et al. Performance assessment of two commercial amplification assays for direct detection of Mycobacterium tuberculosis complex from respiratory and extrapulmonary specimens. J Clin Microbiol 2002;40:4138-42.
5. Hasegawa N, Miura T, Ishii K, et al. New simple and rapid test for culture confirmation of Mycobacterium tuberculosis complex: a multicenter study. J Clin Microbiol 2002;40:908-12.
6. Magdalena J, Vachee A, Supply P, et al. Identification of a new DNA region specific for members of Mycobacterium tuberculosis complex. J Clin Microbiol 1998;36:937-43.
7. WHO (2013). Global tuberculosis report.
8. Bai GH, Park YK, Choi YW, et al. Trend of anti-tuberculosis drug resistance in Korea, 1994-2004. Int J Tuberc Lung Dis 2007;11:571-6.
9. El-Hajj HH, Marras SA, Tyagi S, et al. Detection of rifampin resistance in Mycobacterium tuberculosis in a single tube with molecular beacons. J Clin Microbiol 2001;39:4131-7.
10. Tyagi S, Kramer FR. Molecular beacons in diagnostics. F1000 Med Rep 2012;4:10.
11. Pai M, Minion J, Sohn H, et al. Novel and improved technologies for tuberculosis diagnosis: progress and challenges. Clin Chest Med 2009;30:701-16, viii.
12. Van Deun A, Martin A, Palomino JC. Diagnosis of drug-resistant tuberculosis: reliability and rapidity of detection. Int J Tuberc Lung Dis 2010;14:131-40.
13. Yang XY, Li YP, Mei YW, et al. Time and spatial distribution of multidrug-resistant tuberculosis among Chinese people, 1981-2006: a systematic review. Int J Infect Dis 2010;14:e828-37.
14. Aryan E, Makvandi M, Farajzadeh A, et al. Clinical value of IS6110-based loop-mediated isothermal amplification for detection of Mycobacterium tuberculosis complex in respiratory specimens. J Infect 2013;66:487-93.
15. Aryan E, Makvandi M, Farajzadeh A, et al. A novel and more sensitive loop-mediated isothermal amplification assay targeting IS6110 for detection of Mycobacterium tuberculosis complex. Microbiol Res 2010;165:211-20.
16. Singh A, Kashyap VK. Specific and Rapid Detection of Mycobacterium tuberculosis Complex in Clinical Samples by Polymerase Chain Reaction. Interdiscip Perspect Infect Dis 2012;2012:654694.
17. Chang K, Lu W, Wang J, et al. Rapid and effective diagnosis of tuberculosis and rifampicin resistance with Xpert MTB/RIF assay: a meta-analysis. J Infect 2012;64:580-8.
18. Helb D, Jones M, Story E, et al. Rapid detection of Mycobacterium tuberculosis and rifampin resistance by use of on-demand, near-patient technology. J Clin Microbiol;48:229-37.
19. Morehead RS. Tuberculosis of the pleura. South Med J 1998;91:630-6.
20. Bastian I, Rigouts L, Van Deun A, et al. Directly observed treatment, short-course strategy and multidrug-resistant tuberculosis: are any modifications required? Bull World Health Organ 2000;78:238-51.
21. Bunsow E, Ruiz-Serrano MJ, López Roa P, et al. Evaluation of GeneXpert MTB/RIF for the detection of Mycobacterium tuberculosis and resistance to rifampin in clinical specimens. J Infect 2014;68:338-43.
22. Das S, Paramasivan CN, Lowrie DB, et al. IS6110 restriction fragment length polymorphism typing of clinical isolates of Mycobacterium tuberculosis from patients with pulmonary tuberculosis in Madras, south India. Tuber Lung Dis 1995;76:550-4.
23. Kent L, McHugh TD, Billington O, et al. Demonstration of homology between IS6110 of Mycobacterium tuberculosis and DNAs of other Mycobacterium spp.? J Clin Microbiol 1995;33:2290-3.
24. Kwak N, Choi SM, Lee J, et al. Diagnostic Accuracy and Turnaround Time of the Xpert MTB/RIF Assay in Routine Clinical Practice. PLoS One 2013;8:e77456.
25. Choi JC, Lim SY, Suh GY, et al. Drug resistance rates of Mycobacterium tuberculosis at a private referral center in Korea. J Korean Med Sci 2007;22:677-81.

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Original Article

Home-based exercise: promising rehabilitation for symptom relief, improved functional status and quality of life for post-surgical lung cancer patients

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Background: Post-thoracotomy non-small cell lung cancer (NSCLC) patients report cancer-related fatigue (CRF) as a severe symptom that may increase the occurrence and severity of other symptoms while decreasing functional status and quality of life (QOL). The aim of this pilot study was to describe the effects of a home-based rehabilitative exercise intervention on CRF, other symptoms, functional status, and QOL for post-surgical NSCLC patients starting within days after hospital discharge.

Methods: Seven post-thoracotomy NSCLC patients completed the Brief Fatigue Inventory (BFI) measuring CRF severity, and the M.D. Anderson Symptom Inventory measuring symptom severity at pre- and post-surgery, and at the end of each week of the six-week intervention. Additionally, the Medical Outcomes Short-Form-36 measuring physical and mental functional status; and the Quality of Life Index (QLI) measuring QOL were completed pre- and post-surgery, after week 3, and at the end of the intervention (week 6).

Results: Participants had a mean age of 65 years, a mean of 6 co-morbid conditions, and initiated the intervention within 4 days after hospital discharge. Participants' CRF severity scores were reduced to mild levels while the mean number of symptoms decreased from 10.4 post-surgery to 7.0 at week 6 with lower levels of severity and interference. Likewise, participants' post-intervention functional status and QOL improved to near or above pre-surgical levels.

Conclusions: The exercise intervention for post-surgical NSCLC patients showed promising preliminary efficacy in improving CRF, other symptom severity, functional status, and QOL. Further testing via a two-arm randomized controlled trial is being conducted.

Keywords: Lung cancer; exercise; cancer-related fatigue (CRF); symptoms; functional status; quality of life (QOL)

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Individuals with non-small cell lung cancer (NSCLC) face difficult challenges in symptom control and management on a daily basis (1). In fact, troublesome symptoms are often present at the time of diagnosis with NSCLC (2). Unfortunately, NSCLC treatment may not always relieve the symptoms that patients endure (3). Instead, treatment may initiate an onset of new symptoms and/or exacerbate the severity and distress of existing symptoms (4).

Furthermore, lung cancer often presents in older populations that have a history of tobacco dependence and other co-morbid conditions such as hypertension, heart disease, and chronic obstructive pulmonary disease. Co-morbidities are often accompanied by symptoms that worsen because of cancer and its treatment (5). Unfortunately, concomitant symptoms from cancer, cancer treatment, and co-morbidities persist throughout cancer treatment and further negatively affect the functional status and quality of life (QOL) of individuals with lung cancer (1). Moreover, individuals with lung cancer are less likely to be directed to rehabilitation programs for self-management or defined recovery regimens (6-9). As a result, symptom self-management strategies need to be developed and tested in order to preserve and maximize the functional status and QOL of individuals with lung cancer. The purpose of this pilot study was to describe the effects of a post-surgical home exercise intervention implemented immediately after hospital discharge on cancer-related fatigue (CRF), other symptoms, functional status, and QOL in individuals with NSCLC.

Background

Thoracotomy provides the best treatment for potential cure of individuals with early stage NSCLC (10). However, symptom knowledge related to symptoms, functional status, and QOL outcome research following thoracotomy is limited, and the reports are not optimistic.

Cancer-related fatigue (CRF)

CRF is a debilitating symptom that is infamous for its prevalence, severity, and persistence causing a profound, negative effect on functional status and QOL (11). As defined by the National Comprehensive Cancer Network, CRF is a distressing, persistent, subjective sense of tiredness or exhaustion related to cancer or cancer treatment that is not proportional to recent activity and interferes with usual functioning (12). Individuals with cancer report that CRF is the most distressing symptom related to their cancer and cancer treatment (13-15). Likewise, a single underlying cause to CRF has eluded researchers complicating CRF screening and leaving health care professionals puzzled in their ongoing efforts to address this symptom (12). The 2013 National Comprehensive Cancer Network Guidelines reiterated that screening practices for CRF in the clinical setting are not systematic or effective for a variety of reasons leaving CRF to be underreported, under-diagnosed, and under-treated (12). As a result, CRF is commonly known to be surrounded by a constellation of symptoms interfering with a person's functional status and QOL (16).

Symptoms

For individuals undergoing thoracotomy for NSCLC, limited symptom information exists spanning the period from diagnosis to immediately after thoracotomy and throughout the recovery trajectory. What is known regarding post-operative recovery in this population suggest that patients suffer from myriad of symptoms that are multifactorial and attributed to the cancer, cancer treatment, and existing co-morbidities (17,18). Moreover, symptoms have been found to be severe and persistent for post-thoracotomy patients (7,17,18). Also, symptoms have been found in clusters for post-thoracotomy NSCLC patients and the clusters include the symptom of fatigue (7,18). Consequently, symptoms place an additional burden on the lives of individuals post-thoracotomy for lung cancer with multiple symptoms reported as severe and persisting well into the post-surgical recovery period and beyond. Unfortunately, unmanaged fatigue and other symptoms have a direct negative effect on functional status and QOL (3,16).

Functional status

Few longitudinal studies exist that measure changes in functional status and QOL for post-thoracotomy NSCLC patients. Kurtz reported that symptom severity was a significant predictor of physical functional status (19). One symptom, fatigue, has been reported to be an independent predictor of physical functioning (20). Moreover, CRF has been found to have a negative direct effect on physical functioning and a negative indirect effect on physical functioning by worsening the severity of other symptoms in individuals with cancer including lung cancer (16) Furthermore, post-operative declines in physical and mental functional status scores of at least 10% have been found to be associated with an 18% and 13% higher risk of mortality, respectively (21). Among an age-matched healthy population, pre-operative lung cancer patients not only had worse physical functioning, but they reported worse mental functional status scores than their healthy counterparts (3). Six months post-operatively when compared to the age-matched healthy population, individuals with lung cancer showed continued worsening in the area of physical and mental functional status (3). Thus, findings demonstrated

that pre-operative functional status in individuals after lung resection is impaired, and impairment often persists over time (22). Consequently, individuals with lung cancer often express concern about living with the aftermath following surgical resection (23).

Quality of life (QOL)

While people are concerned with how long they are going to live they are also concerned with how well they are going to live in terms of their functional status and the QOL. Most research on post-thoracotomy patients focuses on post-surgical morbidity, mortality, and survival. When QOL has been investigated within the post-surgical NSCLC population, surrogate instruments such as those focused on functional health status combined with varying symptom assessments are typically used to characterize QOL. Using a QOL measure, the Quality of Life Index (QLI), Handy found that diffusion capacity of the lung for carbon monoxide was a predictor for a reduced level of QOL at six months post-thoracotomy (3). Likewise, Handy recommends further exploration of symptoms relative to QOL because in this study pain and dyspnea worsened six months post-thoracotomy (3). Likewise, in two studies conducted by Schulte, post-thoracotomy NSCLC patients failed to make a complete recovery over a 24-month period where neither younger (<70 years of age) nor older (>70 years of age) patients returned to pre-operative QOL levels (22,24). Expansion of research is needed to document QOL in earlier stage lung cancer prior to surgery, immediately after surgery, and on a periodic basis through the recovery trajectory.

Exercise and CRF

With CRF and other symptom severity strongly associated with functional status and QOL, developing an intervention addressing CRF for the post-thoracotomy NSCLC population was a priority of our research team. To date, exercise has been found to be an effective treatment for CRF (25); however, there is little evidence to support the timing, type, frequency, duration, and intensity of exercise for this population (26,27). In July 2010, the American College of Sports Medicine convened a panel of cancer and exercise specialists to develop guidelines for cancer survivors (28). The guidelines conveyed an important message for cancer survivors: avoid inactivity, and return to normal daily activities as quickly as possible after surgery. Specific issues outlined as challenges to exercise by this panel included patients with co-morbidities, decreased range of motion, and/or substantial deconditioning. All of these are major challenges faced by the post-surgical NSCLC population. This article reports results of the preliminary efficacy of the first home-based, light-intensity exercise intervention implemented immediately post-discharge from the hospital after thoracotomy that targeted CRF and its effect on other symptoms, functional status, and QOL in this vulnerable NSCLC population.

Methods

Design, setting, and sample

This pilot study assessed patterns of change in CRF, other symptoms, functional status, and QOL from pre-surgery to the end of a 6-week home exercise program. Measures were obtained prior to surgery, post-surgery at first home visit when the exercise program had begun, and at weeks 3 and 6 of the exercise program. The study was approved by the institutional review boards at Michigan State University, Grand Valley State University, and a university teaching hospital in Michigan. Enrollment commenced in March 2011 with participants being recruited from two sites at the university teaching hospital. Individuals at least 21 years of age with suspected NSCLC were eligible to participate with: (I) a Karnofsky Performance Status Score at least 70% before surgery; (II) medical clearance from thoracic surgeon after surgery; (III) stable co-morbid conditions; and, (IV) willingness to follow health and safety rules. Participants were excluded with: (I) severe visual, hearing or speaking deficits; (II) oxygen therapy for activities of daily living; (III) weight greater than 300 pounds; (IV) photosensitive seizures or diagnosed dementia; and (V) conditions hindering safe participation. Thirteen individuals were invited to participate, and seven agreed. All seven completed the 6-week exercise intervention.

Pre-intervention procedures

An oncology registered nurse recruiter identified and recruited participants meeting the eligibility criteria. After signing informed consent, participants completed baseline questionnaires before the start of the intervention.

Intervention

After study enrollment, a registered nurse intervener

(nurse) taught participants to self-manage CRF using the National Comprehensive Cancer Network Guidelines and to participate in exercise after hospital discharge. Within 72 hours of discharge from the hospital, the nurse performed an assessment of core symptoms (pain, nausea, vomiting, and dyspnea) to ensure exercise readiness. Once adequate symptom control was confirmed, the nurse conducted the first home visit (mean time from hospital discharge to first home visit was 66 hours, range 22-100 hours) and implemented the exercise protocol, which included warm-up exercises and light-intensity (less than 3.0 metabolic equivalents) walking and balance exercises with the Nintendo Wii. Walking started at 5 minutes per day for 5 days a week during week 1 and increased toward the goal of 30 minutes a day at the start of week 6. Participants achieved a mean of 26 (SD, 4.6) minutes per day during the last week of the exercise intervention. Walking duration was increased 5 minutes a day each week if the participant's confidence for achieving a specified duration was 70% or greater on a 0- to 100-point scale, with 100% having high confidence. Participants also completed balance exercise 5 days a week for the study duration. Within 24 hours after the first home visit, the nurse made a phone visit to answer any questions about the exercise intervention. At the start of week 2, the nurse assessed the exercise prescription via a home visit with subsequent phone visits at the start of weeks 3 to 6. Additional home visits by the nurse were available upon participant request.

Additional details about the intervention components, safety, and feasibility have been reported elsewhere (29).

Outcome measures

CRF severity was measured using the Brief Fatigue Inventory (BFI) (30). Nine items were rated on an 11-point scale (0-10, 10= most severe) focusing on CRF severity at its worst in the past 24 hours. Reliability and validity of the BFI is well established. The Cronbach α for this study ranged from 0.89 to 0.98.

Symptom severity and interference were measured using the M.D. Anderson Symptom Inventory Core and Lung Module (MDASI) (31). The MDASI measures the severity of 16 symptoms and 6-items measure the interference from symptoms on daily living at its worst in the past 24 hours. In this study, assessment of fatigue was excluded with the use of the MDASI. The 15 items were rated on an 11-point scale (0-10, 10= most severe and most interference). The Cronbach α for the study ranged from 0.71 to 0.92 with most scores .84 or greater with scores less than 0.70 at week 5 (Cronbach α, 0.67).

Functional Status was measured using the well-established Medical Outcomes Short Form-36 Version 2 Acute Recall (SF-36) (32). Principle component analysis demonstrates that 80-85% of the variance in the eight subscales was accounted for by two factors, physical and mental health. The subscale scores of the SF-36 are linearly transformed providing normative scores from 0 to 100, with higher scores representing better levels of functional status (32). Similar to reported reliabilities, internal consistency reliability for the Physical Health Component (PHC) summary scale ranged from 0.78 to 0.90 except for week 3 (Cronbach's α, 0.61) of our study. Likewise, internal consistency reliability for the Mental Health Component (MHC) summary scale ranged from 0.78 to 0.92 except for week 3 (Cronbach's α, 0.51).

QOL was measured using Ferrans and Powers QLI (33-35). The QLI is a two-part questionnaire with reliability and validity established in the cancer population. The QLI assesses both satisfaction and importance of aspects of life to the person. Each part is comprised of 32 items that assess overall QOL via the following four domains: *health and functioning*, *social and economic*, *psychological and spiritual*, and *family*. Participants respond to a 6-point rating scale ranging from very dissatisfied to very satisfied for the satisfaction items and from very unimportant to very important for the importance items. Scores for the QLI range from 0 to 30, with higher scores indicating better QOL.

Participant characteristics were obtained from a demographic questionnaire and a medical chart review.

Statistical analyses

Analyses were completed using IBM SPSS Statistics version 19.0 (36). The patterns of change in CRF and symptom outcomes were longitudinally graphed from pre- and post-surgery and at the end of each week of the 6-week intervention. Likewise, results for functional status and QOL outcomes were graphed pre- and post-surgery, at week 3, and at the end of the intervention (6 weeks).

Results

Five women and two men (mean age 64.6; SD 6.5; range 53-73 years) participated, and all underwent a thoracotomy for a lobectomy for NSCLC. Stage of cancer ranged from IA to IIIA. Participants had on average 5.9 (SD 3.4; range 2-12)

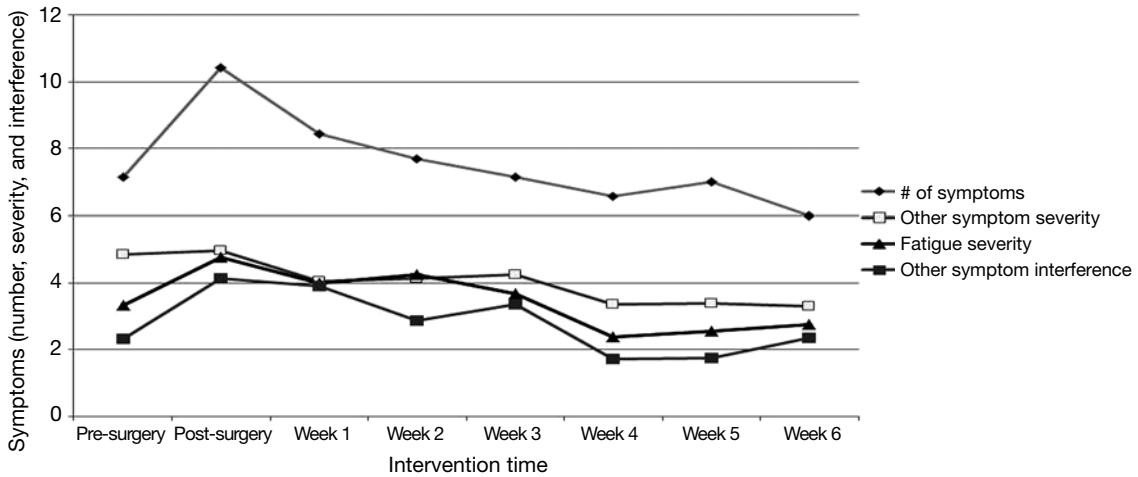

Figure 1 Number of symptoms. Symptom severity and interference (MDASI) with scores ranging from 0 to 10, with higher scores indicating greater severity and interference. Cancer-related fatigue severity (BFI) with scores ranging from 0 to 10, with higher scores indicating greater severity.

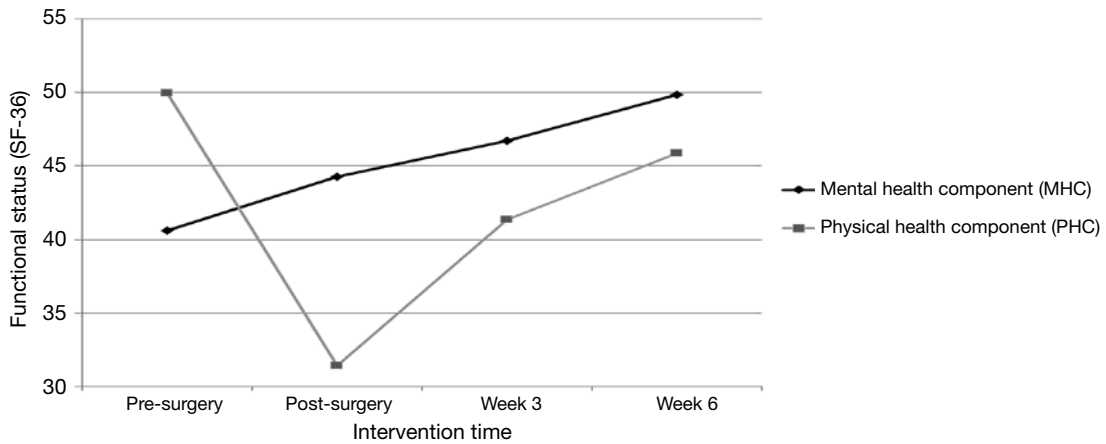

Figure 2 Functional status (SF-36) both physical and mental health component at pre-surgery, post-surgery, and 3 and 6 weeks after the start of the exercise intervention with scores from 0 to 100, with higher scores indicating better levels of functional status .

co-morbid conditions.

Cancer-related fatigue and other symptoms

Figure 1 shows the overall pattern of symptom mean scores from pre-surgery to week 6 of the exercise program. On average, participants experienced seven symptoms pre-surgery that increased to ten symptoms post-surgery and declined to six symptoms by week 6. CRF severity increased from 3.5 to 4.8 pre- to post-surgery and then decreased to 2.8 by week 6. Other symptom severity went from 4.9 to 5.0 pre- to post-surgery and decreased to 3.5 by week 6. Other symptom interference increased from 2.5 to 4.1 pre- to post-surgery and decreased to 2.2 at week 6. Five of seven participants started chemotherapy at week 5 of the home exercise program.

Functional status

Physical functional status

As seen in *Figure 2*, the MOS-36 PHC score indicating overall physical functional status pre-surgery was 49.8, decreased to 31.5 post-surgery, and increased to 41.4 and

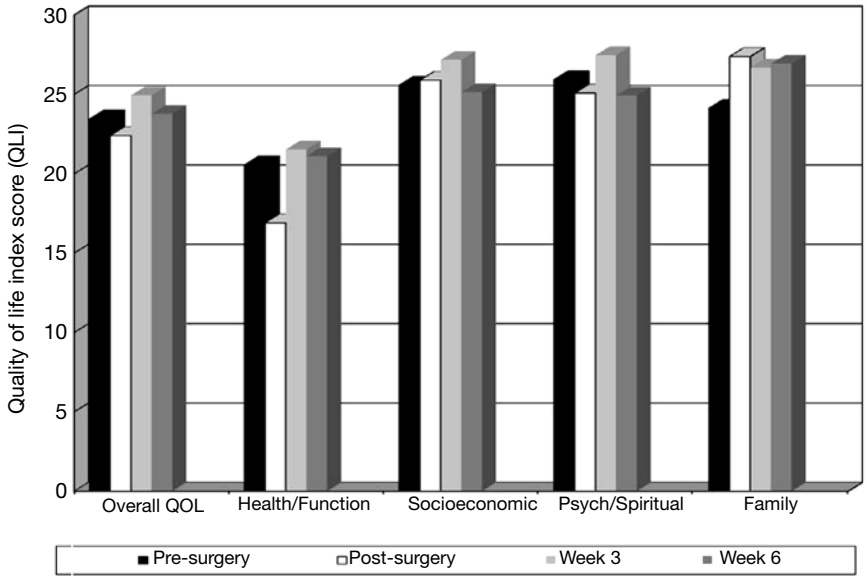

Figure 3 Quality of life index (QLI) at pre-surgery, post-surgery, and 3 and 6 weeks after the start of the exercise intervention with scores ranging from 0 to 30, with higher scores indicating better quality of life.

45.9 at weeks 3 and 6, respectively. Consistent with the overall PHC mean score, all physical functional status subscale mean scores (physical functioning; role-physical functioning; bodily pain, and general health) decreased post-surgery and increased by the end of weeks 3 and 6.

Mental functional status

As seen in *Figure 2*, the MOS-36 MHC mean score indicating mental functional status pre-surgery was 40.6. Post-surgery and at weeks 3 and 6, the MHC mean scores rose to 44.3, 46.7, and 49.8, respectively. The patterns of change in mean scores of the mental functional status domains of vitality, social functioning, role-emotional functioning, and mental health were consistent with the overall MHC with each of the post-intervention scores increasing over pre-surgery scores: vitality (48.9 to 52.4); social functioning (41.9 to 52.5); role-emotional functioning (39.8 to 44.2); and mental health (41.8 to 47.8).

Quality of life (QOL)

As seen in *Figure 3*, overall QOL scores decreased from 23.5 pre-surgery to 22.4 post-surgery and increased to a high of 24.9 at week 3 and decreased to 23.8 at week 6 post-intervention. *Health and functioning* domain score patterns pre-surgery, post-surgery, and at weeks 3 and 6 were 20.6, 16.9, 21.5, and 21.1, respectively. *Psychological and spiritual* domain means score patterns were 25.9, 25.1, 27.5, and 24.9, respectively. S*ocioeconomic* domain score patterns were 25.6, 25.9, 27.2, and 25.1, respectively, while *family* score patterns were 24.1, 27.4, 26.7, and 26.9, respectively. All showed improvement at week 3 over the pre-surgical baseline and a slight decrease at week 6 with 5 out of 7 participants starting chemotherapy at week 5.

Hospital readmissions and unplanned acute care visits

After discharge from the hospital through week 6 post-intervention, there were no re-hospitalizations or unplanned acute care visits.

Discussion

In this first study to assess outcomes of a self-managed home exercise program in NSCLC patients immediately post-thoracotomy, participants mirrored those in previously reported research. They were older with a similar mean age of 65 years, and had a high number of co-morbid conditions (mean of 6). Given that the intervention was shown to be feasible and safe, the next critical step was to determine if positive outcomes in CRF, other symptoms, functional status, and QOL could be achieved in this high acuity

population.

Prior to surgery, participants reported a high number of other symptoms (mean of 7) in addition to CRF that increased post-surgery. From post-surgery to week 6, there was an overall pattern of decline in CRF severity and other symptom mean scores with a slight increase after week 5 of the exercise intervention when chemotherapy was initiated on average for 5 out of 7 participants. By week 4, mean scores for CRF severity and severity and interference of other symptoms were below pre-surgical baseline mean scores. In addition, the average number of symptoms was below the pre-surgical baseline by week 6. These findings are similar to other reports of the number of symptoms and co-morbidities of individuals with NSCLC, but this is the first report showing improvement of symptoms with a home-based exercise program immediately post-thoracotomy.

Pre-operatively, the PHC and MHC representing overall physical and mental functional status were below the mean of 50 for the general U.S. population, 49.8 and 40.6 respectively. Post-surgically, as anticipated, the PHC score fell dramatically to 31.1. Conversely, the MHC score showed improvement post-surgery and continued to improve in weeks 3 and 6 surpassing pre-surgical scores finishing at 49.8 and approaching the general U.S. population mean mental functional status score of 50. Also, PHC scores showed marked improvement in both weeks 3 and 6 nearly achieving pre-surgical baseline levels at post-intervention (week 6) despite the start of adjuvant therapy for 5 of 7 participants at week 5. While research on functional status in the NSCLC population pre- and immediately post-surgery is limited; the findings in this study parallel those reports. However, this is the first study to demonstrate improvements in functional status through a home-based exercise program immediately after hospital discharge. Moreover, this is the first study to demonstrate improvements over the surgical treatment trajectory, establishing a trend from pre-surgery, immediate post-surgery after hospital discharge prior to initiation of exercise, after three weeks of the intervention, and later after a total of six weeks of the intervention.

Last, overall QOL scores decreased from pre-surgery to post-surgery and increased to their highest level exceeding pre-surgery scores at week 3. Despite 5 of 7 participants undergoing adjuvant therapy at week 5, overall QOL scores remained slightly above pre-surgery levels at post-intervention, week 6. Relative to the individual domains of QOL, at post-surgery, the *health and functioning* and *psychological and spiritual* domains of QOL worsened over pre-surgery scores while *socioeconomic* and *family* increased over pre-surgery scores. All domains of QOL exceeded pre-surgery levels at week 3 of the exercise intervention. The domains of *health and functioning*, *socioeconomic*, and *family* remained above pre-surgery levels at post-intervention while the *spiritual* domain of QOL dropped slightly below pre-surgery baseline. Therefore, nearly all of the improvements of the domains of QOL were sustained at the close of the exercise intervention (end of week 6) even as 5 of 7 participants started adjuvant therapy at week 5 suggesting that a home-based exercise intervention to self-manage CRF may have a positive effect on QOL for post-thoracotomy NSCLC patients. While little is known about the evolution of QOL in NSCLC patients who have undergone thoracotomy, the majority of studies incorporating QOL measures in individuals with lung cancer occur much later after surgery or during late stage disease. Also, symptom severity is closely related to QOL in patients with lung cancer and post-surgical NSCLC patients tend to deteriorate related to unmanaged symptoms such as CRF. This is the first known study to document the preliminary efficacy of a home-based exercise intervention for the severity of CRF and subsequent QOL. In addition, QOL measurements were taken regularly prior to surgery and throughout the immediate recovery period to assess the impact of the exercise intervention on QOL with preliminary results indicating positive effects on QOL.

This small pilot study was the first to implement a self-managed, home-based exercise program immediately upon hospital discharge after thoracotomy with resulting performance outcomes that were very promising in this challenging population. The design of this study captured timely, focused data to inform researchers and clinicians of the status of CRF, other symptoms, functional status, and QOL immediately before and after surgery upon hospital discharge for individuals with NSCLC. Likewise, focused assessment of CRF, other symptoms, and health care utilization were assessed on a weekly basis with assessment of functional status and QOL at regular intervals throughout the six-week post-surgical recovery period. Note that 5 out of 7 participants initiated chemotherapy on average at week 5 of the intervention. Further study using a comparative clinical trial design is warranted and is underway by the authors. With the promising efficacy demonstrated in this study, the potential clinical implications of this practical, home-based exercise program would be a major step forward for the recovery and

rehabilitation of this population whose surgical treatment is aimed at cure of their NSCLC.

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References

1. Yang P, Cheville AL, Wampfler JA, et al. Quality of life and symptom burden among long-term lung cancer survivors. J Thorac Oncol 2012;7:64-70.
2. Gift AG, Jablonski A, Stommel M, et al. Symptom clusters in elderly patients with lung cancer. Oncol Nurs Forum 2004;31:202-12.
3. Handy JR Jr, Asaph JW, Skokan L, et al. What happens to patients undergoing lung cancer surgery? Outcomes and quality of life before and after surgery. Chest 2002;122:21-30.
4. Akin S, Can G, Aydiner A, et al. Quality of life, symptom experience and distress of lung cancer patients undergoing chemotherapy. Eur J Oncol Nurs 2010;14:400-9.
5. Given CW, Given B, Azzouz F, et al. Predictors of pain and fatigue in the year following diagnosis among elderly cancer patients. J Pain Symptom Manage 2001;21:456-66.
6. Sanders SL, Bantum EO, Owen JE, et al. Supportive care needs in patients with lung cancer. Psychooncology 2010;19:480-9.
7. Sarna L, Cooley ME, Brown JK, et al. Symptom severity 1 to 4 months after thoracotomy for lung cancer. Am J Crit Care 2008;17:455-67; quiz 468.
8. Brunelli A, Socci L, Refai M, et al. Quality of life before and after major lung resection for lung cancer: a prospective follow-up analysis. Ann Thorac Surg 2007;84:410-6.
9. Granger C, Denehy L. Exercise interventions following surgery for non-small cell lung cancer (NSCLC): the need for future randomised controlled trials. Lung Cancer 2010;70:228-9.
10. Howington JA, Blum MG, Chang AC, et al. Treatment of stage I and II non-small cell lung cancer: Diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines. Chest 2013;143:e278S-313S.
11. Minton O, Berger A, Barsevick A, et al. Cancer-related fatigue and its impact on functioning. Cancer 2013;119 Suppl 11:2124-30.
12. National Comprehensive Cancer Network (NCCN). NCCN clinical practice guidelines in oncology: cancer-related fatigue version 1.2013. Fort Washington, PA2012: http://www.nccn.org/professionals/physician_gls/f_guidelines.asp#supportive, accessed November 1, 2013.
13. Kenny PM, King MT, Viney RC, et al. Quality of life and survival in the 2 years after surgery for non small-cell lung cancer. J Clin Oncol 2008;26:233-41.
14. Braun DP, Gupta D, Staren ED. Quality of life assessment as a predictor of survival in non-small cell lung cancer. BMC Cancer 2011;11:353.
15. Hung R, Krebs P, Coups EJ, et al. Fatigue and functional impairment in early-stage non-small cell lung cancer survivors. J Pain Symptom Manage 2011;41:426-35.
16. Hoffman AJ, von Eye A, Gift AG, et al. Testing a theoretical model of perceived self-efficacy for cancer-related fatigue self-management and optimal physical functional status. Nurs Res 2009;58:32-41.
17. Sarna L, Cooley ME, Brown JK, et al. Women with lung cancer: quality of life after thoracotomy: a 6-month prospective study. Cancer Nurs 2010;33:85-92.
18. Brown JK, Cooley ME, Chernecky C, et al. A symptom cluster and sentinel symptom experienced by women with lung cancer. Oncol Nurs Forum 2011;38:E425-35.
19. Kurtz ME, Kurtz JC, Stommel M, et al. Predictors of physical functioning among geriatric patients with small cell or non-small cell lung cancer 3 months after diagnosis. Support Care Cancer 1999;7:328-31.
20. Given B, Given C, Azzouz F, et al. Physical functioning of elderly cancer patients prior to diagnosis and following initial treatment. Nurs Res 2001;50:222-32.
21. Möller A, Sartipy U. Associations between changes in quality of life and survival after lung cancer surgery. J Thorac Oncol 2012;7:183-7.
22. Schulte T, Schniewind B, Dohrmann P, et al. The extent of lung parenchyma resection significantly impacts long-term quality of life in patients with non-small cell lung cancer. Chest 2009;135:322-9.
23. Maliski SL, Sarna L, Evangelista L, et al. The aftermath of lung cancer: balancing the good and bad. Cancer Nurs 2003;26:237-44.
24. Schulte T, Schniewind B, Walter J, et al. Age-related impairment of quality of life after lung resection for non-small cell lung cancer. Lung Cancer 2010;68:115-20.
25. Cramp F, Byron-Daniel J. Exercise for the management of cancer-related fatigue in adults. Cochrane Database Syst Rev 2012 Nov 14;11:CD006145.

26. Thompson W. ACSM's Guidelines for Exercising Testing and Prescription. 8th ed. Philadelphia, PA: Lippincott Williams & Wilkins, 2010.
27. Jones LW, Eves ND, Kraus WE, et al. The lung cancer exercise training study: a randomized trial of aerobic training, resistance training, or both in postsurgical lung cancer patients: rationale and design. BMC Cancer 2010;10:155.
28. Schmitz KH, Courneya KS, Matthews C, et al. American College of Sports Medicine roundtable on exercise guidelines for cancer survivors. Med Sci Sports Exerc 2010;42:1409-26.
29. Hoffman AJ, Brintnall RA, Brown JK, et al. Too sick not to exercise: using a 6-week, home-based exercise intervention for cancer-related fatigue self-management for postsurgical non-small cell lung cancer patients. Cancer Nurs 2013;36:175-88.
30. Mendoza TR, Wang XS, Cleeland CS, et al. The rapid assessment of fatigue severity in cancer patients: use of the Brief Fatigue Inventory. Cancer 1999;85:1186-96.
31. Cleeland CS, Mendoza TR, Wang XS, et al. Assessing symptom distress in cancer patients: the M.D. Anderson Symptom Inventory. Cancer 2000;89:1634-46.
32. McHorney CA, Ware JE Jr, Raczek AE. The MOS 36-Item Short-Form Health Survey (SF-36): II. Psychometric and clinical tests of validity in measuring physical and mental health constructs. Med Care 1993;31:247-63.
33. Ferrans CE, Powers MJ. Quality of life index: development and psychometric properties. ANS Adv Nurs Sci 1985;8:15-24.
34. Ferrans CE, Powers MJ. Psychometric assessment of the Quality of Life Index. Res Nurs Health 1992;15:29-38.
35. Ferrans CE. Development of a quality of life index for patients with cancer. Oncol Nurs Forum 1990;17:15-9; discussion 20-1.
36. SPSS 15.0 [computer program]. Chicago, IL: SPSS Inc, 2008.

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Original Article

Is uniportal thoracoscopic surgery a feasible approach for advanced stages of non-small cell lung cancer?

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Objectives: Conventional video-assisted thoracoscopic (VATS) lobectomy for advanced lung cancer is a feasible and safe surgery in experienced centers. The aim of this study is to assess the feasibility of uniportal VATS approach in the treatment of advanced non-small cell lung cancer (NSCLC) and compare the perioperative outcomes and survival with those in early-stage tumors operated through the uniportal approach.

Methods: From June 2010 to December 2012, we performed 163 uniportal VATS major pulmonary resections. Only NSCLC cases were included in this study (130 cases). Patients were divided into two groups: (A) early stage and (B) advanced cases (>5 cm, T3 or T4, or tumors requiring neoadjuvant treatment). A descriptive and retrospective study was performed, comparing perioperative outcomes and survival obtained in both groups. A survival analysis was performed with Kaplan-Meier curves and the log-rank test was used to compare survival between patients with early and advanced stages.

Results: A total of 130 cases were included in the study: 87 (A) *vs.* 43 (B) patients (conversion rate 1.1 *vs.* 6.5%, P=0.119). Mean global age was 64.9 years and 73.8% were men. The patient demographic data was similar in both groups. Upper lobectomies (A, 52 *vs.* B, 21 patients) and anatomic segmentectomies (A, 4 *vs.* B, 0) were more frequent in group A while pneumonectomy was more frequent in B (A, 1 *vs.* B, 6 patients). Surgical time was longer (144.9±41.3 *vs.* 183.2±48.9, P<0.001), and median number of lymph nodes (14 *vs.* 16, P=0.004) were statistically higher in advanced cases. Median number of nodal stations (5 *vs.* 5, P=0.165), days of chest tube (2 *vs.* 2, P=0.098), HOS (3 *vs.* 3, P=0.072), and rate of complications (17.2% *vs.* 14%, P=0.075) were similar in both groups. One patient died on the 58th postoperative day. The 30-month survival rate was 90% for the early stage group and 74% for advanced cases

Conclusions: Uniportal VATS lobectomy for advanced cases of NSCLC is a safe and reliable procedure that provides perioperative outcomes similar to those obtained in early stage tumours operated through this same technique. Further long term survival analyses are ongoing on a large number of patients.

Keywords: Advanced lung cancer; uniportal; thoracoscopy; video-assisted thoracoscopic(VATS) lobectomy; minimally invasive surgery; non-small cell lung cancer (NSCLC)

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Introduction

Despite the multiple advantages of video-assisted thoracoscopic (VATS) compared with thoracotomy (1) as decreased postoperative pain, decreased hospitalization, diminished inflammatory response or faster access to chemotherapy, the thoracoscopic approach for advanced stages of lung cancer is still infrequent. The concern about an intraoperative thoracoscopic major bleeding or the technical complication of performing a radical oncologic resection by VATS in advanced cases are the main reasons for the low adoption.

There are few studies reporting perioperative results and survival of patients with advanced disease operated by thoracoscopic approach (2,3). These cases are operated by using conventional VATS. However the same procedure can be performed by using a single incision approach. Since we developed our uniportal technique for VATS lobectomies in 2010 (4) we have increased the application of this technique to more than 90% of cases in our routine surgical practice. The experience we acquired with the uniportal technique during the last years (5), as well as technological improvements in high definition cameras, development of new instruments, vascular clips and more angulated staplers have made this approach safer, incrementing the indications for single-port thoracoscopic resections. We believe it is important to minimize the surgical aggressiveness especially in advanced stage lung cancer patients where the immune system is weakened by the disease or by neoadjuvant treatments. The minimally invasive surgery represents the least aggressive form to operate lung cancer and the single-port or uniportal technique is the final evolution in these minimally invasive surgical techniques.

The objective of this study is to assess the feasibility of uniportal VATS approach in the treatment of advanced non-small cell lung cancer (NSCLC) and to compare the perioperative outcomes and overall survival with early-stage tumors.

Methods

A retrospective descriptive prevalence study was performed on patients undergoing single-port approach for major pulmonary resections at Coruña University Hospital and Minimally Invasive Thoracic Surgery Unit (UCTMI) between June 2010 and December 2012. This study was approved by the review board at Coruña University Hospital and UCTMI. All patients were informed and had a written consent before surgery. A total of 163 surgical interventions (major pulmonary resections) were performed using this technique during the study period. Most were conducted by surgeons experienced with the uniportal approach for minor and major resections.

Only NSCLC were included in the study. Advanced clinical stage NSCLC were considered as tumors bigger than 5 cm, T3 or T4 and/or tumors that received neoadjuvant chemotherapy or radiotherapy. Most of the patients underwent routine preoperative pulmonary function testing, bronchoscopy, computed tomography, and fused positron emisión tomography-computed tomography.

Patients were divided into two groups: (A) early stage (T1 and T2) and (B) advanced clinical stages. A descriptive and retrospective study was performed, comparing perioperative outcomes and survival obtained in both groups.

Thanks to our previous VATS experience with conventional and double-port VATS (6), the indications and contraindications have changed overtime. The only absolute contraindication considered was surgeon discomfort and huge tumors impossible to remove without rib spreading.

Variables studied in each patient were age, sex, smoking habits, COPD, pulmonary function (FEV1 and FVC), presence of associated comorbidities, how the lesion is presented, tumor type and location, type and duration of surgical intervention, surgery-associated adhesions, stage, histology, tumor size, lymph nodes affected (number of lymph nodes retrieved and number of nodal stations explored), duration of chest tube, length of hospital stay, postoperative complications, 60-day mortality and survival.

Statistical analysis

A descriptive analysis of the variables studied was carried out. The quantitative variables are expressed as mean ± standard deviation, median and range. Qualitative variables are expressed by means of frequencies and the corresponding percentages. SPSS 17 for Windows for statistical analysis.

To compare the postoperative course according to perioperative characteristics, the Mann Whitney test was used for quantitative variables and Chi square test or Fisher exact test was used for qualitative variables.

A survival analysis was performed with Kaplan-Meier curves and the log-rank test was used to compare survival between patients with early and advanced stages.

Surgical technique

All patients in both groups were operated by using a single-incision VATS approach with no rib spreading and no wound protector (7). No epidural catheter was used. The 4-5 cm incision was placed in the fifth intercostal space. Anatomic major pulmonary resections were performed in all patients. Following anatomical resection, a complete mediastinal lymphadenectomy was performed in the patients with a diagnosis of malignancy. Instruments used were long and curved with proximal and distal articulation to allow the insertion of 3 or 4 instruments simultaneously and the camera used was 10 mm HD scope 30 degree. Intercostal infiltration was performed at the end of the

Table 1 Patient characteristics

	Mean ± SD [range] or No. (%)	
	A	B
Age	64.87±10.41 [38-84]	65.05±8.99 [47-81]
Sex		
Male	61 (70.1)	35 (81.4)
Female	26 (29.9)	8 (18.6)
Smoking history		
Yes	68 (78.2)	68 (78.2)
No	19 (21.8)	19 (21.8)
Comorbidity		
COPD	34 (39.1)	34 (39.1)
Cardiovascular risk factor	73 (83.9)	73 (83.9)
Cardiac disease	21 (24.1)	21 (24.1)
Previous cancer	22 (25.3)	22 (25.3)
Symptoms		
Casual finding	58 (66.7)	58 (66.7)
Hemoptysis	8 (9.2)	8 (9.2)
General syndrome	7 (8.0)	7 (8.0)
Cough	5 (5.7)	5 (5.7)
Pneumonia	6 (6.9)	6 (6.9)
Chest pain	1 (1.1)	1 (1.1)
Endobronchial tumor	6 (6.9)	6 (6.9)
Preoperative histology	13 (13.8)	13 (13.8)
Neoadyuvant treatment	0	29 (67.4)
Chemotherapy alone		24 (55.8)
Chemo-radiotherapy		5 (11.6)
Pulmonary function		
FEV1	89.06±25.35 [27-134]	79.56±19.87 [45-126]
FVC	94.24±21.04 [57-139]	90.62±14.75 [67-121]

surgery under thoracoscopic view and only one chest tube was placed in all patients.

We always start all lung operations with uniportal VATS to assess the extent of the disease and to rule out any pleuro-pulmonary metastasis. Conversions were defined as operations that began with a thoracoscopic dissection-division of hilar structures and were concluded as ribspreading thoracotomies. The cases that required conversion to open surgery were performed by extending the existing incision and continuing surgery through an anterior minithoracotomy with rib spreading and support of optics (like hybrid VATS).

Table 2 Anatomic pulmonary resections (n=130)

	No. (%)	
	A	B
Right upper lobectomy	26 (29.9)	17 (39.5)
Right middle lobectomy	5 (5.7)	3 (7.0)
Right lower lobectomy	11 (12.6)	7 (16.3)
Left upper lobectomy	26 (29.9)	4 (9.3)
Left lower lobectomy	13 (14.9)	5 (11.6)
Typical segmentectomy	4 (4.6)	0
Right pneumonectomy	0	1 (2.3)
Left pneumonectomy	1 (1.1)	5 (11.6)
Bilobectomy	1 (1.1)	1 (2.3)

Results

Since the start of the Uniportal VATS program in June 2010 until December 2012, we have performed 163 major lung resections using this technique (That is now, December 2013, a total of 323 major resections). Only NSCLC cases were included in this study so a total of 130 cases were studied: 87 (group A) *vs.* 43 patients (group B). The demographic characteristics of the patients in both groups are described in *Table 1*. There were no significant differences in terms of patient age, sex, smoking status, past medical history or associated comorbidity between the two groups. The lesions were most often casual findings (66.7% in group A and 37.2% in B). From the patients in group B, 67.4% of them received chemo or chemo-radiotherapy induction treatment before surgery.

The types of resection performed and their frequency are shown in *Table 2*. Upper lobectomies (A, 52 *vs.* B, 21 patients) and anatomic segmentectomies (A, 4 *vs.* B, 0) were more frequent in group A while pneumonectomy was more frequent in B (A, 1 *vs.* B, 6 patients).

In group A, 68.3% of the patients and 40% of those in group B showed no adherences following lung collapse. In contrast, significant adherences complicating surgery were recorded in 15.4% of the cases in group A and 28.9% in group B.

The advanced group included very complex cases like bronchial sleeve resections, lobectomies with vascular reconstruction, chest wall resection, lobectomies after high

Table 3 Intraoperative data

	Mean ± SD; median [range]		P
	A	B	
Surgical time (minutes)	144.94±41.34; 140.0 [60-300]	183.26±48.97; 180.0 [100-310]	<0.001
Number of lymph nodes	14.36±6.71; 14.0 [5-38]	16.52±6.16; 16.0 [7-29]	<0.001
Number of explored nodal stations	4.76±1.11; 5.0 [3-7]	4.97±1.31; 5.0 [1-8]	NS
Tumor size (cm)	2.55±1.02; 2.5 [0.5-4.8]	3.92±2.38; 4.0 [0-9]	NS

Table 4 Postoperative results

	Mean ± SD; Median [range] or No. [%]		P
	A	B	
Days of chest tube	2.92±2.4	3.42±2.66	
	2.0 [1-16]	2.0 [1-14]	NS
ICU	2.0±6.54	1.15±0.36	
	1.0 [0-54]	1.0 [1-2]	NS
HOS	4.45±7.27	4.26±2.87	
	3.0 [1-58]	3.0 [2-14]	NS
Rate of complications	15 [17.2]	6 [14.0]	NS
Prolonged air leak (>5 days)	3	2	
Atelectasis	1	0	
Wound problems	3	0	
Atrial fibrillation	3	2	
Respiratory failure/SIRS	1/1	0	
Postoperative bleeding requiring reoperation	2	2	
Reinsertion of chest tube	1	2	

Table 5 Concordance between clinical and pathological staging (NSCLC)

	Preoperative No. (%)			
	A		B	
Stage				
IA	59 (67.8)	50 (57.5)	1 (2.3)	6 (14.0)
IB	15 (17.2)	16 (18.4)	4 (9.3)	5 (11.6)
IIA	6 (6.9)	7 (8.0)	7 (16.3)	9 (20.9)
IIB	1 (1.1)	2 (2.3)	1 (2.3)	4 (9.3)
IIIA	3 (3.4)	9 (10.3)	26 (60.5)	9 (20.9)
IIIB	0	0	0	0
IV	0	0	2 (4.7)	2 (4.7)
pT0N0M0				6 (14.0)
Concordance N (%)	74 (85.1)		20 (46.5)	
Downstaging chemo N (%)			16 (55.2)	

NSCLC, non-small cell lung cancer.

doses of chemo-radiotherapy, redo-VATS, completion pneumonectomy or sulcus tumor after induction treatment.

The intraoperative results are described in *Table 3*. Conversion rate was higher in group B (1.1% *vs.* 6.5%, P=0.119). Also in group B, surgical time was longer (144.9±41.3 *vs.* 183.2±48.9, P<0.001) and median number of lymph nodes (14 *vs.* 16, P=0.004) was statistically higher in advanced cases.

The postoperative results are described in *Table 4*. There were no significant differences in terms of days of stay in the intensive care unit, days of chest tube, HOS and rate of complications. One patient died on the 58th postoperative day due to a respiratory failure (group A).

In both groups the majority of the patients (A, 82.8% and B, 86%) suffered no postoperative complications. From the patients in group A, 65.5% of them were discharged in the first 72 hours versus 51.2% of patients in group B. All patients (100%) were discharged without any nursing assistance at home.

The most common histological type in group A (48, 55.1%) was adenocarcinoma while in group B (24, 55.8%) it was squamous cell carcinoma. The concordance between clinical and pathological stages is described in *Table 5*. A total of 85.1% of patients (A) and 46.5% (B) presented concordance between preoperative and postoperative staging. From the patients receiving chemotherapy 55.2% (16 patients) were pathologically downstaged (six of them were down-staged to pT0N0M0, total tumoral regression).

The survival rates are described in *Table 6*. The 30-month survival was 90.4% for early stages (group A) and 73.7% for advanced cases (group B). The 30-month overall survival of the 130 pacients was 85% (Kaplan-Meier).

Discussion

Since the first lobectomies using VATS were reported 20 years ago (8), the thoracoscopic approach has experienced an exponential increase for lung cancer treatment, especially for early stages. The majority of publications on VATS

Table 6 Survival data (n=130)

	Mean ± SD (range) or No. (%)	
	A	B
Postoperative mortality	1 (1.1)	0
Actual survival		
Alive	82 (94.3)	33 (76.7)
Deceased during following	5 (5.7)	10 (23.3)
Estimated survival (months)	39.02±1.02 (37.02-41.01)	25.25±1.48 (22.34-28.16), P<0.002
30 months-estimated survival (Kaplan-Meier)	90.4%	73.7%

lobectomy focus on patients with early stages of NSCLC, showing less postoperative pain, lower stress responses and improved outcomes, when compared with thoracotomy (9). However the role of VATS for treatment of advanced stages of lung cancer is not clear and has been questioned.

Thanks to the advances in the field of thoracoscopic surgery the indications and contraindications for lung cancer treatment have been changed overtime. Initially only early stages were considered for VATS approach and advanced NSCLC tumors were considered a contraindication for thoracoscopic surgery (10). Several concerns regarding the radicality of oncologic resection, technical challenges, and safety has reduced the incorporation of VATS for more advanced stages of lung cancer. In cases of extended resections such as vascular or bronchial sleeve, chest wall resection or tumors after high doses of induction chemo-radiotherapy; the VATS approach is even less frequent. However, thoracoscopic major lung resection for advanced stage lung cancer is now gaining wide acceptance in experienced VATS departments (11). Skilled VATS surgeons can perform 90% or more of their lobectomies thoracoscopically, reserving thoracotomy only for huge tumors or complex broncho-vascular reconstructions.

Despite the increasing implementation of the techinque by experienced surgeons to deal with advanced tumors, the number of publications showing results is still insignificant. Hennon and colleagues, showed similar outcomes of advanced cases performed by VATS when compared with open surgery (2). In this study the perioperative complications were equal in patients undergoing thoracoscopic resection when compared to those having a thoracotomy. No difference was observed for disease-free and overall survival.

In another multi-institutional experience, more than 400 patients with stage III or IV disease were treated with a VATS approach over a period of 8 years. The preliminary analysis indicate no significant difference in overall survival between VATS and open thoracotomy groups, with a conversion rate of approximately 5% in the cohort of patients with advanced stage NSCLC (12).

The incidence of surgical complications after neoadjuvant therapy has been reported in the literature to be high (13). VATS lobectomy has been usually avoided in patients undergoing preoperative chemotherapy or radiotherapy due to concerns regarding the propensity of induction therapy to increase the difficulty of hilar and mediastinal dissection, especially around vessels. In our series of patients the induction treatment increased the complexity of hilar and lymph node dissection but these were performed successfully, most likely due to our previous thoracoscopic experience (5,6). There are publications reporting that pulmonary resection may be performed safely after induction chemo or high doses of radiotherapy (14,15). However, recent publications showed prior chemotherapy as one of the significant predictors of morbidity in a multivariable analysis (16). The rate of complications in our study in patients receiving induction treatment had not increased, being similar to perioperative results in early stage tumors.

Recently, Huang J and colleagues published a study of 43 locally advanced NSCLC patients (including nine sleeves and four pneumonectomies) undergoing VATS following neoadjuvant therapy with good posotperative results (3). Lee and colleagues report that thoracoscopic pulmonary resection for NSCLC showed better compliance with adjuvant chemotherapy, allowing to apply the thoracoscopic procedure not only to patients with early stage NSCLC but also to patients who need adjuvant chemotherapy (17).

Uniportal VATS has become an increasingly popular and effective approach in our unit to manage early and advanced stages of NSCLC, because of the reduced access trauma, advantages in view and more anatomic instrumentation and good perioperative results. The success in performing uniportal complex VATS lobectomies is a result of skills

and experience accumulated over time from performing uniportal VATS surgery (5). With gained experience with the uniportal VATS technique the most complex cases can be performed in the same manner as with double or triple port approach. We have performed advanced NSCLC cases via single-port VATS including lobectomies with chest wall resection (18), redo-VATS and completion pneumonectomies, cases after high doses of chemo-radiotherapy, vascular reconstruction (19), bronchial sleeve lobectomies (20) and complex pneumonectomies (21).

Mean operation time for advanced uniportal VATS resections was higher than for early stage tumors (188 *vs.* 141 m), as expected. However our surgical time is less than other authors by using more number of thoracoscopic incisions (11). We found several advantages of the single incision technique especially for advanced cases. The geometrical explanation of the approach could explain our results (22). The advantage of using the camera in coordination with the instruments is that the vision is directed to the target tissue, bringing the instruments to address the target lesion from a straight perspective, thus we can obtain similar angle of view as in open surgery. Bimanual instrumentation also facilitates the surgery for complex cases. Conventional three port triangulation makes a forward motion of VATS camera to the vanishing point. This triangulation creates a new optical plane with genesis of dihedral or torsional angle that is not favorable with standard two-dimension monitors. Instruments inserted parallel to the videothoracoscope also mimic inside the chest maneuvers performed during open surgery. There is a physical and mathematical demonstration about better geometry obtained for instrumentation and view in the uniportal VATS over conventional approach (22). This fact in combination with the expierence obtained so far as well as recent improvements in surgical instruments, new energy devices and modern high definition cameras enable us to be very confident with the instrumentation and the manipulation of tissue even in very complex and advanced procedures.

The rate of pneumonectomies was logically higher in patients with advanced stages.

Pneumonectomy is only considered in cases where it is not possible to perform a sleeve resection. In our unit it is mandatory to do a careful assessment of the location of the tumor in order to proceed with a uniportal VATS pneumonectomy. Sleeve resections are also performed via single-incision VATS with no need to convert to thoracotomy allowing patients a better postoperative recovery (23). This is especially important in patients receiving induction chemo-radiotherapy as performing a pneumonectomy would increase the rate of postoperative complications. The uniportal thoracoscopic resection of the whole lung is technically easier to perform than a lobectomy because the fissure doesn't need to be managed. However extra care must to be taken during dissection and division of the main artery and transection of the main bronchus (21). There are several studies reporting that pneumonectomies performed thoracoscopically or via thoracotomy resulted in equivalent survival rates (24). Further studies and follow-up is needed to verify the benefits of VATS pneumonectomy for lung cancer.

From the literature, conversion rates from VATS lobectomy to open surgery have been reported to be from 2% to 23%, with these higher rates coming from patients with more advanced tumors (2). Most frequently the conversion to thoracotomy was considered necessary because of bleeding during dissection or oncological reasons, such as centrally located tumors requiring sleeve resection, or unexpected tumors that infiltrate the mediastinum or chest wall. In our series, the rate of conversion for advanced cases is low (only 6.5%) compared with other series (2,3). Furthermore, no patient was converted to conventional thoracotomy in our study (enlarged incision to antherior thoracotomy and Hybrid VATS).

Also in our study, the incidence of postoperative complications in early stages and advanced stages were similar. The uniportal technique was developed in 2010 by one of the surgeons of the department and sequentially taught to a total of four consultant surgeons and two trainees. Most of the advanced cases were performed by the surgeon who developed the technique, and with more thoracoscopic experience. This surgeon's experience in managing complex and highly difficult procedures under uniportal VATS and the advantages of the minimally invasive approach (small incision, no rib retractor and only one intercostal space opened) is also important to reduce the prevalence of postoperative complications, especially in the advanced group.

We believe that minimize the surgical aggression is particularly important given the large number of frail patients with advanced stage disease who require multimodality therapy, sometimes being difficult to tolerate in older patients or patients with severe comorbidity. Several articles in the literature suggest that the immune response is better preserved after VATS surgery than thoracotomy (1). Given that immune function is an important factor in controlling tumor growth and recurrence, we have hypothesized that the reduced inflammatory response associated with

thoracoscopy, especially with uniportal VATS (which represents the minimal invasive approach) may be associated with improved long-term survival. Further studies to analyze inflamatory response and long term survival on uniportal VATS are ongoing.

This study is limited by its retrospective design and absence of comparative subjects with open approach. Most of the data except on present survival were collected from chart review, with the limitations accompanying a retrospective study. Also, the follow-up duration was relatively short, and the free-disease period was not studied making it difficult to conclude whether survival rates were favorable for patients undergoing uniportal VATS lobectomy.

Another limitation of the study is the absence of an analysis of the results based on the cases performed by surgeons with a greater experience in the technique (those who have performed most operations), compared to those surgeons who started the technique later.

There are few reports regarding perioperative results and survival of advanced cases of NSCLC operated by thoracoscopic approach. According to the VATS Consensus Statement (agreement among 50 international experts to establish a standardized practice of VATS lobectomy after 20 years of clinical experience), eligibility for VATS lobectomy should include tumour size ≤7 cm and N0 or N1 status. Chest wall involvement was considered a contraindication for VATS lobectomy, while centrality of tumour was considered a relative contraindication when invading hilar structure (25). The Consensus Group acknowledged the limitations of VATS lobectomy based on their individual experiences with a recommendation to convert to open thoracotomy in cases of major bleeding, significant tumour chest wall involvement and the need for bronchial and/or vascular sleeve procedures. However, these recommendations are directed at the general thoracic surgical community, and indications for VATS lobectomy and conversion to thoracotomy should depend on each surgeons experience.

In conclusion, Uniportal VATS lobectomy for advanced cases of NSCLC is a safe and reliable procedure that provides perioperative outcomes similar to those obtained in early stage tumours operated through this same technique. Our 30-month survival rate is acceptable and similar to survival rates reported in other studies performed by conventional VATS. Further analyses of long term survival for advanced cases operated by uniportal VATS needs to be performed with a large number of patients to validate the oncologic outcomes of the technique.

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References

1. Nagahiro I, Andou A, Aoe M, et al. Pulmonary function, postoperative pain, and serum cytokine level after lobectomy: a comparison of VATS and conventional procedure. Ann Thorac Surg 2001;72:362-5.
2. Hennon M, Sahai RK, Yendamuri S, et al. Safety of thoracoscopic lobectomy in locally advanced lung cancer. Ann Surg Oncol 2011;18:3732-6.
3. Huang J, Xu X, Chen H, et al. Feasibility of complete video-assisted thoracoscopic surgery following neoadjuvant therapy for locally advanced non-small cell lung cancer. J Thorac Dis 2013;5:S267-73.
4. Gonzalez D, Paradela M, Garcia J, et al. Single-port video-assisted thoracoscopic lobectomy. Interact Cardiovasc Thorac Surg 2011;12:514-5.
5. Gonzalez-Rivas D, Paradela M, Fernandez R, et al. Uniportal video-assisted thoracoscopic lobectomy: two years of experience. Ann Thorac Surg 2013;95:426-32.
6. Gonzalez D, de la Torre M, Paradela M, et al. Video-assisted thoracic surgery lobectomy: 3-year initial experience with 200 cases. Eur J Cardiothorac Surg 2011;40:e21-8.
7. Gonzalez-Rivas D, Fieira E, Delgado M, et al. Uniportal video-assisted thoracoscopic lobectomy. J Thorac Dis 2013;5:S234-5.
8. Roviaro G, Rebuffat C, Varoli F, et al. Videoendoscopic pulmonary lobectomy for cancer. Surg Laparosc Endosc 1992;2:244-7.
9. Hanna WC, de Valence M, Atenafu EG, et al. Is video-assisted lobectomy for non-small-cell lung cancer oncologically equivalent to open lobectomy? Eur J Cardiothorac Surg 2013;43:1121-5.
10. Yang X, Wang S, Qu J. Video-assisted thoracic surgery (VATS) compares favorably with thoracotomy for the treatment of lung cancer: a five-year outcome comparison. World J Surg 2009;33:1857-61.
11. Hennon MW, Demmy TL. Video-assisted thoracoscopic surgery (VATS) for locally advanced lung cancer. Ann Cardiothorac Surg 2012;1:37-42.
12. Cao C, Zhu ZH, Yan TD, et al. Video-assisted thoracic surgery versus open thoracotomy for non-small-cell

lung cancer: a propensity score analysis based on a multi-institutional registry. Eur J Cardiothorac Surg 2013;44:849-54.
13. Venuta F, Anile M, Diso D, et al. Operative complications and early mortality after induction therapy for lung cancer. Eur J Cardiothorac Surg 2007;31:714-7.
14. Petersen RP, Pham D, Toloza EM, et al. Thoracoscopic lobectomy: a safe and effective strategy for patients receiving induction therapy for non-small cell lung cancer. Ann Thorac Surg 2006;82:214-8; discussion 219.
15. Maurizi G, D'Andrilli A, Anile M, et al. Sleeve lobectomy compared with pneumonectomy after induction therapy for non-small-cell lung cancer. J Thorac Oncol 2013;8:637-43.
16. Villamizar NR, Darrabie M, Hanna J, et al. Impact of T status and N status on perioperative outcomes after thoracoscopic lobectomy for lung cancer. J Thorac Cardiovasc Surg 2013;145:514-20; discussion 520-1.
17. Lee JG, Cho BC, Bae MK, et al. Thoracoscopic lobectomy is associated with superior compliance with adjuvant chemotherapy in lung cancer. Ann Thorac Surg 2011;91:344-8.
18. Gonzalez-Rivas D, Fernandez R, Fieira E, et al. Single-incision thoracoscopic right upper lobectomy with chest wall resection by posterior approach. Innovations (Phila) 2013;8:70-2.
19. Gonzalez-Rivas D, Delgado M, Fieira E, et al. Single-port video-assisted thoracoscopic lobectomy with pulmonary artery reconstruction. Interact Cardiovasc Thorac Surg 2013;17:889-91.
20. Gonzalez-Rivas D, Fernandez R, Fieira E, et al. Uniportal video-assisted thoracoscopic bronchial sleeve lobectomy: first report. J Thorac Cardiovasc Surg 2013;145:1676-7.
21. Gonzalez-Rivas D, de la Torre M, Fernandez R, et al. Video: Single-incision video-assisted thoracoscopic right pneumonectomy. Surg Endosc 2012;26:2078-9.
22. Bertolaccini L, Rocco G, Viti A, et al. Geometrical characteristics of uniportal VATS. J Thorac Dis 2013;5:S214-6.
23. Gonzalez-Rivas D, Delgado M, Fieira E, et al. Left lower sleeve lobectomy by uniportal video-assisted thoracoscopic approach. Interact Cardiovasc Thorac Surg 2014;18:237-9.
24. Nwogu CE, Yendamuri S, Demmy TL. Does thoracoscopic pneumonectomy for lung cancer affect survival? Ann Thorac Surg 2010;89:S2102-6.
25. Yan TD, Cao C, D'Amico TA, et al. Video-assisted thoracoscopic surgery lobectomy at 20 years: a consensus statement. Eur J Cardiothorac Surg 2014;45:633-9.

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Original Article

Circulating endothelial microparticles involved in lung function decline in a rat exposed in cigarette smoke maybe from apoptotic pulmonary capillary endothelial cells

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Background: Plasma levels of endothelial microparticles (EMPs), small membrane vesicles, shed from activated or apoptotic endothelial cells are elevated in patients with COPD and in smokers with normal lung function. Whether plasma EMPs levels are elevated in a rat exposed in cigarette smoke, whether the elevated EMPs derived from pulmonary endothelial cell apoptosis, and the relationship between EMP and lung function are obscure.

Methods: All 60 wister rats were divided into six groups, three groups of ten rats were exposed to cigarette smoke of ten non-filter cigarettes per day, 5 days a week, using a standard smoking machine (Beijing BeiLanBo Company, China) for a period of 2, 4 and 6 months (n=10, respectively). Age-matched three control groups were sham-smoked. Pulmonary function parameters, including the ratio of forced expiratory volume in 0.3 second over forced vital capacity (FEV0.3/FVC) and dynamic compliance (Cdyn), were tested at the end of each period (2, 4, 6 months). Blood samples were collected and platelet-free plasma was isolated. Then CD42b–/CD31+ EMPs were analysed by flow cytometry. In parallel, lungs were removed and Colocalization with terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL), Hoeschts and CD31 was performed to evaluate pulmonary capillaries-specific apoptosis and identify the origins of the EMPs.

Results: At 2, 4 and 6 months, in comparison with control groups, rats in cigarette smoke exposed groups had a significant increase in CD42b–/CD31+ EMPs ($P<0.001$, $P<0.001$, $P<0.001$, respectively), and Pulmonary function indicated that FEV0.3/FVC ($P<0.05$, $P<0.01$, $P<0.01$, respectively) and Cdyn ($P<0.01$, $P<0.001$, $P<0.001$ respectively) decreased. At the same time, CD42b–/CD31+ EMP counts were negatively correlated with Cdyn ($P<0.05$). Moreover, *in vivo*, TUNEL-positive cells co-localized with CD31 in whole lung tissue demonstrated a sequence of apoptosis signal in the cigarette smoke exposed groups.

Conclusions: CD42b–/CD31+ EMPs may be a potential biomarker for indicating the severity of impairment of pulmonary function in the rats exposed cigarette smoke. The increased EMPs may derive from pulmonary capillaries-specific apoptosis.

Keywords: Cigarette smoke; circulating endothelial microparticle; pulmonary capillary; pulmonary function; apoptosis

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Background

Long-term cigarette smoke exposure induces an inflammatory process in the lung that may underlie the development of chronic obstructive pulmonary disease (COPD), which involves a mixture of pathomorphological changes (airways narrowing, emphysema and vascular abnormalities) (1,2). The pathogenesis of COPD is complex and includes the balance of proteases and antiproteases in the lung, tilted toward an excess of unopposed proteases that destroy the connective tissue backbone of the lung parenchyma (2-6). Despite major abnormalities taking place in the airway side, changes in pulmonary vessels represent an important component of the disease. In recent years, increased evidence has demonstrated that endothelial damage is closely connected to the pathophysiology of COPD (7-9). It was noted that there was a paucity of the pulmonary microvessel in lung parenchyma with emphysema, and alterations in pulmonary endothelial injury and apoptosis are highly prevalent.

Circulating endothelial microparticles (EMPs), small membrane vesicles (100 nm-1 μm in diameter), are shed into circulating blood that originate from activated and apoptotic endothelial cells (10,11). The number of EMPs increases in patients with vascular disorders, such as acute coronary disease (12), renal failure (13) and metabolic diseases (14), and it reflects endothelial damage occurring in these patients. Gordon and coworkers presented their finding that smokers, even in the absence of obvious radiographic, spirometric, or clinical evidence of emphysema or airflow obstruction, had increased levels of circulating endothelial micro-particles (CD42b–/CD31+), which demonstrate an apoptotic phenotype (reduced CD42b–/CD62+ or CD42b–/CD31+ ratio and increased annexin V1 expression), and appeared to originate from the pulmonary vasculature (evident by positive immunostaining for angiotensin-converting enzyme) (15). In addition, Takahashi *et al.* found that VE-cadherin (CD144), PECAM (CD31) and E-selectin (CD62E) EMPs were significantly more numerous in the stable COPD patients than in the healthy non-COPD volunteers, and their numbers further increased in the exacerbated phase. And higher E-selectin EMP levels may predict the COPD patients who are susceptible to exacerbation (16).

Based on the above knowledge, we hypothesize that the number of EMPs is elevated in rats exposed in cigarette smoke, and the elevated EMPs are derived from pulmonary capillaries. By observing changes in EMP levels and pulmonary function of two cohorts of laboratory rats (cigarette smoke exposure groups and sham exposure groups), and calculated the correlations between levels of EMP and pulmonary function parameters (FEV0.3/FVC and Cdyn). EMP, quantified in plasma as particles that are CD31 (PECAM) possitive, but CD42b (the constitutive platelet-specific glycoprotein Ib) negative. We also explored the role of pulmonary endothelial injury and apoptosis on the pathogenesis of COPD.

Some of the results from this study have been previously presented in abstracts.

Methods

Animals

Sixty male wistar rats, 8 weeks old, and initially weighing (180±5) g, were purchased from Nantong University Laboratory Animal Center. Upon arrival, all animals were given a diet of standard chow and water, and were acclimated to the new housing environment for 1 week before cigarette smoke exposure began. Rats were removed from the study due to lethargy and significant weight loss at the end of each time period. All procedures involving animals and their care were approved by the Ethics Committee of Nantong University and were conducted following institutional guidelines that comply with national laws and policies.

Cigarette smoke exposure

Rats were divided into six groups , three groups of ten rats was exposed to the smoke of ten nonfilter cigarettes per day, 5 days a week, using a standard smoking machine (Beijing BeiLanBo Company, China) for a period of 2, 4 and 6 months (n=10, respectively). Age-matched control groups were sham-smoked.

Pulmonary function tests

At the end of each time period, the rats were anesthetized intraperitoneally with 12 % urethane at a dose of 1 mL per 100 mg. After their tracheas were cannulated, the rats were placed supine in a small animal plethysmograph (Beijing BeiLanBo Company, China). Lung function tests were performed using the modification method of Wright *et al.* (17). The lungs were inflated with air at a volume of 5-fold tidal volume and then rapidly deflated to a pressure

of –35 cm H_2O. The ratio of forced expiratory volume in 0.3 second to forced vital capacity (FEV0.3/FVC) and dynamic compliance (Cdyn) were calculated by computer.

Assay of EMP

After all pulmonary function tests were completed, blood samples were collected by the heart punctures in 5 mL sodium citrate tubes and, within 1 hour, centrifuged 10 minutes (160 g, 23 ℃) to prepare platelet rich plasma. Within 5 minutes, the supernatant was further centrifuged 8 minutes (1,000 g, 23 ℃) to obtain platelet-poor plasma. Within 5 minutes, 50 μL aliquots of platelet-poor plasma were incubated (45 min, 4 ℃) with 4 μL of fluorescein-conjugated anti-rat PECAM (FITC) and 4 μL of phycoallocyanine-conjugated anti-rat CD42b (PE) was added (45 min, 4 ℃) to each sample to exclude platelet-derived microparticles. CD42b–/CD31+ microparticle levels were corrected for correlating isotype control antibodies. EMP phenotype analysis was performed within 15 minutes based on size and fluorescence. The method of flow cytometry were performed using the method of Gordon *et al.* (15).

Co-localization assay for apoptosis

Co-localization assays were performed referencing the method of Hassoun *et al.* (18). Briefly, after the rats were exsanguinated, the right pulmonary were inflated with 0.5% agarose, fixed in formalin, and embedded in paraffin blocks. Terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) assays for apoptosis were performed referencing per manufacturer's instructions (Roche, Indianapolis IN, USA), and subsequent staining with rat anti-CD31 monoclonal antibody (1:1,000, BD, USA) and Hoeschts dye (BisBenzimide, 1 mg/100 mL) were co-localized endothelial cells undergoing apoptosis by confocal microscopy.

Statistical analysis

All data were reported as means ± standard deviation (SD), and statistical analyzed by SPSS *vs.*17.0 (SPSS, Inc, Chicago, USA). For comparison between smoke exposed groups and sham-smoked groups, a paired student's *t*-test was performed. The Pearson test was used to calculate correlations between levels of EMP and pulmonary function parameters (FEV0.3/FVC and Cdyn). P values of less than 0.05 were considered statistically significant.

Results

Weight and lung function

Two rats from the 2 months smoke exposure group, two from the 4 months smoke exposure group, four from the 6 months smoke exposure group, and each two rats from the 4 months, 6 months control groups were removed from the study due to lethargy and significant weight loss at the end of each time period. At the end of the 6 months of smoke exposure, the remaining six rats were included in flowing analysis, and the six sets of data from each other groups was drawn randomly.

Rats of the smoke-exposed groups weighed less than rats of the sham-smoked groups did. At the end of 2, 4, and 6 months, they weighed 225±10, 250±10, and 285±10 g, respectively, whereas the sham-smoked rats weighed 240±5, 280±8, and 340±8 g, respectively ($P<0.05$, 0.05, and 0.01, respectively).

The pulmonary function test data are shown in *Figures 1,2*. After 2 month of smoke exposure, the smoke-exposed rats began to show low-grade obstructive ventilatory disorder with decrease in the ratio of FEV0.3 to FVC ($P<0.05$). This was accompanied by significantly decreased Cdyn ($P<0.01$). At the end of 4 and at 6 months, the obstructive ventilatory disorder was marked (4 months, $P<0.01$ FEV0.3/FVC and $P<0.001$ Cdyn; 6 months, $P<0.01$ FEV0.3/FVC, and $P<0.001$ Cdyn) with the smoke exposure time.

Levels of EMP

CD42b–/CD31+ EMP were measured at the end of each period. Results are shown in *Figure 3*. This demonstrates that EMPs measured with the special marker discriminate rats of the smoke-exposed from rats of the sham exposure groups.

Figure 3E summarizes data on EMPs detected with CD31and CD42. It shows that, at the end of 2, 4 and 6 months, CD42b–/CD31+ EMP levels are markedly higher in rats of the smoke-exposed groups than in rats of the control groups ($P<0.001$, respectively). It also illustrates the significant elevation in rats of the 6 months exposed group with the 2, 4 months exposed groups ($P<0.001$, respectively), and 4 months exposed group with the 2 months exposed group ($P<0.001$). The CD42b–/CD31+ EMP level was highest in 6 months exposed group, followed

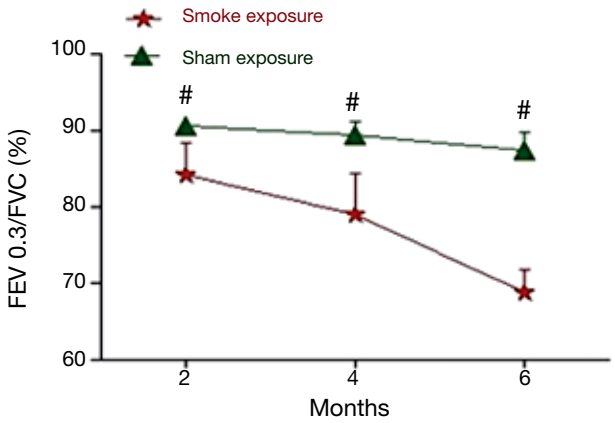

Figure 1 FEV0.3/FVC% are shown expressed in percentage for each of the 2-, 4-, and 6-month time periods. Values = mean ± SD. #, indicate P<0.05 or greater significant difference between sham exposed (n=6) and smoke-exposed (n=6) rats; SD, standard deviation.

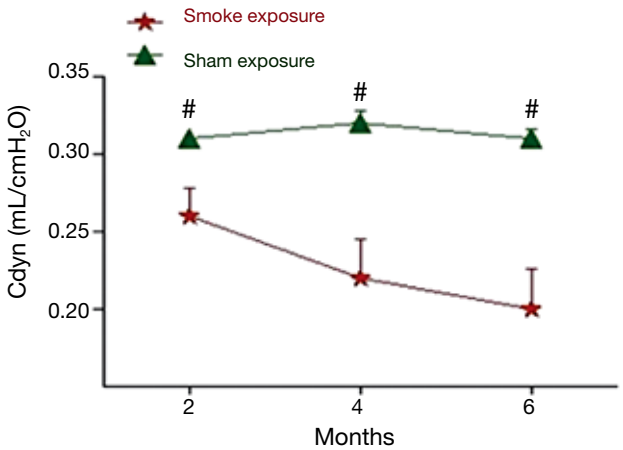

Figure 2 Cdyn are shown expressed in mL/cm H_2O for each of the 2-, 4-, and 6-month time periods. Values = mean ± SD. #, indicate P<0.05 or greater significant difference between sham exposed (n=6) and smoke-exposed (n=6) rats; SD, standard deviation.

by 4 months exposed group and 2 months exposed group.

Co-localization assay for pulmonary endothelium cells apoptosis

TUNEL-positive cells co-localized with CD31 in rat lung tissue after cigarette smoke exposed 2 months, demonstrating an increase signal of cell death of pulmonary endothelium cells, but negative in lung tissue of sham exposure rats (*Figure 4*).

Correlations between levels of EMP and pulmonary function parameters (FEV0.3/FVC and Cdyn)

There were no correlations between CD42b–/CD31+ EMP counts and FEV0.3/FVC (r=–0.907, P=0.276). CD42b–/CD31+ EMP counts were negatively correlated with Cydn (r=–0.998, P=0.038) (*Figure 5*).

Discussion

Cigarette smoke exposure will induce emphysema and vascular alterations (17,19). Despite pulmonary hypertension is a major component of vascular abnormalities of COPD patients, pulmonary endothelial injury and apoptosis are becoming more and more important in the aetiopathogenesis of COPD/emphysema (2,4,8,20-24). On the basis of previous research (15,16), we have rats exposed to a standard smoking machine for a period of 2, 4 and 6 months. To evaluate the alterations of pulmonary function (FEV0.3/FVC and Cdyn) and the endothelial damage induced by cigarette smoke exposure in rats, we compared circulating EMP numbers in rats of the smoke exposure groups and the sham exposure groups. In this study, we found that exposure of rats to cigarette smoke induced decreases in FEV0.3/FVC and Cdyn compared with rats of sham exposure groups, similar to what has been described of Wright *et al.* (17). By measuring levels of EMP with the special marker, we found that exposure of rats had high levels of circulating EMPs compared with rats of sham exposure groups, and the levels of circulating EMPs higher with the time of smoke exposure. The EMPs likely derived from endothelial cells undergoing apoptosis, and likely mostly from pulmonary endothelium.

Microparticles are submicron membrane vesicles shed from the plasma membranes of different cell types in response to cell activation, injury, and/or apoptosis (25-28). As what mentioned before, there are various subtypes of EMPs. Reviewing previous researches, apoptosis-induced EMPs are more likely to express only CD31 and show the presence of phosphatidylserine (annexin V) as an apoptotic parameter. Healthy smokers and symptomatic smokers with normal spirometry and DLco had mildly elevated levels of circulating EMPs compared with healthy nonsmokers. Strikingly, however, healthy smokers with normal spirometry but an isolated reduction in DLco had high levels of circulating EMPs compared with all other groups (15). In our study, the levels of circulating EMPs in rats are higher with the time of smoke exposure.

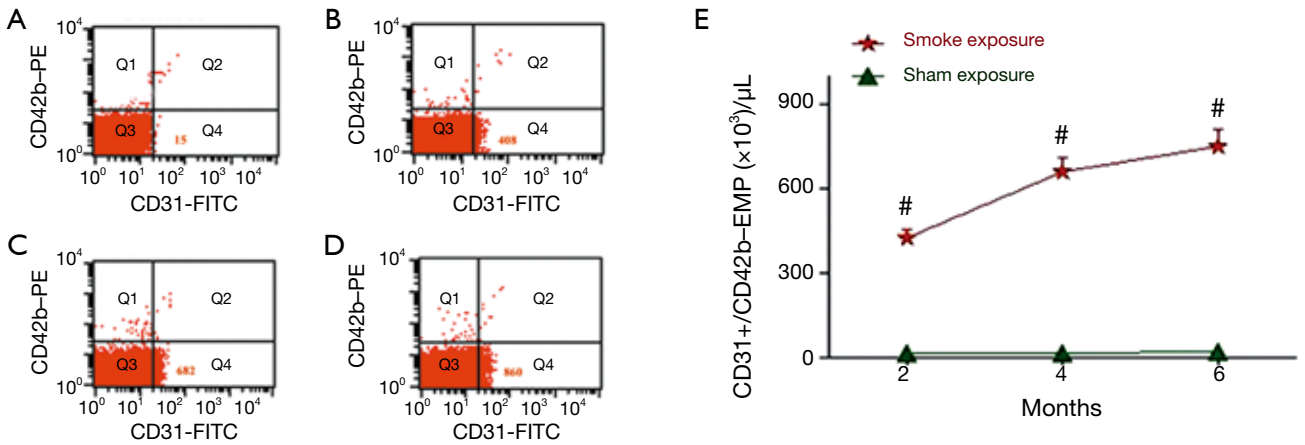

Figure 3 Representative examples of flow cytometry of CD42b–/CD31+ EMP in a rat exposed to smoke 2 months (B) 4 months (C) and 6 months (D) and a rat of sham exposure groups (A). The points represented in region Q4 of both examples are CD31+ (positive) and CD42- (negative); therefore they are considered to be EMPs. In region Q4 (lower right of histogram), there is a visibly more dense cluster of EMPs in the rat exposed to smoke (B, C, D) compared with in the rat of sham exposure groups (A). In these examples, the rat exposed to smoke 2 months had 408 EMPs (B), the rat exposed to smoke 4 months had 682 EMPs (C), the rat exposed to smoke 6 months had 860 EMPs (D), and the rat of sham exposure groups had 15 EMPs (A). (E) CD42b–/CD31+ EMP counts are shown for each of the 2-, 4-, and 6-month time periods. Values = mean ± SD. #, indicate P<0.05 or greater significant difference between sham exposed (n=6) and smoke-exposed (n=6) rats; EMP, endothelial microparticle; SD, standard deviation.

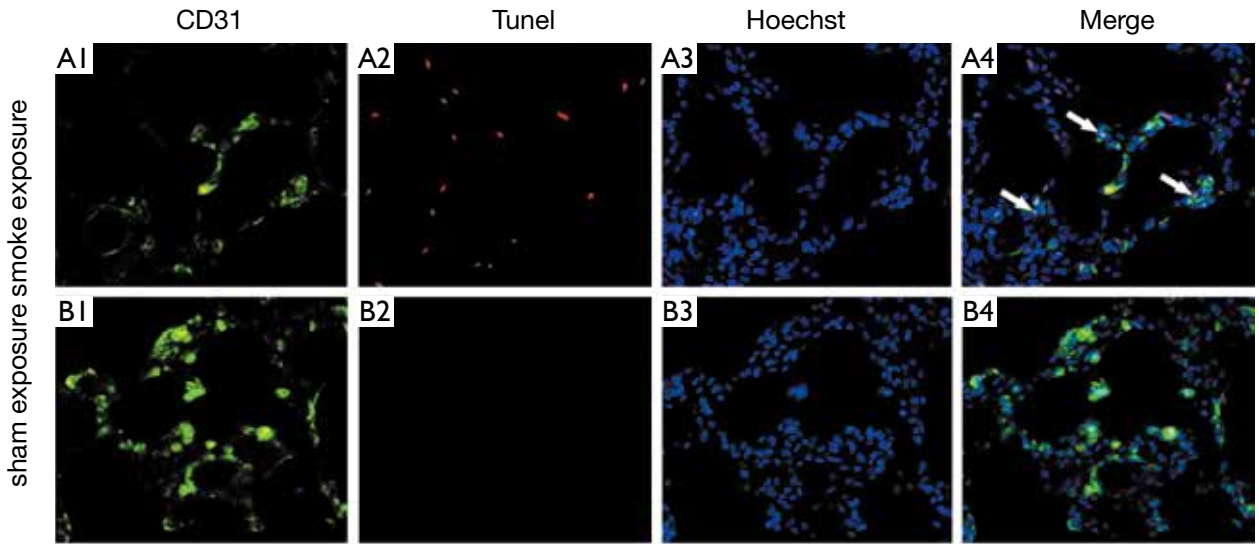

Figure 4 Lung endothelial cell apoptosis after smoke exposure and sham exposure. Lung immunofluorescence micrographs (×200) stained with CD31 (green), TUNEL (red), Hoechst (blue), and merged CD31-TUNEL- Hoechst. The pink signals indicated by the arrows suggesting lung endothelial cell apoptosis in representative examples of smoke exposed rats (A1, A2 and A3). No signal indicated in the lung stain in sham exposed rats (B1, B2 and B3). TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling.

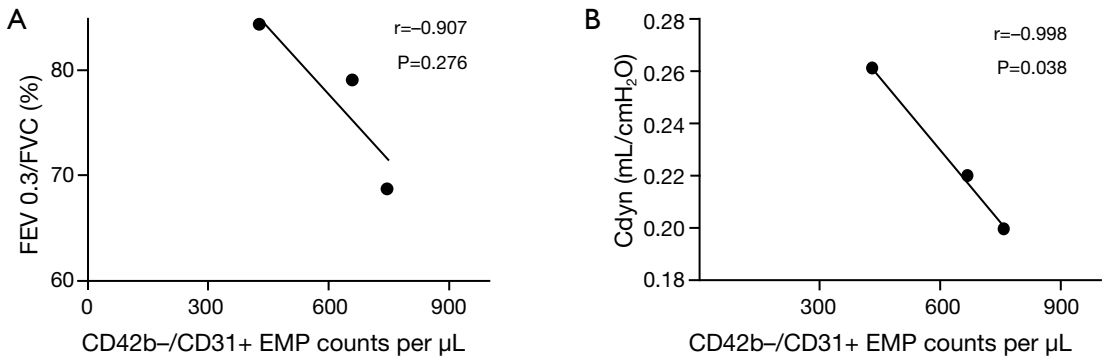

Figure 5 Correlations between levels of EMP and pulmonary function parameters [FEV0.3/FVC (A) and Cdyn (B)] are shown and P values of less than 0.05 were considered statistically significant. EMP, endothelial microparticle.

The following correlations between the Levels of EMP and lung function parameters were observed: (I) there were no correlations between CD42b–/CD31+ EMP counts and FEV0.3/FVC; (II) CD42b–/CD31+ EMP counts were negatively correlated with Cydn.

FEV0.3/FVC expressed the pulmonary ventilation function well, especially in COPD rat model (15,16), whereas CD42b–/CD31+ EMP counts showed no correlations with FEV0.3/FVC. Because we only made rat models of the early period of COPD, it may require a longer time of study.

Cdyn is the lung compliance measured in respiratory cycle when the flow is not blocked. In small airway disease, such as the early period of COPD, Cdyn declined, while the rising of intrapleural pressure with the Specific changes in lung capacity. CD42b–/CD31+ EMP counts showed negative correlations with Cdyn. These indicate that the high levels of circulating EMPs may reflect the decline of small airway function indirectly in the early of COPD.

Through co-localization stain of pulmonary endothelium cells, we also demonstrated that the evaluated EMPs mostly derived from pulmonary endothelial cells undergoing apoptosis. CD31 EMPs may measure as a preferable marker of pulmonary endothelium injury and destruction of pulmonary function.

Pulmonary endothelial apoptosis as a primary mechanism in the development of COPD is supported by the observation of endothelial apoptosis in the lungs of humans with emphysema (8,9,20-23). Therefore, high EMP levels may indicate a role for endothelial damage in COPD progression. Because quicker responses can be seen in circulating EMP levels compared with an annual lung function decline, monitoring EMP levels is valuable for estimating COPD progression, and can be a useful index for drug discovery.

There are limitations in this study, we used experimental groups of only six animals because of the disqualification of weight and the spirit. While this somewhat limits statistical power, for almost all the measurements we made there were clearly significant differences between smoke and sham smoke. Overall, however, we do not believe that using a group size of six has posed a serious problem to our data interpretation.

In conclusion, pulmonary endothelial damage occurs during the smoke exposure, and has been a primary mechanism in the development of COPD. Plasm levels of CD42b–/CD31+ EMPs is useful for evaluating the degree of COPD progression.

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References

1. Rivera RM, Cosio MG, Ghezzo H, et al. Comparison of lung morphology in COPD secondary to cigarette and biomass smoke. Int J Tuberc Lung Dis 2008;12:972-7.
2. Roth M. Pathogenesis of COPD. Part III. Inflammation in COPD. Int J Tuberc Lung Dis 2008;12:375-80.
3. Abboud RT, Vimalanathan S. Pathogenesis of COPD. Part I. The role of protease-antiprotease imbalance in emphysema. Int J Tuberc Lung Dis 2008;12:361-7.
4. Hogg JC, Senior RM. Chronic obstructive pulmonary disease - part 2: pathology and biochemistry of

emphysema. Thorax 2002;57:830-4.
5. Moraes TJ, Chow CW, Downey GP. Proteases and lung injury. Crit Care Med 2003;31:S189-94.
6. Spurzem JR, Rennard SI. Pathogenesis of COPD. Semin Respir Crit Care Med 2005;26:142-53.
7. Barnes PJ. Mediators of chronic obstructive pulmonary disease. Pharmacol Rev 2004;56:515-48.
8. Kasahara Y, Tuder RM, Cool CD, et al. Expression of 15-lipoxygenase and evidence for apoptosis in the lungs from patients with COPD. Chest 2000;117:260S.
9. Morissette MC, Parent J, Milot J. Alveolar epithelial and endothelial cell apoptosis in emphysema: what we know and what we need to know. Int J Chron Obstruct Pulmon Dis 2009;4:19-31.
10. Diamant M, Tushuizen ME, Sturk A, et al. Cellular microparticles: new players in the field of vascular disease? Eur J Clin Invest 2004;34:392-401.
11. Freyssinet JM. Cellular microparticles: what are they bad or good for? J Thromb Haemost 2003;1:1655-62.
12. Nozaki T, Sugiyama S, Koga H, et al. Significance of a multiple biomarkers strategy including endothelial dysfunction to improve risk stratification for cardiovascular events in patients at high risk for coronary heart disease. J Am Coll Cardiol 2009;54:601-8.
13. Amabile N, Guérin AP, Leroyer A, et al. Circulating endothelial microparticles are associated with vascular dysfunction in patients with end-stage renal failure. J Am Soc Nephrol 2005;16:3381-8.
14. Pirro M, Schillaci G, Paltriccia R, et al. Increased ratio of CD31+/CD42- microparticles to endothelial progenitors as a novel marker of atherosclerosis in hypercholesterolemia. Arterioscler Thromb Vasc Biol 2006;26:2530-5.
15. Gordon C, Gudi K, Krause A, et al. Circulating endothelial microparticles as a measure of early lung destruction in cigarette smokers. Am J Respir Crit Care Med 2011;184:224-32.
16. Takahashi T, Kobayashi S, Fujino N, et al. Increased circulating endothelial microparticles in COPD patients: a potential biomarker for COPD exacerbation susceptibility. Thorax 2012;67:1067-74.
17. Wright JL, Churg A. Cigarette smoke causes physiologic and morphologic changes of emphysema in the guinea pig. Am Rev Respir Dis 1990;142:1422-8.
18. Hassoun HT, Lie ML, Grigoryev DN, et al. Kidney ischemia-reperfusion injury induces caspase-dependent pulmonary apoptosis. Am J Physiol Renal Physiol 2009;297:F125-37.
19. Wright JL, Sun JP. Effect of smoking cessation on pulmonary and cardiovascular function and structure: analysis of guinea pig model. J Appl Physiol (1985) 1994;76:2163-8.
20. Kasahara Y, Tuder RM, Taraseviciene-Stewart L, et al. Inhibition of VEGF receptors causes lung cell apoptosis and emphysema. J Clin Invest 2000;106:1311-9.
21. Kasahara Y, Tuder RM, Cool CD, et al. Endothelial cell death and decreased expression of vascular endothelial growth factor and vascular endothelial growth factor receptor 2 in emphysema. Am J Respir Crit Care Med 2001;163:737-44.
22. Aoshiba K, Yokohori N, Nagai A. Alveolar wall apoptosis causes lung destruction and emphysematous changes. Am J Respir Cell Mol Biol 2003;28:555-62.
23. Plataki M, Tzortzaki E, Rytila P, et al. Apoptotic mechanisms in the pathogenesis of COPD. Int J Chron Obstruct Pulmon Dis 2006;1:161-71.
24. Vestbo J, Hurd SS, Agustí AG, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. Am J Respir Crit Care Med 2013;187:347-65.
25. Jimenez JJ, Jy W, Mauro LM, et al. Endothelial cells release phenotypically and quantitatively distinct microparticles in activation and apoptosis. Thromb Res 2003;109:175-80.
26. Chironi GN, Boulanger CM, Simon A, et al. Endothelial microparticles in diseases. Cell Tissue Res 2009;335:143-51.
27. Garcia S, Chirinos J, Jimenez J, et al. Phenotypic assessment of endothelial microparticles in patients with heart failure and after heart transplantation: switch from cell activation to apoptosis. J Heart Lung Transplant 2005;24:2184-9.
28. Wilson DO, Weissfeld JL, Balkan A, et al. Association of radiographic emphysema and airflow obstruction with lung cancer. Am J Respir Crit Care Med 2008;178:738-44.

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Original Article

Effect of pharmaceutical care on medication adherence and hospital admission in patients with chronic obstructive pulmonary disease (COPD): a randomized controlled study

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Background: Poor adherence leads to a high rate of exacerbation and poor health-related quality of life (HRQoL) in patients with chronic obstructive pulmonary disease (COPD). However, few strategies are acceptable and effective in improving medication adherence. We investigated whether pharmaceutical care by clinical pharmacists could reinforce medication adherence to reduce exacerbation and improve HRQoL.

Methods: A randomized controlled study was carried out at The First Affiliated Hospital of Guangzhou Medical University from February 2012 to January 2014. Non-adherence patients were randomly assigned to receive pharmaceutical care or to usual care. The pharmaceutical care consisted of individualized education and a series of telephone counseling for 6 months provided by clinical pharmacists. Medication adherence was measured by pill counts plus direct interview at 1- and 6-month pharmaceutical care and one-year follow-up. Severe exacerbations were defined as events that led to hospitalization for acute COPD attack. An interview was conducted to investigate hospital admissions and evaluate severe exacerbations at one-year follow-up. HRQoL was measured by St George's Respiratory Questionnaire at 6 months.

Results: At 6-month pharmaceutical care and one-year follow-up, the pharmaceutical care group exhibited higher medication adherence than the usual care group (73.4±11.1 *vs.* 55.7±11.9, P=0.016 and 54.4±12.5 *vs.* 66.5±8.6, P=0.039, respectively). There are 60 acute exacerbations resulted in a hospital admission in the usual group while 37 ones in the pharmaceutical care group during one-year follow-up (P=0.01). Hospital admissions due to acute exacerbation in the pharmaceutical care group were 56.3% less than the usual care group (P=0.01). There was a significant difference in the symptoms and impact subscales respectively at 6-month pharmaceutical care between two groups (P=0.032, P=0.018).

Conclusions: Individualized pharmaceutical care improved medication adherence, reduced hospitalization and elevated HRQoL in patients with COPD.

Keywords: Pharmaceutical care; medication adherence; chronic obstructive pulmonary disease (COPD); hospitalization

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Introduction

Chronic obstructive pulmonary disease (COPD) is a progressive disease and a main cause of morbidity and mortality worldwide (1). In China increasingly high prevalence of COPD will impose greater and greater burden on whole society in years to come as Chinese is aging and pollution is deteriorating (2,3). Although COPD can be treated by medications, poor drug adherence can dramatically counteract the therapeutic efficacy of medications. It is common in patients with COPD that they

fail to keep an appointment with their physician, fail to take drugs as prescribed, or even do not take them at all. Because COPD is not reversible, the purpose of pharmacological treatment is to reduce exacerbations and symptoms. A recent systematic review revealed that more than 40% of the COPD patients were not compliant to prescribed drugs (4), leading to a high rate of exacerbation and poor health related quality of life (HRQoL) (5,6).

Despite its importance, medication adherence is an individual patient behavior that is difficult to improve (7). Previous clinical randomized controlled trials examining the effects of on adherence in patients with COPD have shown conflicting results. Khdour MR *et al.* (8) and Jarab AS *et al.* (9) reported clinical pharmacists with an individualized education program and telephone follow-up continued one year can improve adherence and reduce hospitalization. Garcia-Aymerich J *et al.* (10) reported nurses with an individualized care plan and telephone follow-up at discharge for one year can also improve adherence. However, there was no significance on adherence between the control and intervention group in two studies (11,12). Gallefoss and colleagues (11) developed face-to-face education program delivered by a nurse and physiotherapist, no significant difference in medication adherence was found compared usual general practitioner care at 12-month follow-up. Solomon and colleagues (12) used a face-to-face and telephone pharmaceutical care completed by a pharmacist and pharmacy residents, but there was no improvement on adherence between intervention and control groups at 6-month follow-up.

A systematic review found that educational interventions and memory aids were two major types of strategies in older patients to optimize medication adherence (13). Ross JS. held that successful interventions on adherence are often labor intensive and comprehensive, and direct advice to COPD patients from pharmacists may be particularly promising because of their specialized training and knowledge of medications and availability to the patients (7). In this study, we developed our pharmaceutical care program and investigated whether and how our interventions improve the current poor adherence in COPD patients.

Methods

Patients

Participants were enrolled from The First Affiliated Hospital of Guangzhou Medical University, Guangzhou, from February 7 to August 10, 2012. The entrance criteria were as follow: (I) stable COPD (respiratory symptoms and medication unchanged for at least 4 weeks before enrollment); (II) a post-bronchodilator forced expiratory volume in 1 s (FEV_1) to forced vital capacity (FVC) ratio of less than 0.70 and an FEV_1 between 25% and 79% of predicted value; (III) at least two consecutive visits to our hospital for the treatment of COPD; (IV) no participation in a respiratory rehabilitation in the past year; and (V) no previous diagnosis of asthma, dementia, uncontrolled psychiatric disease and severe heart, liver, and kidney disease. The exclusion criteria were: (I) adherence patients (taken above 80% of the daily dose prescribed); (II) refusal to participate the study. All COPD patients having fulfilled the entrance criteria, and having no exclusion criteria present, were invited to participate. The study was approved by local ethics committees and was done in compliance with the Declaration of Helsinki.

Study design

This was a prospective randomized controlled study completed by January 31, 2014. The pharmacists were blinded to the randomization codes, which were computer generated and sealed in envelopes labeled with consecutive numbers. The envelopes were opened and patients were allocated to the control or pharmaceutical care group.

A comprehensive pharmaceutical care program had been developed by our clinical pharmacists based on relative researches (14,15). It was chiefly composed of provision of individualized education and a series of telephone counseling. Patients were individualized educated (20-30 minutes per session, 5-6 sessions) in a structured fashion step by step during the clinic visit. The interview contents included effective use of respiratory devices, pathophysiology of the disease, interpretation of medical testing and rationale for medication. After each interview, medication management records for patients were established that evaluated each participant's preferences and analyzed possible barriers to medication adherence. The frequency of a 10-minute telephone call was based on the results of last interview (maximum reach 12 sessions, weekly call at first month in 25% patients of pharmaceutical care group), but generally telephone call (4-5 sessions) at the midpoint between two clinic visits. During telephone counseling, the pharmacist asked about the patient's treatment effects, clarified any misconceptions, explained the nature of any side effects

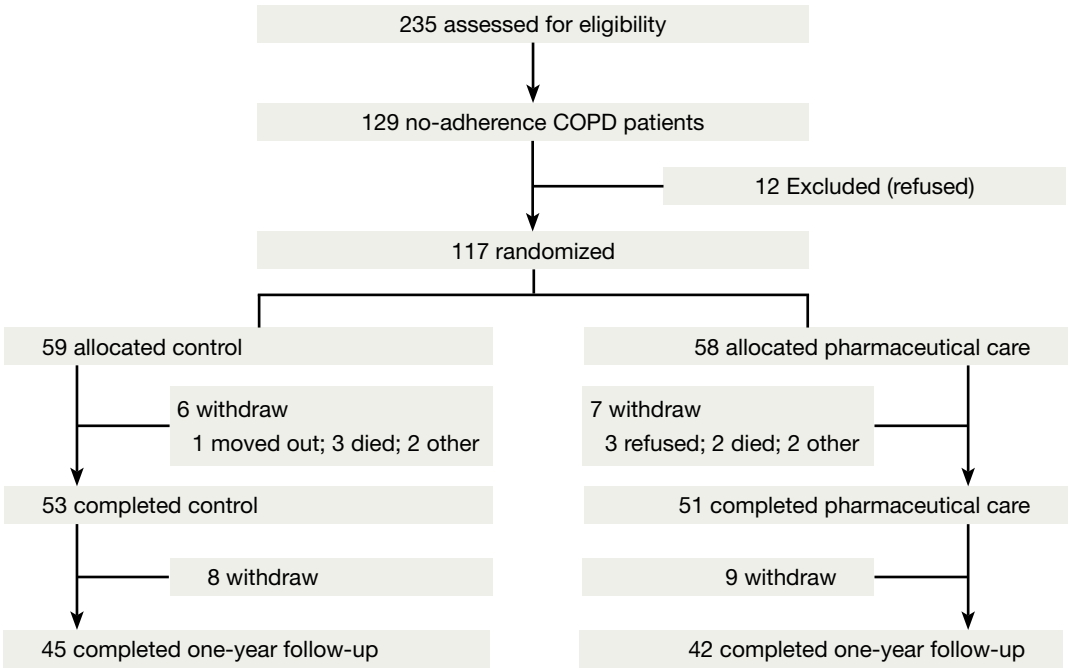

Figure 1 Flow of patients through the study.

and reminded patients of their next clinical appointment. The pharmaceutical care program continued for half a year. Patients in the control group received general counseling, but no individualized education and follow-up telephone counseling.

An interview was held by clinical pharmacist to investigate hospital admissions and evaluate severe exacerbations at one-year follow-up (*Figure 1*).

Outcome measures

The primary endpoint was medication adherence which was measured by pill counts plus direct interview. Our main measurement method of medication adherence is pill counts. In order to increasing its accuracy, structured questionnaire was developed based on Wu *et al.*'s study (14). The pharmacist asked the patient to describe their regimen prescribed at their last visit by drug dosages (number of pills) and frequency when patients were given drug samples. They were asked whether they had missed any medication, or changed their regimens in terms of dose and frequency. This information was checked against the dispensing information and gave the pharmacist a reasonable impression of the patient's degree of adherence. We defined those as adherence patients if they had taken beyond 80% of the daily dose prescribed, and those as non-adherence patients if they failed to do so. Patient adherence was scored by the total number of prescribed drugs a patient took for a month and expressed as a percentage. A patient who complied with all prescribed drug had an adherence score of 100%, whereas one who complied with only three of the six drugs had an adherence score of 50%. Medication adherence was assessed by clinical pharmacists who have been specifically trained for the study before intervention, at 1- and 6-month pharmaceutical care, and at one-year follow-up.

Secondary endpoints included the severe exacerbation rate and HRQoL. A severe exacerbation of COPD was defined as a hospitalization due to an acute COPD attack (16). Hospital admission was defined as (I) hospital stay of any duration in an acute care bed; (II) or day hospital stay of over 2 consecutive days (17). HRQoL was assessed by disease-specific St George's Respiratory Questionnaire (SGRQ, using a validated Chinese version) (18). SGRQ consists of three domains: (I) respiratory symptoms; (II) activities (a measure of the activities that cause or are limited by breathlessness); and (III) impacts (a measure of the overall disturbance of daily life, social function, and well-being). The scores range from 0 to 100, with a lower score indicating a better quality of life. SGRQ was measured by clinical pharmacists before intervention and at 6-month pharmaceutical care, respectively.

Table 1 Patient demographics and baseline characteristics

	Control (n=59)	Pharmaceutical care (n=58)
Age	63.9±6.2	65.2±8.1
Female, n (%)	19 (32.2)	20 (34.5)
Spouse is the main caregiver, n (%)	41 (69.5)	43 (74.1)
<6 years' education, n (%)	27 (45.8)	31 (53.4)
Current smoker, n (%)	21 (35.6)	17 (29.3)
Duration of COPD (years)	7.26±4.98	6.35±5.12
FEV_1 (L)	1.15±0.47	1.07±0.41
FEV_1/FVC (%)	54±9.68	52±10.36
SGRQ total scores	51.50±14.23	51.12±17.25
Comorbid conditions, n (%)	20 (33.9)	24 (41.2)
No. of medications	6.6±5.0	6.2±4.3
Medications for COPD, n (%)		
Anticholinergic agents	31 (52.5)	27 (46.6)
Long acting β_2 agonist	18 (30.5)	15 (25.9)
Inhaled corticosteroids	9 (15.3)	11 (20.0)
Xanthines	27 (45.8)	24 (41.2)
Carbocisteine	37 (62.7)	40 (69.0)

COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; SGRQ, St George's Respiratory Questionnaire.

Statistical analysis

Accurate calculation of sample size had been an issue of great attention when planning the trial, but we were unable to do so because of a scarcity of reference data from previously published studies. Consequently, the sample size was determined on the basis of experiences of Chinese clinical pharmacists. The sample size was estimated to be powered for this study.

All statistical analyses for baseline characteristics and outcomes were done on an intention-to-treat base. We used SPSS10.0 for all analyses. Data are presented as means ± SD or as percentages within groups. The student's sample *t*-test and χ^2 test were used to compare groups. Exacerbation rate was also analysed with a regression model including interactions between intervention and covariates (such as history of exacerbation, FEV_1, the number of taken inhaled corticosteroids and long acting bronchodilators, and quality of life). A two sided value of P<0.05 was considered to be significant.

Results

We interviewed 235 eligible patients with COPD from the outpatient clinic, 106 (45%) of them were adherence patients. One hundred and twenty-nine non-adherence COPD patients were recruited for inclusion in the study, 12 patients declined to participate. Their main reason for refusal was that they felt the evaluation a serious inconvenience. One hundred and seventeen patients are randomized evenly to a control group (59 patients) and a pharmaceutical care group (58 patients) (*Figure 1*).

The two groups were similar in socio-demographic, clinical, functional variables, and respiratory medication (P>0.10, *Table 1*). The number of patients taking medications that decrease exacerbation (inhaler corticosteroids, anti-cholinergic agents, and long-acting β_2 agonist) was similar between the pharmaceutical care group and control group (41 *vs.* 37, P=0.21). During 6-month intervention, the patients in the pharmaceutical care group received 5.48±3.62 sessions individualized education and 5.76±4.12 sessions telephone counseling.

At 6-month intervention and 1-year follow-up, the pharmaceutical care group exhibited significant higher adherence score (%) than the control group, but there was no such a significant difference between the two groups at 1-month (*Table 2*). In the pharmaceutical care group, the adherence score at 1-year follow-up was significantly decreased compared with that at 6-month intervention (73.4±11.1 *vs.* 66.5±8.6, P=0.042).

Patients admissions due to acute exacerbations of COPD in the year preceding study entry were similar between the both groups (41 *vs.* 38, P=0.45). At 1-year follow-up, 46 severe acute exacerbation in the control group resulted in a hospital admission while 20 ones in the pharmaceutical care group, yielding a 56.5% reduction in hospital admissions (P=0.01). Significantly more patients in the control group had one hospital admission and two hospital admissions during the 1-year follow-up respectively (*Table 3*). By analysing the covariance factors in the regression model, no factors were found to significantly affect COPD exacerbation.

Baseline HRQoL scores on the SGRQ were comparable between the control and pharmaceutical care groups on each of the subscales and the total scores (*Table 4*). At 6-month intervention, scores on symptoms and impacts subscales in the pharmaceutical care group were significantly improved compared with those in the control group respectively, while activities subscale and total scores were not significant difference between the two groups (*Table 4*).

Table 2 Compliance scores in pharmaceutical care and control groups

	Control		Pharmaceutical care		P value
	No	Mean	No	Mean	
Before pharmaceutical care	59	54.2±11.5	58	58.6±12.6	0.559
After pharmaceutical care					
1 month	57	56.8±10.6	57	62.1±9.7	0.142
6 months	53	52.7±21.9	51	73.4±11.1	0.016
1-year follow-up	45	54.4±12.5	42	66.5±8.6	0.039

We defined patients as compliant with drug if they had taken beyond 80% of the prescribed daily dose. A compliance score of a patient is calculated by the total number of prescribed drugs and expressed it as a percentage. A patient who complied with all prescribed drug had a compliance score 100%.

Table 3 Hospital admissions for acute COPD attack during 1-year follow-up

	Control (n=45)	Pharmaceutical care (n=42)	P value
No. of admissions	46	20	0.01
Patients admitted, n (%)			
Once	31 (68.9)	13 (31.0)	0.01
Twice	6 (13.0)	2 (4.8)	0.01
Thrice	1 (2.2)	1 (2.4)	–
Hospital days per patient	11.11±18.16	5.56±9.68	0.01

COPD, chronic obstructive pulmonary disease.

Discussion

Our study showed that individualized 6-month pharmaceutical care improved the medication adherence in patients with COPD. We found that the medication adherence of the pharmaceutical care group was superior to that of the control group at 1-year follow-up, suggesting the therapeutic effects might be sustainable.

Some studies used questionnaires to measure medications adherence for patients with COPD (8-10). Khdour MR *et al.* (8) and Jarab AS *et al.* (9) administered Morisky Scale used widely in many kind of diseases, while Garcia-Aymerich J *et al.* (10) used Inhaler Adherence Scale suited for inhaler drugs. Morisky Scale measures adherence through four Yes/No response items, reflects the number of ways medication omission can occur: forgetting, carelessness, stopping when feeling better and stopping when feeling worse (19). Although these scales are relative easy to measure, questioning the patients can be biased by inaccurate patient recall or social desirability resulting in the health care provider's overestimating the patient's adherence (20). Pill counts measurement is a relative objective and one of the most commonly used methods for evaluating adherence, but this method may provide wrong information (i.e., switch medicines between bottles before visits in order to be following the regime), and provides no information on other aspects of taking drug (i.e., dose timing) (21). In our study pill counts plus direct interview maximized the accuracy of measuring medication adherence (22).

There are many barriers to medication adherence for patients with COPD. In response to a questionnaire, typical reasons cited by patients for not taking their drugs included high costs for drugs and taking medication based on their feeling (e.g., "it does not do any good") (23). This intentional non-adherence is often due to the patient's misunderstanding the clinician's instructions. Although proper education ensure the patient is fully informed about the important aspects of their treatment regimen, generally educational interventions, such as mailed patient educational material and brief interaction, do not consistently improve medication adherence (13). No education effects on medication adherence by Gallefoss and colleagues (11) may be due to the short term intervention which consisted of two 2-hour group sessions and 1 to 2 individual sessions. Our study showed that medication adherence improved significantly at 6-month pharmaceutical care, but not at 1-month pharmaceutical care, suggesting that long-time intervention might be sufficient to alleviate patients' concern. Besides the patients' understanding, their motivation and expectation about the success likelihood of medical intervention are critical factors to medications adherence (24). As patients were intervened by outpatient pharmacists mainly in community or ambulatory care settings, no significant changes in adherence scores by Solomon and colleagues (12) may be due to lacking of motivation and expectation of patients. In our face-to-face

Table 4 SGRQ scores in pharmaceutical care and control groups

	Control		Pharmaceutical care		P value
	No	Mean	No	Mean	
Total					
Baseline	59	51.12±17.25	58	51.50±14.23	0.921
After treatment	53	52.16±13.59	51	48.86±12.54	0.139
Symptoms					
Baseline	59	48.25±19.56	58	47.39±21.37	0.801
After treatment	53	47.25±20.96	51	40.68±18.59*	0.032
Activities					
Baseline	59	53.96±20.14	58	52.66±24.63	0.625
After treatment	53	52.59±23.15	51	50.10±22.37	0.309
Impacts					
Baseline	59	40.68±24.56	58	41.59±25.61	0.562
After treatment	53	41.36±25.66	51	32.19±19.22*	0.018

SGRQ, St George's Respiratory Questionnaire. *P<0.05 compared with baseline.

pharmaceutical care intervention, one of the goals is help the patients understand and believe the treatment is good and fit for him.

After the patients understand and are convinced of the treatment of COPD, some of them still exhibit poor medication adherence. This difficulty may be attributable to a busy schedule, lack of attention to detail, and inadvertently forgetting to take their medications. Periodic reinforcements are necessary to improve medication adherence. Marteau TM *et al.* (25) held that much automatic behavior is cued by environmental stimuli, resulting in actions that are largely unaccompanied by conscious reflection. In our study, medication adherence in the pharmaceutical care group at 1-year follow-up decreased compared with that at 6-month pharmaceutical care, suggesting that continuous support from clinical pharmacist is indispensable to changing negative health behaviors of patients.

An important finding in the present study was the 56.5% reduction in hospitalization over the control group at 1-year follow-up. Since exacerbation of COPD can worsen health status and fasten disease progression, reduction of exacerbation rate is a key target for intervention (16). Some researchers reported a reduction in hospital admission as a result of educational interventions (8-10,12,17). In education plan of these studies, researchers emphasized prompt initiation of antibiotic and oral corticosteroid medication for COPD exacerbations besides improving medication adherence. Rees PJ argued the self-management program might be the major reason for the reduction in hospital admissions with exacerbations (26). In our study, reduction in hospital admissions might have been more attributable to reduction in severe exacerbation.

In addition to preventing COPD exacerbations, pharmaceutical care was shown to improve HRQoL. A meta-analysis of education interventions in COPD did not find consistently effectiveness in HRQoL (27). Our study illustrated that significant improvements over the control group in impact and symptoms scores of SGRQ were achieved at 6-month pharmaceutical care.

Our findings highlight for the first time the significance of pharmaceutical care for medication adherence in non-compliant patients with COPD. We report beneficial effects of medication adherence on reducing hospitalization and enhancing HRQoL. To confirm the generalizability of our findings, a multi-centre prospective randomized controlled study is warranted in large samples of COPD patients from other geographical areas.

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References

1. Mannino DM, Buist AS. Global burden of COPD: risk factors, prevalence, and future trends. Lancet 2007;370:765-73.
2. Lamprecht B, McBurnie MA, Vollmer WM, et al.

COPD in never smokers: results from the population-based burden of obstructive lung disease study. Chest 2011;139:752-63.
3. Grouse L. The rise of a non-communicable disease epidemic. J Thorac Dis 2012;4:238-9.
4. Bryant J, McDonald VM, Boyes A, et al. Improving medication adherence in chronic obstructive pulmonary disease: a systematic review. Respir Res 2013;14:109.
5. Vestbo J, Anderson JA, Calverley PM, et al. Adherence to inhaled therapy, mortality and hospital admission in COPD. Thorax 2009;64:939-43.
6. Antoniu SA. Adherence to inhaled therapy in COPD: effects on survival and exacerbations. Expert Rev Pharmacoecon Outcomes Res 2010;10:115-7.
7. Simpson RJ Jr. Challenges for improving medication adherence. JAMA 2006;296:2614-6.
8. Khdour MR, Kidney JC, Smyth BM, et al. Clinical pharmacy-led disease and medicine management programme for patients with COPD. Br J Clin Pharmacol 2009;68:588-98.
9. Jarab AS, Alqudah SG, Khdour M, et al. Impact of pharmaceutical care on health outcomes in patients with COPD. Int J Clin Pharm 2012;34:53-62.
10. Garcia-Aymerich J, Hernandez C, Alonso A, et al. Effects of an integrated care intervention on risk factors of COPD readmission. Respir Med 2007;101:1462-9.
11. Gallefoss F, Bakke PS. How does patient education and self-management among asthmatics and patients with chronic obstructive pulmonary disease affect medication? Am J Respir Crit Care Med 1999;160:2000-5.
12. Solomon DK, Portner TS, Bass GE, et al. Clinical and economic outcomes in the hypertension and COPD arms of a multicenter outcomes study. J Am Pharm Assoc (Wash) 1998;38:574-85.
13. Schlenk EA, Bernardo LM, Organist LA, et al. Optimizing Medication Adherence in Older Patients: A Systematic Review. J Clin Outcomes Manag 2008;15:595-606.
14. Wu JY, Leung WY, Chang S, et al. Effectiveness of telephone counselling by a pharmacist in reducing mortality in patients receiving polypharmacy: randomised controlled trial. BMJ 2006;333:522.
15. Lee JK, Grace KA, Taylor AJ. Effect of a pharmacy care program on medication adherence and persistence, blood pressure, and low-density lipoprotein cholesterol: a randomized controlled trial. JAMA 2006;296:2563-71.
16. Wedzicha JA, Seemungal TA. COPD exacerbations: defining their cause and prevention. Lancet 2007;370:786-96.
17. Bourbeau J, Julien M, Maltais F, et al. Reduction of hospital utilization in patients with chronic obstructive pulmonary disease: a disease-specific self-management intervention. Arch Intern Med 2003;163:585-91.
18. Jones PW. Health status measurement in chronic obstructive pulmonary disease. Thorax 2001;56:880-7.
19. Morisky DE, Green LW, Levine DM. Concurrent and predictive validity of a self-reported measure of medication adherence. Med Care 1986;24:67-74.
20. Ho PM, Bryson CL, Rumsfeld JS. Medication adherence: its importance in cardiovascular outcomes. Circulation 2009;119:3028-35.
21. Osterberg L, Blaschke T. Adherence to medication. N Engl J Med 2005;353:487-97.
22. Turner BJ, Hecht FM. Improving on a coin toss to predict patient adherence to medications. Ann Intern Med 2001;134:1004-6.
23. George J, Kong DC, Thoman R, et al. Factors associated with medication nonadherence in patients with COPD. Chest 2005;128:3198-204.
24. Rand CS. Patient adherence with COPD therapy. Eur Respir Rev 2005;14:97-101.
25. Marteau TM, Hollands GJ, Fletcher PC. Changing human behavior to prevent disease: the importance of targeting automatic processes. Science 2012;337:1492-5.
26. Rees PJ. A disease-specific self-management program reduced hospital utilization and improved health status in COPD. ACP J Club 2003;139:65.
27. Monninkhof E, van der Valk P, van der Palen J, et al. Self-management education for patients with chronic obstructive pulmonary disease: a systematic review. Thorax 2003;58:394-8.

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Original Article

Video-assisted mediastinoscopic resection compared with video-assisted thoracoscopic surgery in patients with esophageal cancer

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Objective: The purpose of this study was to explore the indications of radical vedio-assisted mediastinoscopic resection for esophageal cancer.

Methods: The data of 109 patients with T1 esophageal cancer who underwent video-assisted mediastinoscopic resection (VAMS group) in Third Affiliated Hospital of Soochow University Hospital from December 2005 to December 2011 were collected in the study for comparison with the 58 patients with T1 esophageal cancer who underwent video-assisted thoracoscopic surgery (VATS group) in Zhongshan Hospital, Fudan University. The perioperative safety and survival were compared between the two groups.

Results: All operations were successful in both groups. One perioperative death was noted in the VATS group. The incidences of post-operative complications were not significantly different between these two groups, whereas the VAMS group was favorable in terms of operative time (P<0.001) and blood loss (P<0.001), and a significantly larger number of chest lymph nodes were dissected in the VATS group compared with the VAMS group (P<0.001). Long-term follow-up showed that the overall survival was not significantly different between these two groups (P=0.876).

Conclusions: T1N0M0 esophageal cancer can be as the indication of VAMS radical resection. VAMS radical resection can be considered as the preferred option for patients with poor pulmonary and cardiac function or a history of pleural disease.

Keywords: Esophageal cancer; mediastinoscopy; video-assisted thoracoscopic surgery (VATS)

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Introduction

Esophageal cancer is a malignancy that poses a serious threat to human health, with a mortality of up to over 150,000 people each year, accounting for the vast majority of the world's deaths of esophageal cancer (1,2). Surgery is currently the preferred option with which cure of early esophageal cancer can be expected (3-5). The introduction of endoscopic-assisted minimally invasive esophageal surgery provides a new way with reduced damage to chest associated with invasive thoracic operation, decreased surgical mortality and improved postoperative quality of life for patients (6). As mediastinoscopy has altered the traditional surgical approach, chest operation is avoided, thus maintaining the integrity of the pleural cavity with less injury (7). This technique is therefore carried out in a large number of medical centers (7-9). Currently, the indications and contraindications for video-assisted mediastinal endoscopic resection of esophageal cancer have remained controversial and any widely accepted standards are yet to come (10). The purposes of this study were to

compare the advantages and disadvantages of video-assisted mediastinoscopic and thoracoscopic esophageal surgery, and to provide a preliminary summary of the indications and contraindications for the mediastinoscopic esophageal resection.

Patients and methods

Patients

The data of 109 patients with T_1 esophageal cancer who underwent video-assisted mediastinoscopic resection (VAMS group) in Third Affiliated Hospital of Soochow University Hospital from December 2005 to December 2011 were collected in the study for comparison with the 58 T_1 esophageal cancer patients who underwent video-assisted thoracoscopic surgery (VATS group) in Zhongshan Hospital, Fudan University. In total, there were 60 men and 49 women aged 54 to 78 years (with a median age of 62 years) in the VAMS group; and 32 men and 26 women aged 55-72 years (with a median age of 62 years) in the VATS group. There was no significant difference in terms of age, sex and other demographic parameters between the two groups.

The same inclusion criteria were applied for both groups: diagnosis of esophageal squamous cell carcinoma by endoscopy; no obvious lymph node enlargement on preoperative CT examination of the chest and upper abdominal ultrasound; and no obvious enlargement of mediastinal lymph nodes on preoperative EUS, and esophageal tumor infiltration not exceeding than T_1. Exclusion criteria were as the following: with a previous history of malignancy; obvious lymph node enlargement on preoperative CT examination of the chest and upper abdominal ultrasound; obvious enlargement of mediastinal lymph nodes on preoperative EUS, and esophageal tumor infiltration exceeding than T_1.

Pre-operative preparation

All patients underwent an upper gastrointestinal series, enhanced chest CT and EUS for confirmation of early esophageal cancer without significantly enlarged mediastinal lymph nodes. The preoperative staging was $T_{1\text{-}2}N_0M_0$. Routine preoperative examination was conducted to rule out any obvious contraindication for surgery.

Surgical techniques and postoperative treatment

Two concurrent operations were performed for patients in VAMS group. For the neck surgery, an incision was made along the left anterior sternocleidomastoid edge (up to the midpoint of the sternocleidomastoid and down to the jugular notch, about 5 cm long). The upper and lower esophageal segments were separated respectively through video-assisted mediastinoscopy and the diaphragm hiatus. To avoid damage to the recurrent laryngeal nerve, the separation went down along the left posterior region of the esophagus with an attempt to divide the vagus nerve, followed by the upper esophageal segment, to prevent pulling of the recurrent laryngeal nerve. The nutritional support branch from the aorta for the esophagus was clipped using a titanium clip down to the level of the pulmonary veins. Paraesophageal mediastinal lymph node dissection was carried out during this process. In the case of ruptured pleural cavity or the need of thoracic lymph node dissection, closed thoracic drainage was used to drain the pleural fluid. A silicone ball was placed for drainage after surgery, which was removed after 2-3 days as soon as the mediastinal drainage was significantly reduced.

The patients were placed in a lateral position in the VATS group. The four-port technique was then used, with a 10-mm incision in the 7th intercostal space at the midaxillary line for thoracoscope placement, a 5-mm and a 10-mm incision in the 8th intercostal space at the subscapularis angle and the scapular line, respectively, for placement of a endoscopic grasper and scalpel for dividing the esophagus, and the last 5-mm incision in the 3rd intercostal space at the anterior axillary line as the third working port for pulling of the lung and esophageal exposure. The mediastinal pleuron was cut along the esophagus longitudinally to separate the esophagus. The arch of the azygos vein was divided and the vein was cut with an endoscopic vein vascular stapler or tissue clamp. The esophagus was then pulled and separated along the surgical plane, and the surrounding lymph nodes, adipose tissue around the esophagus, subcarinal lymph nodes and lymph nodes next to the recurrent laryngeal nerve on both sides were also resected completely. The esophagus was exposed down to the diaphragmatic hiatus and up to the neck.

For the abdominal operation, laparotomy or laparoscopic incision was made to both groups to separate the stomach, which was pulled via the transesophageal bed to the neck position for resection of the affected esophagus and connection of the esophagus-stomach anastomosis. The patients were admitted to ICU after surgery, and received anti-inflammatory, hemostatic, phlegm-resolving treatment and nutritional support, as well as continuous infusion of

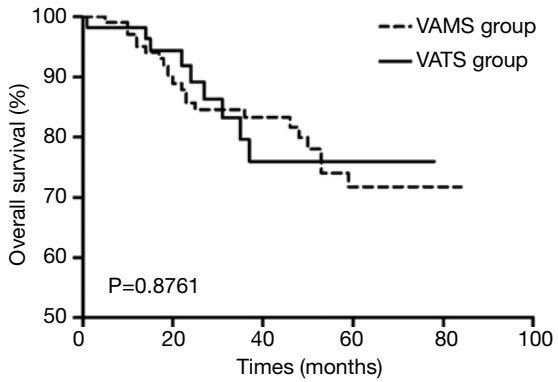

Figure 1 Overall survival in video-assisted mediastinoscopic resection (VAMS group) and video-assisted thoracoscopic surgery (VATS group).

morphine to relieve pain.

Statistical analysis

The statistical indicators included operative time, intraoperative blood loss, number of dissected chest lymph nodes, postoperative complication rate and so on. Long-term follow-up was carried out after surgery to compare the overall survival and survival time. The statistical analysis was performed in GraphPad Prism 5.0. Data between the two groups were compared using Mann Whitney test, and survival analysis was conducted using Log-rank test. $P<0.05$ was considered significantly different.

Results

All operations were successful in both groups. An incidence of intraoperative bleeding was observed in the VAMS group, with which the surgery was completed following an additional right chest incision for bleeding control. The average time was 43.91 minutes for thoracic surgery, with a median of 50 minutes. The average blood loss was 115.16 mL, with a median of 100 mL. The number of dissected chest lymph nodes was 511, with an average of 4.69/cases. Thoracic lymph node metastasis was observed in 2 cases, with a positive rate of 1.8%. There were 12 cases of anastomotic leakage, one case of mediastinal chyle, seven cases of arrhythmia, and nine cases of hoarseness after surgery, and all were cured and discharged following symptomatic treatment.

The thoracic surgery lasted an average of 76.15 minutes for the VATS group, with a median of 80 minutes; blood loss was 144.5 mL, with a median of 150 mL. The number of dissected chest lymph nodes was 506, with an average of 8.72/cases. Thoracic lymph node metastasis was observed in 2 cases, with a positive rate of 3.4%. There were seven cases of anastomotic leakage, two cases of chylothorax, two cases of arrhythmia, one case of pulmonary embolism, and one case of postoperative thoracic bleeding which required a second surgery to stop. All the subjects with complications were cured and discharged following symptomatic treatment. There was one case of postoperative stump fistula leading to perioperative death due to septic shock.

Comparing the two groups, there was no significant difference in postoperative complications ($P=0.7284$) or the incidence of anastomotic fistula ($P=0.8373$). The VAMS group was favorable in terms of operative time ($P<0.001$) and blood loss ($P<0.001$), and a significantly larger number of chest lymph nodes were dissected in the VATS group compared with the VAMS group ($P<0.001$). Long-term follow-up in both groups revealed no significant difference in the overall survival ($P=0.876$; *Figure 1*).

Discussion

Minimally invasive esophageal surgery has been developing rapidly in just over a decade (6,7,11). As the continuous improvement of the technology and application techniques, the potential of becoming a preferred option to conventional surgery has gradually appeared (12). Since thoracoscopic esophagectomy still takes the traditional approach, despite reduced surgical injury, it still undermines the integrity of the pleural cavity, which is intolerable for certain patients. In recent years, we have adopted the video-assisted mediastinoscopy for the treatment of esophageal cancer, and achieved good outcomes (13). Similar reports have preliminarily demonstrated the feasibility, safety and long-term efficacy of mediastinoscopic resection of esophageal cancer (11-13). Due to limited space and vision under mediastinoscopy, it has remained controversial as to whether lymph node dissection can be accomplished with microscope. Therefore, there are still no unified, definite standards for the indication for mediastinoscopic esophageal resection (12,13). This study compared the efficacy of video-assisted mediastinoscopic and thoracoscopic esophageal surgery with an attempt to summarize the indications for the mediastinoscopic esophageal resection.

Due to limited vision and space, mediastinal endoscopic resection demands for more stringent indications, and therefore it was not suitable for cases with significant tumor

invasion or evident mediastinal lymph node involvement. Previous studies have demonstrated that for patients with early esophageal cancer (T_2 and before), video-assisted mediastinoscopy can achieve similar therapeutic effect as thoracotomy (11,14). There was no difference in the long-term efficacy for patients with stage T_1 after surgery in the both groups. This indicates that esophageal cancer at the T_1 stage as confirmed by preoperative endoscopic ultrasound and chest enhanced CT scan, without significantly enlarged mediastinal lymph nodes, can be treated with VAMS esophageal resection, with which a similar therapeutic effect can be achieved as thoracoscopic surgery.

Some investigators reported that in patients with non-small cell lung cancer, cervical mediastinoscopy can be used to dissect all mediastinal lymph nodes except groups 9 and 4L, including the lymph nodes near bilateral recurrent laryngeal nerve (8,15-17). In our experience, the amplification effect of video-assisted mediastinoscopy was conducive to fine operation and thus the detection rate of enlarged mediastinal lymph nodes for complete resection, helping to achieve the purpose of cure. Our results also demonstrated that thoracoscopic surgery has an advantage in the number of dissected mediastinal lymph nodes. Hence, there needs to be a larger-scale multi-center, prospective study to draw more scientific conclusions for mediastinal lymph node dissection under mediastinoscopy.

During neck operation, injury to the recurrent laryngeal nerve is likely to occur. In the VAMS group, there were nine cases of hoarseness, though all of them were healed spontaneously afterwards. In our experience, the use of suction cautery is associated with a high likelihood of recurrent laryngeal nerve injury. Therefore, when separating the upper esophagus during the later part of the surgery, the instrument should be avoided and changed to ultrasonic electrotome for the operation.

In conclusion, $T_1N_0M_0$ esophageal cancer is the surgical indication for mediastinoscopic resection. Mediastinoscopic esophageal resection is done through the mediastinal pathway, which does no injury to the pleural cavity. Therefore, despite the controversy over mediastinal lymph node dissection, this technique can be considered as the preferred option for patients with poor pulmonary and cardiac function or a history of pleural disease.

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References

1. Chen W, Zheng R, Zhang S, et al. Report of incidence and mortality in China cancer registries, 2009. Chin J Cancer Res 2013;25:10-21.
2. Chen W, He Y, Zheng R, et al. Esophageal cancer incidence and mortality in China, 2009. J Thorac Dis 2013;5:19-26.
3. Saglam S, Arifoglu A, Saglam EK, et al. Neoadjuvant hyperfractionated-accelerated radiotherapy with concomitant chemotherapy in esophageal cancer: phase II study. J Gastrointest Oncol 2013;4:380-7.
4. Lu J, Tao H, Song D, et al. Recurrence risk model for esophageal cancer after radical surgery. Chin J Cancer Res 2013;25:549-55.
5. Ling TC, Kang JI, Slater JD, et al. Proton therapy for gastrointestinal cancers. Transl Cancer Res 2012;1:150-8.
6. Tapias LF, Morse CR. Minimally invasive ivor lewis esophagectomy: description of a learning curve. J Am Coll Surg 2014;218:1130-40.
7. Navarro-Ripoll R, Córdova H, Rodríguez-D'Jesús A, et al. Cardiorespiratory Impact of Transesophageal Endoscopic Mediastinoscopy Compared With Cervical Mediastinoscopy: A Randomized Experimental Study. Surg Innov 2014. [Epub ahead of print].
8. Binţinţan V, Gutt CN, Mehrabi A, et al. Gas-chamber mediastinoscopy for dissection of the upper esophagus. Chirurgia (Bucur) 2009;104:67-72.
9. Mimatsu K, Oida T, Kawasaki A, et al. A novel technique of mediastinoscopy-assisted esophagectomy with a flexible laparoscope and endoscopic overtube. Surg Laparosc Endosc Percutan Tech 2010;20:e44-6.
10. Wu B, Xue L, Qiu M, et al. Video-assisted mediastinoscopic transhiatal esophagectomy combined with laparoscopy for esophageal cancer. J Cardiothorac Surg 2010;5:132.
11. Verhage RJ, Hazebroek EJ, Boone J, et al. Minimally invasive surgery compared to open procedures in esophagectomy for cancer: a systematic review of the literature. Minerva Chir 2009;64:135-46.
12. Venissac N, Pop D, Mouroux J. Video-assisted mediastinoscopy as a therapeutic tool. Surg Endosc 2009;23:2466-72.
13. Bonavina L, Incarbone R, Bona D, et al. Esophagectomy via laparoscopy and transmediastinal endodissection. J Laparoendosc Adv Surg Tech A 2004;14:13-6.
14. Pop D, Venissac N, Mouroux J. Video-assisted mediastinoscopy improved radical resection for cancer

in transhiatal esophagectomy. J Thorac Cardiovasc Surg 2007;133:267-8.
15. Zieliński M. Transcervical extended mediastinal lymphadenectomy: results of staging in two hundred fifty-six patients with non-small cell lung cancer. J Thorac Oncol 2007;2:370-2.
16. Binţinţan VV, Mehrabi A, Fonouni H, et al. Evaluation of the combined laparoscopic and mediastinoscopic esophagectomy technique. Chirurgia (Bucur) 2009;104:187-94.
17. Ikeda Y, Niimi M, Kan S, et al. Mediastinoscopic esophagectomy using carbon dioxide insufflation via the neck approach. Surgery 2001;129:504-6.

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Original Article

Size of solitary pulmonary nodule was the risk factor of malignancy

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Objective: The purpose of this study was to analyze the role of the sizes of solitary pulmonary nodules (SPNs) in predicting their potential malignancies.

Methods: A total of 379 patients with pathologically confirmed SPNs were enrolled in this study. They were divided into three groups based on the SPN sizes: ≤10, 11-20, and >20 mm. The computed tomography (CT) findings of these SPNs were analyzed in these three groups to identify the malignant and benign SPNs. The risk factors were analyzed using binary logistic regression analysis.

Results: Of these 379 patients, 120 had benign SPNs and 259 had malignant SPNs. In the ≤10 mm SPN group, air cavity density was the risk factor for malignancy, with the sensitivity, specificity, and accuracy being 77.8%, 75.0%, and 76.3%. In the 11-20 mm SPN group, age, glitches and vascular aggregation were the risk factors for malignancy, with the sensitivity, specificity, and accuracy being 91.3%, 56.9%, and 81.5%. In the >20 mm SPN group, age, lobulation, and vascular aggregation were the risk factors for malignancy, with the sensitivity, specificity, and accuracy being 88.6%, 57.1%, and 79.1%.

Conclusions: According to CT findings of SPNs, age, glitches, lobulation, vascular aggregation, and air cavity density are the risk factors of malignancy, whereas calcification and satellite lesions are the protective factors. During the course of development from small to large nodules, air cavity density could be firstly detected in early stages, followed by glitches and vascular aggregation. Lobulation is associated with relatively large lesions.

Keywords: Solitary pulmonary nodules (SPNs); computed tomography (CT); logistic regression; risk factors

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Introduction

Solitary pulmonary nodule (SPN) is a single mass in the lung less than or equal to 3 cm in diameter, without concomitant pneumonia and atelectasis of involved lung segments and lobes (1-3). Diagnoses of benign and malignant SPN has been concerned and become a challenge for radiological studies (4-7). Some SPNs are indicated pathologically in the early stages of lung cancers (8,9). Therefore, it is utmost important to utilize preoperative radiography in the characterization of SPN (10,11).

Computed tomography (CT) is the preferred radiological approach to examine SPNs. CT scans can clearly show the size, internal features, edge changes, changes of adjacent structures, and enhancement of pulmonary nodules and could provide comprehensive radiological evidences for

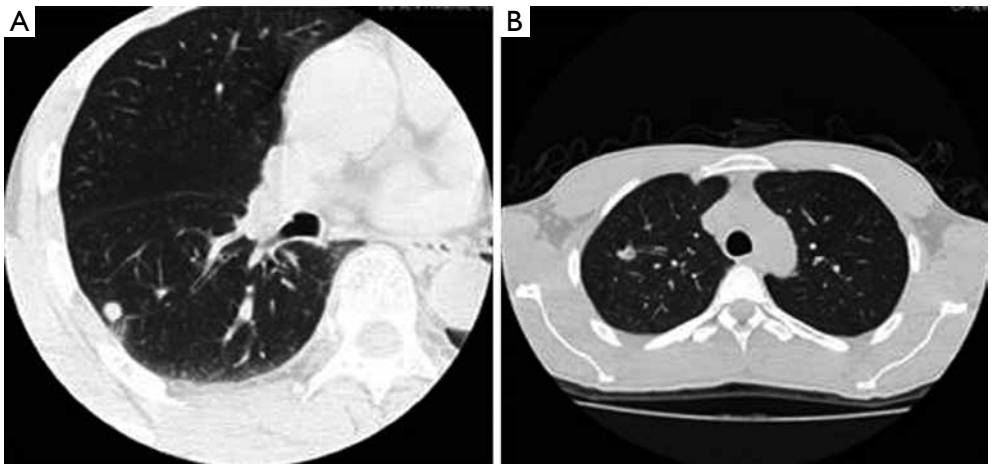

Figure 1 <10 mm lung nodules: (A) right inferior pulmonary nodules of a 57-year-old man, axial CT image showed: smooth nodule edge, no lobulation or glitches, local pleural stretch, and adjacent few fiber lesions. The prediction P value was 0.312, and lesion was considered to be a benign pulmonary nodule. It was later identified to be a pulmonary tuberculosis mass in the right inferior lung, as demonstrated by pathological examinations; (B) left upper lung nodule in a 37-year-old man, axial CT image showed: The nodule was lobular, with air cavity density and adjacent vascular aggregation inside it. The prediction P value was 0.647, and lesion was considered to be a malignant pulmonary nodule. It was pathologically confirmed to be the organizing pneumonia in the right upper lung. CT, computed tomography.

the differential diagnosis between benign and malignant diseases (12,13). However, since most radiological signs were present in both benign and malignant lesions, it is necessary to balance the weight of various radiological signs in the identification of pulmonary nodules, in order to further understand the role of CT in the diagnosis and differential diagnosis of pulmonary nodules.

Known as one of the most common methods for medical statistical analysis, logistic regression model could be constructed to predict benign and malignant probability of pulmonary nodules as well as relevant features based on various CT signs. In literature described previously, logistic regression model was established to predict the CT diagnosis of SPN (14,15). However, no stratification in the size of pulmonary nodules was used. In this study, the size of pulmonary nodules was stratified to analyze the relevant risk factors of malignant pulmonary nodules, in order to improve the knowledge of CT signs for malignant pulmonary nodules in various sizes.

Patients and methods

General data

Totally 379 cases of SPNs, including 120 benign lesions and 259 malignant lesions were collected and confirmed by clinical pathology or biopsy. There were 38 cases of pulmonary nodules with the size ≤10 mm (20 benign lesions and 18 malignant lesions; *Figure 1*), 178 cases of pulmonary nodules with the size of 11-20 mm (51 benign lesions and 127 malignant lesions; *Figure 2*) and 163 cases of pulmonary nodules with the size >20 mm (49 benign lesions and 114 malignant lesions; *Figure 3*). There were 211 men and 168 women aged 21 to 87 years (median: 53 year). CT scanning conditions: helical scan; slice thickness of 2-5 mm, the interlamination of 2-5 mm, scanning from pulmonary apex to the basis.

Radiological examinations

CT images were examined by two radiologists with relevant experience for more than five years, and variables examined include the size of lesions, edge sharpness, glitches, air cavity density (including vacuole sign and inflatable bronchioles), calcification, pleural indentation, satellite lesions, vascular aggregation and extent of enhancement. In case of inconsistent results, the final decision was to be made after discussion.

Statistical analysis

SPSS 17.0 software was employed for statistical analysis, and comparison of different age groups was conducted by *t*-test.

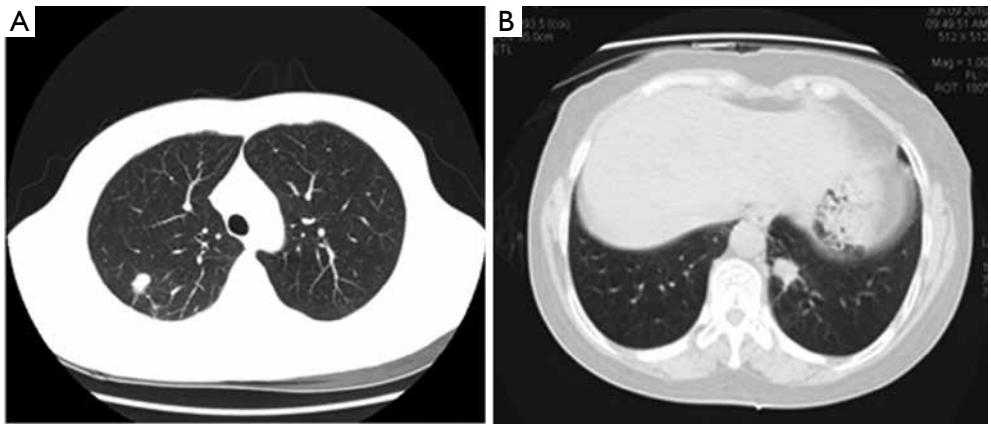

Figure 2 11-20 mm lung nodules: (A) right upper pulmonary nodules in a 34-year-old man. Axial CT image showed: the nodule was slightly lobular, with glitches at its edge and adjacent pleural stretch. No vascular aggregation was found. The prediction P value was 0.354, and lesion was considered to be a benign pulmonary nodule. It was pathologically confirmed to be a pulmonary tuberculosis mass in the right upper lung; (B) left inferior nodules in a 53-year-old women. Axial CT image showed: the nodule was slightly lobular, with glitches at its edge and adjacent pleural stretch. No vascular aggregation was found. The prediction P value was 0.659, and the lesion was considered to be a malignant pulmonary nodule. The lesion was pathologically confirmed to be caused by mycosis and fungal infections at the left lower lung pulmonary. CT, computed tomography.

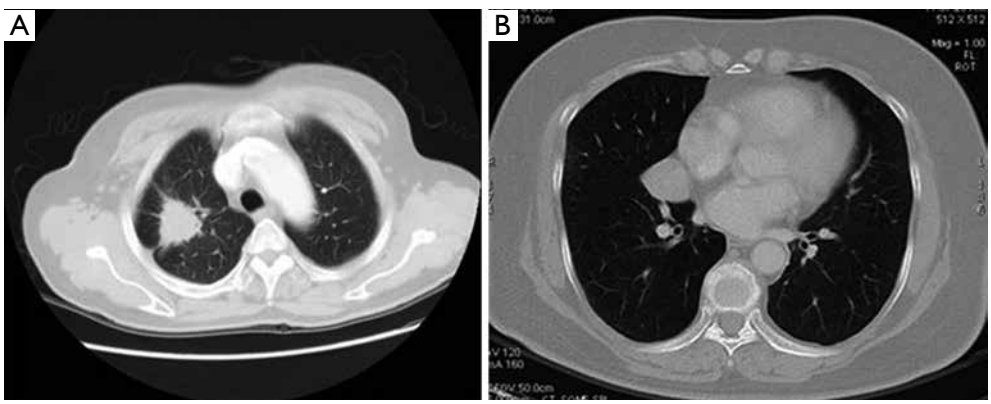

Figure 3 >20 mm lung nodules: (A) right upper pulmonary nodules in a 61-year-old man. Axial CT image showed: the nodule was lobular, along with glitches at its edge, pleural indentation, and vascular aggregation. The prediction P value was 0.930, and the lesion was considered to be a malignant pulmonary nodule. It was pathologically confirmed to be a poorly-differentiated adenocarcinoma in the right upper lung; (B) right middle pulmonary nodules in a 66-year-old man. Axial CT image showed: smooth nodule edge, but without lobulation, glitches, vascular aggregation, or pleural indentation. The prediction P value was 0.297, and lesion was considered to be a benign pulmonary nodule. The lesion was pathologically confirmed to be a spindle cells carcinoid in the right middle lung. CT, computed tomography.

Comparisons of gender, size and enumeration data were performed by chi-square test. For variables with statistical significance, logistic regression method was employed to make a differential diagnosis between benign and malignant pulmonary nodules. Forward stepwise regression analysis was used based on the maximum likelihood estimate for Logistic regression method, and a $P \leq 0.05$ was considered as the criteria for variable inclusion. Dependent variables include benign and malignant pulmonary nodules (number of malignant nodules, 1; number of benign nodules, 0), with gender (male, 1; female, 0), age (based on actual age), size (≤10 mm, 0; 11-20 mm, 1; >20 mm, 2), lobulation sign (presence, 1; absence, 0), edge profile (clear, 1; ambiguous, 0), pleural indentation (presence, 1; absence, 0), satellite lesions

Table 1 Comparison of the ages between patients with benign or malignant lesions

Size (mm)	Age		P
	Malignant pulmonary nodules	Benign pulmonary nodules	
≤10	55.44±10.48	50.70±13.44	0.231
11-20	59.26±10.73	50.65±13.25	<0.001
>20	61.03±11.45	51.90±14.70	<0.001
Total	59.77±11.09	51.17±13.79	<0.001

Table 2 Comparison of the gender, nodule size and CT findings between patients with benign or malignant lesions

	Malignant pulmonary nodules	Benign pulmonary nodules	P
Gender			0.180
Men	12	9	
Women	6	11	
Lobulation			0.856
Yes	5	4	
None	13	16	
Edge			0.083[a]
Clear	13	19	
Ambiguous	5	1	
Pleural indentation			0.944
Yes	7	8	
None	11	12	
Air cavity density			0.004*
Yes	13	5	
None	5	15	
Calcification			0.107[a]
Yes	0	4	
None	18	16	
Pleural indentation			0.454[a]
Yes	3	6	
None	15	14	
Satellite lesions			0.048*,[a]
Yes	0	5	
None	18	15	
Vascular aggregation			0.804
Yes	7	7	
None	11	13	

*, P<0.05; [a], Fisher's exact test. CT, computed tomography.

(presence, 1; absence, 0) and vascular aggregation (presence, 1; absence, 0).

Results

Analysis of relevant risk factors

There were 379 cases of plain scans, with the age of 59.77±11.09 years for the group of malignant lesion and 51.17±13.79 years for the group of benign lesion. According to the results of *t*-test, there was statistically significant difference in the age between the two groups (*Table 1*).

Pulmonary nodules with various sizes

The size of nodules had a significant effect on diagnosis. Changes of risk factors could be monitored during the course of size changes from small to large lesions, in order to better understand the significance of various signs for the diagnosis and differential diagnosis of pulmonary nodules.

The group of ≤10 mm pulmonary nodules

There were 38 cases in the group of ≤10 mm pulmonary nodules. According to chi-square test, there were statistically significant difference in air cavity density and satellite lesions between benign and malignant pulmonary nodules, but no statistically significant differences were observed for the gender, size of nodules, lobulation, glitches, edge, calcification, pleural indentation and vascular aggregation (*Table 2*).

The variables with statistical significance in Tables including air cavity density, satellite lesions, and age were input into the regression model, yielding the results of regression analysis (*Table 3*).

The regression equation was defined as logit P=–0.875+ 2.342× (air cavity density)–21.699× satellite lesions. Hosmer-Lemeshow fitted index test showed χ^2 <0.001, ν =1, P=1.000, suggesting the statistical significance of model fitting. As demonstrated by the results, the hazard of malignant lesion is as higher as 10.4-fold for the presence of air cavity density in comparison to its absence. According to the prediction results of 38 cases with benign and malignant pulmonary nodules, the sensitivity, specificity and accuracy were determined to be 72.2%, 85.0% and 78.9% respectively.

The group of 11-20 mm pulmonary nodules with plain scans

There were 178 cases with 11-20 mm pulmonary nodules

Table 3 Results of the multivariate logistic regression analysis in the group of ≤10 mm pulmonary nodules with plain scans

	Partial regression coefficient	P	Odds ratio	95.0% confidence interval
Satellite lesions	–21.699	0.999	<0.001	–
Air cavity density	2.342	0.005	10.400	(2.03,53.20)
Constant	–0.875	0.100	0.417	–

Table 4 Comparison of the gender, nodule size and CT findings between patients with benign or malignant lesions

	Malignant pulmonary nodules	Benign pulmonary nodules	P
Gender			0.193
Men	61	30	
Women	66	21	
Lobulation			0.014*
Yes	98	30	
None	29	21	
Rim			0.410[a]
Clear	123	48	
Ambiguous	4	3	
Spiculated			<0.001
Yes	106	23	
None	21	28	
Air cavity density			0.031*
Yes	65	17	
None	62	34	
Calcification			0.410[a]
Yes	4	3	
None	123	48	
Pleural indentation			0.101
Yes	72	22	
None	55	29	
Satellite lesions			0.008*,[a]
Yes	1	5	
None	126	46	
Vascular aggregation			<0.001
Yes	83	11	
None	44	40	

*, P<0.05; [a], Fisher's exact test. CT, computed tomography.

in this group. According to chi-square test, there were statistically significant difference in lobulation, glitches, air cavity density, calcification, satellite lesions and vascular aggregation between benign and malignant pulmonary nodules, but no statistically significant differences were observed for gender, edge, calcification or not and pleural indentation (*Table 4*). The variables of statistically significant difference from chi-square test of *Table 4* and age were input into the regression model to afford the results of regression analysis (*Table 5*).

The regression equation was defined as logit P=–4.233+ 0.066× age +1.380× glitches–2.301× (satellite lesions) + 1.656× (vascular aggregation). Hosmer-Lemeshow fitted index test showed χ^2 =12.316, ν =8, P=0.138, suggesting the statistical significance of model fitting. As demonstrated by the results, the hazard of malignant lesion was increased by 6.8% in proportion to the increase of one year, and as higher as 3.97-fold for the presence of glitches in comparison to its absence; and as higher as 5.24-fold for the presence of vascular aggregation in comparison to its absence. However, the presence of satellite lesions seemed to be protective from malignant pulmonary nodules. According to the differential diagnosis prediction between benign and malignant pulmonary nodules, the sensitivity, specificity and accuracy were determined to be 91.3%, 56.9% and 81.5%.

The group of >20 mm pulmonary nodules

There were 163 cases with >20 mm pulmonary nodules in this group. According to chi-square test, there were no statistically significant difference in gender and edge sharpness, but statistically significant differences were observed for lobulation, glitches, pleural indentation, air cavity density, calcification, satellite lesions and vascular aggregation (*Table 6*). The variables of statistically significant difference from chi-square test of *Table 6* and age were input into the regression model to afford the results of regression analysis (*Table 7*).

The regression equation was defined as logit P=–3.646+ 0.042× age +1.532× glitches +2.134× (vascular aggregation).

Table 5 Results of the multivariate logistic regression analysis in the group of 11-20 mm pulmonary nodules with plain scans (n=178)

	Partial regression coefficient	P	Odds ratio	95.0% confidence interval
Age	0.066	<0.001	1.068	(1.031,1.107)
Spiculated	1.380	0.001	3.974	(1.735,9.104)
Satellite lesions	–2.301	0.063	0.100	(0.009,1.137)
Vascular aggregation	1.656	<0.001	5.240	(2.207,12.442)
Constant	–4.233	<0.001	0.015	–

Table 6 Comparison of the gender and CT findings between patients with benign or malignant lesions

	Malignant pulmonary nodules	Benign pulmonary nodules	P
Gender			0.665
Men	68	31	
Women	46	18	
Lobulation			<0.001
Yes	106	31	
None	8	18	
Rim			1.000[a]
Clear	111	48	
Ambiguous	3	1	
Spiculated			<0.001
Yes	95	25	
None	19	24	
Air cavity density			0.027*
Yes	61	17	
None	53	32	
Calcification			0.007*
Yes	6	10	
None	108	39	
Pleural indentation			<0.001
Yes	80	18	
None	34	31	
Satellite lesions			0.029*,[a]
Yes	1	4	
None	113	45	
Vascular aggregation			<0.001
Yes	77	8	
None	36	41	

*, P<0.05; [a], Fisher's exact test.

Hosmer-Lemeshow fitted index test showed χ^2 =8.216, ν =8, P=0.413, suggesting the statistical significance of model fitting. As demonstrated by the results, the hazard of malignant lesion was increased by 4.3% in proportion to the increase of one year, and as higher as 4.63-fold for the presence of lobulation in comparison to its absence; and as higher as 8.45-fold for the presence of vascular aggregation in comparison to its absence. According to the differential diagnosis prediction between benign and malignant pulmonary nodules in these 163 patients, the sensitivity, specificity and accuracy were determined to be 88.6%, 57.1% and 79.1%.

Discussion

CT method is the most effective method for current diagnosis of pulmonary nodule (16), and the general data and CT signs were analyzed in this study. There were no statistically significant difference in gender and edge sharpness, but statistically significant differences were observed for lobulation, glitches, air cavity density, pleural indentation, calcification, satellite lesions, vascular aggregation and extent of enhancement.

In this study, a close correlation was demonstrated between the incidence of lung cancer and subject age. In the diagnosis model for the group of patients with CT more than 10 mm, the variable of age is a dependent risk factor for malignant pulmonary nodules (17). The incidence of was approximate 2.5-fold in 2009 in comparison to in 1985 for lung cancer in the District Jinsan, Shanghai, representing a continuous tend of higher incidence of this disease (18). However, with age disposition excluded, the growth of normalized incidence of lung caner was insignificant, suggesting the age-dependent risk of malignant lesions.

Based on investigation of 1,000 pulmonary nodules, it was found that, for <10 mm pulmonary nodules, benign lesions represent 67.5%; for 10-20 mm pulmonary nodules, benign and malignant nodules share the equal probability; for

Table 7 Results of the multivariate logistic regression analysis in the group of >20 mm pulmonary nodules with plain scans (n=163)

	Partial regression coefficient	P	Odds ratio	95.0% confidence interval
Age	0.042	0.010	1.043	(1.010,1.077)
Lobulation	1.532	0.004	4.627	(1.623,13.194)
Vascular aggregation	2.134	<0.001	8.445	(3.418,20.862)
Constant	–3.646	<0.001	0.026	–

>20 mm pulmonary nodules, malignant nodules represent 85% (19). In this study, similar findings were observed: for <10 mm pulmonary nodules, malignant nodules represent 47.4%, for >10 mm pulmonary nodules, malignant nodules represent 70.1%, suggesting a close correlation between the size versus benign and malignant characteristics. Smaller nodules are associated with benign lesions, while larger nodules tend to be malignant (20). Therefore, in clinical practice, small nodules also have considerable probability of malignant transformation and should be closely monitored or receive active surgical intervention.

Air cavity density was identified to be a risk factor for <10 mm pulmonary nodules; and aging, glitches and vascular aggregation were risk factors for 11-20 mm pulmonary nodules. For the >20 mm pulmonary nodules, the risk factors were aging, lobulation, and vascular aggregation. The presence of satellite lesions seemed to be protective from malignant pulmonary nodules. In the prediction of malignancies, the <10 mm pulmonary nodules had lower sensitivity and higher specificity, whereas the >10 mm pulmonary nodules had higher sensitivity and remarkably lower specificity.

Air cavity density was defined as vacuole sign and inflatable bronchiole sign. For vacuole sign, there were point-like translucent low-density shadows with the size of 1-2 mm in pulmonary nodules. Inflatable bronchiole sign was defined as the direct involvement of bronchiole in nodules or bronchiole shadows in pulmonary nodules, which are frequently detected in adenocarcinoma and are of importance for the diagnosis of small nodules (19,21). Intralesional fibrosis is the pathological basis for vascular aggregation. Nodules are concomitant with aggregation of few small vascular involvement and invasion towards nodules. Vascular surrounding and interruptions are frequently indicative of malignancy. Therefore, vascular aggregation is one of important signs for the diagnosis of malignant SPNs.

Glitch is a risk factor for 11-20 mm pulmonary nodules, while lobulation is a risk factor for >20 mm pulmonary nodules, which suggested glitch might be developed earlier than lobulation and relatively large size of nodules might be observed for lobulation. Glitch formation is a result of direct invasion of tumors into adjacent bronchial vascular sheath or local expansion of the lymphatic nodules or fibrous lines radiating into the surrounding lung fields which were induced by desmoplastic response in nodules (22). Therefore, glitch formation could be detected in the early stages of pulmonary glitch. Lobulation is associated with the size of tumors (23). Round or round-like appearance of lobulation could be observed in case of small tumors, and lobulation genesis might be induced by 1-1.5 cm tumor. In response to the increase of tumor size, lobulation might become continuously obvious and deepened.

Satellite lesions are point-like or line-like high-density shadow scattered around pulmonary nodules, with proliferation, calcification and fibrosis lesions as their major presentations. The probability of benign lesion is increased if there were satellite lesions around the lesion. Although calcification might also occur in case of lung cancer, a >5 cm calcification is more common and the primary calcification of lung cancer is relatively rare (24). For <3 cm pulmonary nodules, calcification findings are more likely to be correlated to benign lesions. Moreover, there was a statistically significant difference in the probability of pleural indentation sign between benign and malignant pulmonary nodules. However, it has not become a risk factor for the diagnosis of malignant pulmonary nodules. As possible reasons, the images of 2-5 mm in thickness were selected for these patients and some patients did not undergo high-resolution CT scanning. Moreover, some benign pulmonary nodules (e.g., tuberculosis mass) might also develop signs of pleural indentation.

Edge sharpness has been identified not to be a risk or protection factor for the diagnosis of pulmonary nodules. The facts of the sharp edges of most pulmonary nodules, ambiguous edges of some pulmonary nodules and unclear edges of malignant pulmonary nodules might be the results of invasive growth of tumors. Moreover, nodules are

frequently associated surrounding inflammatory reactions. For inflammatory nodules, ambiguous changes might occur in the edges of nodules. Therefore, there was no clinical significance of edge sharpness of pulmonary nodules in the differential diagnosis between malignant and benign lesions.

Conclusions

In conclusion, according to CT-based diagnosis of SPNs, the relevant factors of age, size, glitches, lobulation, vascular aggregation, air cavity density, calcification and satellite lesions should be considered; meanwhile, during the course of development from small to large nodules, air cavity density could be firstly detected in early stages, followed by glitches and vascular aggregation. Lobulation is associated with relatively large lesions. These findings deepened the understandings and knowledge of radiological signs of pulmonary nodules in different sizes.

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References

1. Sim YT, Poon FW. Imaging of solitary pulmonary nodule-a clinical review. Quant Imaging Med Surg 2013;3:316-26.
2. Khan AN, Al-Jahdali H. Value of delayed 18F-FDG PET in the diagnosis of solitary pulmonary nodule. J Thorac Dis 2013;5:373-4.
3. Tofts RP, Lee PM, Sung AW. Interventional pulmonology approaches in the diagnosis and treatment of early stage non small cell lung cancer. Transl Lung Cancer Res 2013;2:316-31.
4. Henzler T, Shi J, Jafarov H, et al. Functional CT imaging techniques for the assessment of angiogenesis in lung cancer. Transl Lung Cancer Res 2012;1:78-83.
5. Shao W, Wang W, Yin W, et al. Nonintubated thoracoscopic lobectomy plus lymph node dissection following segmentectomy for central type pulmonary masses. Chin J Cancer Res 2013;25:124-7.
6. Cao C, Manganas C, Ang SC, et al. A meta-analysis of unmatched and matched patients comparing video-assisted thoracoscopic lobectomy and conventional open lobectomy. Ann Cardiothorac Surg 2012;1:16-23.
7. Zhan P, Qian Q, Wan B, et al. Prognostic value of TTF-1 expression in patients with non-small cell lung cancer: a meta-analysis. Transl Cancer Res 2013;2:25-32.
8. Bar J, Urban D, Borshtein R, et al. EGFR mutation in lung cancer: tumor heterogeneity and the impact of chemotherapy. Chin Clin Oncol 2013;2:2.
9. Shimizu K, Okita R, Nakata M. Clinical significance of the tumor microenvironment in non-small cell lung cancer. Ann Transl Med 2013;1:20.
10. Albert RH, Russell JJ. Evaluation of the solitary pulmonary nodule. Am Fam Physician 2009;80:827-31.
11. Swensen SJ, Jett JR, Hartman TE, et al. CT screening for lung cancer: five-year prospective experience. Radiology 2005;235:259-65.
12. Hashimoto M, Miyauchi T, Heianna J, et al. Accurate diagnosis of peripheral small cell lung cancer with computed tomography. Tohoku J Exp Med 2009;217:217-21.
13. Ost DE, Gould MK. Decision making in patients with pulmonary nodules. Am J Respir Crit Care Med 2012;185:363-72.
14. Li Y, Wang J. A mathematical model for predicting malignancy of solitary pulmonary nodules. World J Surg 2012;36:830-5.
15. Gould MK, Ananth L, Barnett PG, et al. A clinical model to estimate the pretest probability of lung cancer in patients with solitary pulmonary nodules. Chest 2007;131:383-8.
16. Zerhouni EA, Stitik FP, Siegelman SS, et al. CT of the pulmonary nodule: a cooperative study. Radiology 1986;160:319-27.
17. Jemal A, Siegel R, Xu J, et al. Cancer statistics, 2010. CA Cancer J Clin 2010;60:277-300.
18. Chen W, Zheng R, Zhang S, et al. Report of cancer incidence and mortality in China, 2010. Ann Transl Med 2014. [Epub ahead of print].
19. Ost D, Fein AM, Feinsilver SH. Clinical practice. The solitary pulmonary nodule. N Engl J Med 2003;348:2535-42.
20. Stoller JK, Ahmad M, Rice TW. Solitary pulmonary nodule. Cleve Clin J Med 1988;55:68-74.
21. Libby DM, Smith JP, Altorki NK, et al. Managing the small pulmonary nodule discovered by CT. Chest

2004;125:1522-9.
22. Aberle DR, Brown K. Lung cancer screening with CT. Clin Chest Med 2008;29:1-14, v.
23. Lee JW, Goo JM, Lee HJ, et al. The potential contribution of a computer-aided detection system for lung nodule detection in multidetector row computed tomography. Invest Radiol 2004;39:649-55.
24. Mahoney MC, Shipley RT, Corcoran HL, et al. CT demonstration of calcification in carcinoma of the lung. AJR Am J Roentgenol 1990;154:255-8.

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Original Article

PET/CT evaluation of response to chemotherapy in non-small cell lung cancer: PET response criteria in solid tumors (PERCIST) versus response evaluation criteria in solid tumors (RECIST)

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Background: ^{18}F-FDG PET/CT is increasingly used in evaluation of treatment response for patients with non-small cell lung cancer (NSCLC). There is a need for an accurate criterion to evaluate the effect and predict the prognosis. The aim of this study is to evaluate therapeutic response in NSCLC with comparing PET response criteria in solid tumors (PERCIST) to response evaluation criteria in solid tumors (RECIST) criteria on PET/CT.

Methods: Forty-four NSCLC patients who received chemotherapy but no surgery were studied. Chemotherapeutic responses were evaluated using ^{18}F-FDG PET and CT according to the RECIST and PERCIST methodologies. PET/CT scans were obtained before chemotherapy and after 2 or 4-6 cycles' chemotherapy. The percentage changes of tumor longest diameters and standardized uptake value (SUV) (corrected for lean body mass, SUL) before and after treatment were compared using paired *t*-test. The response was categorized into 4 levels according to RECIST and PERCIST: CR (CMR) =1, PR (PMR) =2, SD (SMD) =3, PD (PMD) =4. Pearson chi-square test was used to compare the proportion of four levels in RECIST and PERCIST. Finally the relationship between progression-free survival (PFS) and clinicopathologic parameters (such as TNM staging, percentage changes in diameters and SUL, RECIST and PERCIST results etc.) were evaluated using univariate and multivariate Cox proportional hazards regression method.

Results: The difference of percentage changes between diameters and SUL was not significant using paired *t*-test (t=–1.69, P=0.098). However the difference was statistically significant in the 40 cases without increasing SUL (t=–3.31, P=0.002). The difference of evaluation results between RECIST and PERCIST was not significant by chi-square test (χ^2=5.008, P=0.171). If RECIST evaluation excluded the new lesions which could not be found or identified on CT images the difference between RECIST and PERCIST was significant (χ^2=11.759, P=0.007). Reduction rate of SUL_{peak} (%), RECIST and PERCIST results were significant factors in univariate Cox analysis. But Multivariate Cox proportional hazards regression analysis demonstrated that only PERCIST was a significant factor for predicting DFS [hazard ratio (HR), 3.20; 95% (CI), 1.85-5.54; P<0.001].

Conclusions: PERCIST and RECIST criteria have good consistency and PERCIST (or PET) is more sensitive in detecting complete remission (CR) and progression. PERCIST might be the significant predictor of outcomes. The combination of PERCIST and RECIST would provide clinicians more accurate information of therapeutic response in earlier stage of treatment.

Keywords: Non-small cell lung cancer (NSCLC); treatment response; response evaluation criteria in solid tumors (RECIST); PET response criteria in solid tumors (PERCIST); ^{18}F-FDG PET/CT

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Introduction

Morphological analysis based on CT is primary method in evaluation of treatment response for non-small cell lung cancer (NSCLC) and other solid tumors. Response evaluation criteria in solid tumors (RECIST) is the "gold" criteria in CT evaluation which was established in 2000 and revised in 2009 (RECIST 1.1) (1).With the popularity of PET/CT many researchers have studied the changes of standardized uptake value (SUV) before and after treatment, but there are no uniform criteria for evaluation of treatment response. In 2009 Wahl *et al.* proposed the PET response criteria in solid tumors (PERCIST) as a new method in which the treatment response was evaluated by metabolic changes (2). The present study was designed to evaluate the therapeutic response of forty-four NSCLC patients according to PERCIST protocol and to compare with the RECIST 1.1 criteria. Further to access PERCIST criteria and discuss the advantage of it relative to RECIST.

Methods

Patients information

With the approval of Ethics Review Board in our hospital the records of NSCLC patients on PACS (Picture Archiving and Communication Systems) were retrospectively reviewed who underwent ^{18}F-FDG PET/CT examination twice or more without operation between Jan 2010 and Jun 2013. The patients were histologically confirmed NSCLC and received chemotherapy which consisted of cisplatin and another drug (such as pemetrexed and so on). After the chemotherapy targeted drugs might be used. The first time of PET/CT examination was before the start of treatment and the second was in 15-30 days after 2 or 4-6 cycles' chemotherapy. The images met the criterion of RECIST and PERCIST and at least one target lesion could be confirmed. The SUL_{peak} (SUV normalized to body weight and lean body mass) of target lesions at baseline (pretreatment) must not less than (1.5× mean liver SUL + 2SDs of mean SUL). According to the above requirements 44 patients were collected (33 men and 11 women; median age, 67 years; range, 41-83 years; mean weight, 66.3±12.9 kg, range, 43-98 kg).

PET/CT protocol

PET/CT examination was performed with an integrated scanner (Siemens biograph 16). ^{18}F-fluorodeoxyglucose (^{18}F-FDG) was produced by CTI RDS III cyclotron (GE) and the radiochemical purity was more than 95%. Each patient had to fast for 6 hours at least and the blood glucose level must be less than 200 mg/dL before intravenous injection of ^{18}F-FDG at the dose of 3.7-5.5 MBq/kg body weight and been suggested to drink about 1,000 mL water after injection. PET/CT scan was begun about 60 min after injection and the range was from the skull base to the middle of the femur. CT acquisition parameters were as follows: 120 kV and 200 reference mAs; dynamic dose control mode (Caredose 4D); 1.5-mm detector collimation and 5.0-mm slice thickness. PET parameters: 3D emission scan, 1.5-2 min per bed position; 6-7 beds, ordered-subset expectation maximization (OSEM) reconstruction. CT scan data was used for attenuation correction of PET image. Breath-holding CT images including lung lesions were obtained after PET/CT program and thin-section images were reconstructed.

Target lesions and measurement

The target lesions of patients on PET/CT images were determined by two experienced radiologists. Only one target lesion was chosen in the present study because there is one target lesion in PERCIST protocol, and this might be more comparable for PERCIST and RECIST. The target lesion size (length × width) was measured on breath-holding CT mediastinal window images and recorded as CT baseline data. The peak SUL of hottest single tumor lesion with maximal 1.2-cm diameter volume ROI (SUL_{peak}) was required to measure in PERCIST. The software on Siemens PET/CT wizard workstation had limitations in obtaining the peak SUV directly. In this study we used layer by layer accumulated region of interest (ROI) measurement method by reference to the related literatures (3,4). At the center layer of lesion (including the maximal SUV) the ROI with 1.2-cm diameter was made and the mean SUV of three continuous layers (layer thickness was about 4 mm) adjoin to the centre layer were measured. The average of three mean SUVs was approximately taken as the peak SUV (SUV_{peak}) of volumetric ROI. Then the SUV_{peak} was normalized for the lean body mass and generated SUL_{peak} According to the formula as follows (5): SUL = A/(ID/LBM), LBM (male) = 1.10× BW – 120 $(BW/H)^2$, LBM (female) =1.07× BW – 148 $(BW/H)^2$. Where A is the decay-corrected tissue activity concentration (measured in megabecquerels per milliliter), ID is the net injected dose (in megabecquerels), BW is the patient's body weight (in grams), and LBM is the patient's

Table 1 The diameters and SUL of target lesions before and after treatment (cm, $\bar{x}$±s)

n	RECIST			PERCIST		
	D1	D2	ΔD%	SUL1	SUL2	ΔSUL%
44	3.58±1.71	2.40±1.51	(31±29)%	11.3±5.65	6.36±4.44	(40±40)%
40	3.50±1.53	2.21±1.25	(35±25)%	11.3±5.77	5.52±3.43	(48%±27)%

Note: D1, Diameters before treatment; D2, Diameters after treatment; ΔD% = (D1 – D2)/D1 ×100%; SUL, standardized uptake value corrected for lean body mass; SUL1, SUL before treatment; SUL2, SUL after treatment; ΔSUL% = (SUL1 – SUL2)/SUL1 ×100%. RECIST, response evaluation criteria in solid tumors; PERCIST, PET response criteria in solid tumors.

lean body mass. The longest diameters and SUL_{peak} of target lesions on the PET/CT images before and after treatment were measured and recorded as D1, D2 and SUL1, SUL2.

Response evaluation methods

Objective therapeutic responses according to RECIST 1.1 are as follows (1): complete remission (CR) is disappearance of target lesion for at least 4 wk; partial remission (PR) is a decline of at least 30% in tumor diameter; stable disease (SD) is neither PR nor progressive disease (PD); and PD is at least a 20% increase in tumor diameter and 5-mm absolute increase was required. Objective therapeutic responses according to PERCIST 1.0 are as follows (2): complete metabolic response (CMR) is complete resolution of ^{18}F-FDG uptake within the measurable target lesion so that it is less than mean liver activity and indistinguishable from surrounding background blood-pool levels with no new ^{18}F-FDG-avid lesions. Partial metabolic response (PMR) is reduction of a minimum of 30% in the target tumor ^{18}F-FDG SUL_{peak}. Stable metabolic disease (SMD) is disease other than CMR, PMR, or progressive metabolic disease (PMD); and PMD is a 30% increase in ^{18}F-FDG SUL_{peak} or advent of new ^{18}F-FDG-avid lesions that are typical of cancer.

Statistical and survival analysis

The percentage changes of longest diameters and SUL_{peak} of target lesions in 44 patients before and after treatment were calculated according to the formula as follows: ΔD%= (D1 – D2)/D1 ×100%, ΔSUL% = (SUL1 – SUL2)/SUL1 ×100%. A paired Student's *t*-test method was used to assess the statistical significance of these two changes and the results could evaluate the sensitivity of CT and PET on the response. Then the response was classed into four levels according to RECIST and PERCIST: CR (CMR) =1, PR (PMR) =2, SD (SMD) =3, PD (PMD) =4. Pearson chi-square test was used to compare the proportion of four levels in RECIST and PERCIST. Because the new lesions noted on PET/CT were used for progress in RECIST 1.1, in order to compare PET/CT and CT in the evaluation of treatment response, the new lesions which could not be found or confirmed on routine CT were eliminated in RECIST and compared with PERCIST once more with chi-square test. A P value of less than 0.05 was considered to be significant.

The relationship between progression-free survival (PFS) and clinicopathologic results (such as TNM stage, percentage changes, RECIST and PERCIST results etc.) were evaluated using univariate Cox proportional hazards regression analysis. Significant parameters identified by univariate analysis were included in a multivariate Cox proportional hazards regression analysis [stepwise selection (Wald) method; P≤0.05 was used for entry into the model, and P>0.1 was selected for removal]. The statistical software was SPSS17.0.

Results

The relation between the changes of diameter and SUL

There were 30 adenocarcinoma and 14 squamous cell carcinoma cases in 44 patients. TNM staging were 10 cases in stage II, 7 cases in stage III and 27 cases in stage IV. Twenty-five patients were reviewed at the end of 2 cycles of chemotherapy and 19 patients at the end of 4-6 cycles of chemotherapy. The longest diameters, SUL and percentage changes of target lesions before and after treatment were shown in *Table 1* and *Figure 1*. The difference of percentage changes between diameter and SUL was not significant using paired *t*-test (*t*=–1.69, P=0.098). However if the 40 cases without increasing SUL after treatment were analyzed there was significant difference in the percentage changes between diameter and SUL (*t*=–3.31, P=0.002).

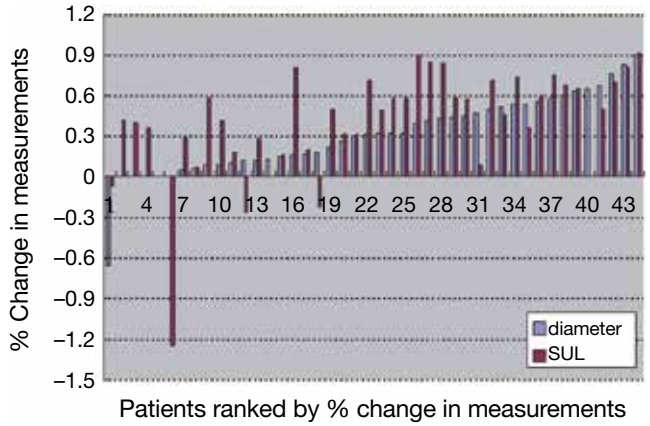

Figure 1 Percentage changes in longest diameters CT measurements by response evaluation criteria in solid tumors (RECIST) 1.1 and SUL by PET response criteria in solid tumors (PERCIST) after therapy in 44 patients.

Table 2 Comparison of treatment response assessments by RECIST (PD with new lesions determined on PET/CT) and PERCIST

RECIST	PERCIST				
	PMD	SMD	PMR	CMR	Total
PD	6	0	0	0	6
SD	1	6	7	1	15
PR	0	2	15	4	21
CR	0	0	0	2	2
Total	7	8	22	7	44

Note: CR, complete remission; PR, partial remission; SD, stable disease; PD, progressive disease; CMR, complete metabolic response; PMR, partial metabolic response; SMD, stable metabolic disease; PMD, progressive metabolic disease; RECIST, response evaluation criteria in solid tumors; PERCIST, PET response criteria in solid tumors.

The response evaluation of RECIST and PERCIST

The response classification for 44 patients according to RECIST and PERCIST criteria was as follows: CR/CMR, 2/7; PR/PMR, 21/22; SD/SMD, 15/8; PD/PMD, 6/7; and 15 patients were not consistent. The difference between RECIST and PERCIST was not significant by chi-square test (Pearson χ^2=5.008, P=0.171). If the new lesions which could not be found or identified on CT images were revaluated in RECIST, the evaluation results were CR/CMR, 2/7; PR/PMR, 22/22; SD/SMD, 19/8; PD/PMD, 1/7.

Table 3 Comparison of treatment response assessments by RECIST (PD without new lesions determined on PET/CT) and PERCIST

RECIST	PERCIST				
	PMD	SMD	PMR	CMR	Total
PD	1	0	0	0	1
SD	5	6	7	1	19
PR	1	2	15	4	22
CR	0	0	0	2	2
Total	7	8	22	7	44

Note: CR, complete remission; PR, partial remission; SD, stable disease; PD, progressive disease; CMR, complete metabolic response; PMR, partial metabolic response; SMD, stable metabolic disease; PMD, progressive metabolic disease; RECIST, response evaluation criteria in solid tumors; PERCIST, PET response criteria in solid tumors.

The grading of 20 patients were not consistent and the difference between RECIST and PERCIST was significant by chi-square test (Pearson χ^2=11.759, P=0.007). The details of evaluation results were summarized in *Tables 2* and *3*.

Survival analysis and prognosis

The PFS of 44 patients was 2-49 months and the average was 14.8 months. Associations between PFS and clinicopathologic results, changes of imaging parameters and chemotherapeutic responses [such as TNM stage, reduction rate of tumor diameter (%), chemotherapy cycles (2 or 4-6), reduction rate of SUL_{peak} (%), RECIST (CR/PR/SD/PD) and PERCIST (CMR/PMR/SMD/PMD)] were assessed using univariate and multivariate Cox proportional hazards regression analysis (*Table 4*). Reduction rate of SUL_{peak} (%), RECIST and PERCIST were significant factors in univariate Cox analysis. But Multivariate Cox proportional hazards regression analysis demonstrated that only PERCIST was a significant factor for predicting DFS [hazard ratio (HR), 3.20; 95% CI: 1.85-5.54; P<0.001]. The survival curve of RECIST and PERCIST produced by SPSS was shown in *Figures 2* and *3*.

Discussion

RECIST criteria is widely applied to evaluate the treatment response for solid tumors, but is known to have limitations because it depends on the morphologic changes (2,6). Now with increasing use of the targeted therapy, such as

Table 4 Univariate cox proportional hazards regression analysis for prediction of DFS

Univariate analysis	DFS			
	n	HR	95% CI	P
TNM (II, III, IV)	10/7/27	1.23	0.84-180	0.280
Reduction rate of tumor diameter (%)	44	0.38	0.10-1.43	0.162
Reduction rate of SUL_{peak} (%)	44	0.22	0.08-0.65	0.006
RECIST (CR/PR/SD/PD)	2/21/15/6	2.55	1.42-4.59	0.002
PERCIST (CMR/PMR/SMD/PMD)	7/22/8/7	3.20	1.85-5.54	<0.001
Chemotherapy cycles: 2 or [4-6]	25/19	0.68	0.32-1.45	0.345

Note: DFS, disease-free survival; HR, hazard ratio; CI, confidence interval; CR, complete remission; PR, partial remission; SD, stable disease; PD, progressive disease; CMR, complete metabolic response; PMR, partial metabolic response; SMD, stable metabolic disease; PMD, progressive metabolic disease; RECIST, response evaluation criteria in solid tumors; PERCIST, PET response criteria in solid tumors.

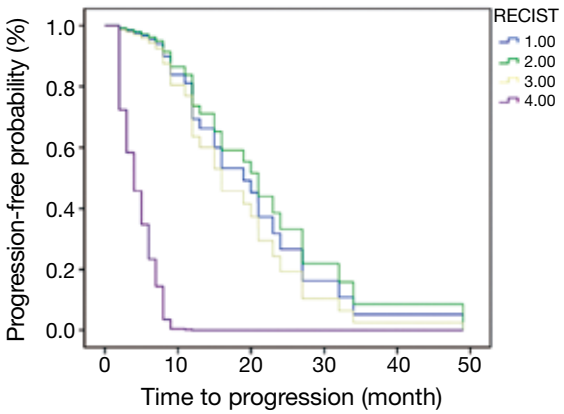

Figure 2 The survival of RECIST evaluation results (CR =1, PR =2, SD =3, PD =4) in 44 patients. RECIST, response evaluation criteria in solid tumors; CR, complete remission; PR, partial remission; SD, stable disease; PD, progressive disease.

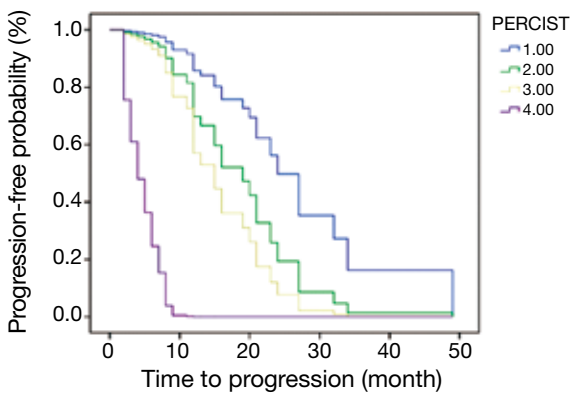

Figure 3 The survival of PERCIST evaluation results (CMR =1, PMR =2, SMD =3, PMD =4) in 44 patients. PERCIST, PET response criteria in solid tumors; CMR, complete metabolic response; PMR, partial metabolic response; SMD, stable metabolic disease; PMD, progressive metabolic disease.

antiangiogenic therapy in clinic, a new evaluation method is necessary to effectively monitor the response of this therapy (6). ^{18}F-FDG PET or PET/CT is considered to overcome such limitations and more suitable for assessment of therapeutic effect because it can better reflect the intrinsic nature of malignant tumor (7). The present study demonstrated that the percentage changes of SUL after treatment for NSCLC monitored by PET were higher than the percentage changes of diameter by CT and the evaluation results by PERCIST were more sensitive and prognostic than the evaluation results by RECIST.

Many studies have confirmed that ^{18}F-FDG PET can monitor the metabolic changes of tumors after treatment when the morphologic changes on CT images can not been detected (8,9). The data of most patients in this study also support this viewpoint in which the reduction percentages of diameter were significantly lower than that of SUL on PET/CT images. But no statistically significant result was obtained in 44 patients' data with paired *t*-test or non parametric test methods (Wilcoxon signed-rank test). The selection bias should be responsible for this inconformity because of the negative data of progression patients. If the increasing SUL cases and decreasing SUL cases were respectively analyzed, the percentage reduction of SUL in 40 patients was significantly higher than that of diameter. To our knowledge there is no research that proposed the similar problem although a considerable proportion of assessment result was progression in clinic. The reason may be that the similar comparison of percentage changes was not studied by the other research. However the classification according to RECIST or PERCIST or others is established by the researchers rather than tumors itself and it may be a problem when the reduction of 29% is compared with the

reduction of 31%. Further research should pay attention to the details of information in the response assessment.

In the present study the evaluation results were not significantly different between PERCIST and RECIST 1.1 (with new lesions determined on PET/CT), but showed significant difference between PERCIST and RECIST 1.0 (without new lesions determined on PET/CT). This result revealed that PERCIST and RECIST 1.1 had good consistency and PERCIST (or PET) was more sensitive in detection the CR and progression patients. In the study of Van Ruychevelt *et al*. 59 NSCLC patients were evaluated by RECIST and EORTC criteria, and the results showed that PET was more sensitive than CT in early detecting the patients of PD (10). In the research of Yanagawa *et al*. Fifty-one patients with locally advanced esophageal cancer who received neoadjuvant chemotherapy were studied. Chemotherapeutic lesion responses were evaluated using ^{18}F-FDG PET and CT according to the RECIST and PERCIST methods. There was significant difference between the PERCIST and RECIST evaluation results and the number of CR cases in PERCIST was much more than which in RECIST (4). All these studies indicated that PERCIST is superior to RECIST in the detection of CR and progression. One possible reason is that the metabolic changes after treatment is more sensitive than morphologic changes in the nature of tumors and PET can just monitor the metabolic changes. The other reason may be that the intrinsic properties of this two criteria because the achievement to CR in RECIST is more difficult. In the ordinary PET/CT work we found that some NSCLC lesions didn't further shrank or disappear when they reduced to a certain degree but no uptake of ^{18}F-FDG. The residual lesions may be the fiber texture or scar tissue and can be confirmed by surgery.

The relationship between the metabolic changes of tumors and prognosis was discussed in many studies. The prognostic value of parameters about SUV and the evaluation results was not in agreement (8-10). In the study of van Ruychevelt *et al*. only a significant reduced survival was observed in progressive patients and no differences among the else (10). The other study about Esophageal Cancer concluded that PERCIST 1.0 (CMR *vs.* non-CMR) was the most significant prognostic factor for predicting DFS and OS in the multivariate Cox proportional hazards regression analysis (4). The results of another study showed that an early metabolic response did not translate into better survival outcome (8). The present study draw a conclusion that only PERCIST evaluation result is a significant prognostic factor and the survival curve suggested that the progressive patients had significantly shorter PFS. The conclusions in the above studies have limitations because of the small number of cases and different classification results. In the further study, the factors affecting the survival and evaluation results [including age, TNM stage, pathological type, subsequent treatment, the basal SUV, Total lesion glycolysis (11) and review time *et al*.] should be taken into account as far as possible in the multivariate Cox proportional hazards regression analysis, thus the conclusion may be more credible.

Limitations in this study included the retrospective nature of patient data collection, the number of target lesion and the different cycles' interval of PET/CT review. In PERCIST only one target lesion was required to evaluate but in RECIST 1.1 no more than five target lesions were included. In order to precisely compare PERCIST with RECIST we evaluated the longest diameter of just one target lesion that was assessed in PERCIST. The study of Darkeh MH *et al*. suggested that measuring fewer than four target lesions might cause discrepancies when more than five target lesions are present in RECIST 1.0 (12). So the evaluation results according to RECIST criteria in the present study might not be accurate. Here a new problem will be proposed that how many target lesions should be chosen when the research aim to compare the PERCIST with RECIST. Another limitation is the time of PET/CT review was not consistent in the present study. Although the cycles' interval (2 or 4-6 cycles) was not significant factor in the univariate Cox proportional hazards regression analyses and the present study is paired analysis, the difference of sensitivity between PERCIST and RECIST will reduce as the time go on. The further research had better separate the different treatment time of patients and analyze respectively.

Conclusions

In conclusion, RECIST criteria are still a "gold standard" in the response evaluation of the solid tumor. The present study indicates that PERCIST and RECIST 1.1 have good consistency and PERCIST (or PET) is more sensitive in detection the CR and progression patients. Combining the PERCIST and RECIST the clinician will acquire more response information relatively early. However because of the small number of patients the selection bias could not be avoided. More researchers are expected to join the study of PERCIST and make it serve for tumor patients better.

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Disclosure: The authors declare no conflict of interest.

References

1. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 2009;45:228-47.
2. Wahl RL, Jacene H, Kasamon Y, et al. From RECIST to PERCIST: Evolving Considerations for PET response criteria in solid tumors. J Nucl Med 2009;50 Suppl 1:122S-50S.
3. Dibble EH, Alvarez AC, Truong MT, et al. 18F-FDG metabolic tumor volume and total glycolytic activity of oral cavity and oropharyngeal squamous cell cancer: adding value to clinical staging. J Nucl Med 2012;53:709-15.
4. Yanagawa M, Tatsumi M, Miyata H, et al. Evaluation of response to neoadjuvant chemotherapy for esophageal cancer: PET response criteria in solid tumors versus response evaluation criteria in solid tumors. J Nucl Med 2012;53:872-80.
5. Abikhzer G, Alabed YZ, Azoulay L, et al. Altered hepatic metabolic activity in patients with hepatic steatosis on FDG PET/CT. AJR Am J Roentgenol 2011;196:176-80.
6. de Langen AJ, van den Boogaart V, Lubberink M, et al. Monitoring response to antiangiogenic therapy in non-small cell lung cancer using imaging markers derived from PET and dynamic contrast-enhanced MRI. J Nucl Med 2011;52:48-55.
7. Juweid ME, Cheson BD. Positron-emission tomography and assessment of cancer therapy. N Engl J Med 2006;354:496-507.
8. Lee DH, Kim SK, Lee HY, et al. Early prediction of response to first-line therapy using integrated 18F-FDG PET/CT for patients with advanced/metastatic non-small cell lung cancer. J Thorac Oncol 2009;4:816-21.
9. Skoura E, Datseris IE, Platis I, et al. Role of positron emission tomography in the early prediction of response to chemotherapy in patients with non--small-cell lung cancer. Clin Lung Cancer 2012;13:181-7.
10. van Ruychevelt V, Garcia C, Meert AP, et al. Positron emission tomography with 18F-FDG and cancer response to chemotherapy. Rev Mal Respir 2011;28:618-25.
11. Chen HH, Chiu NT, Su WC, et al. Prognostic value of whole-body total lesion glycolysis at pretreatment FDG PET/CT in non-small cell lung cancer. Radiology 2012;264:559-66.
12. Darkeh MH, Suzuki C, Torkzad MR. The minimum number of target lesions that need to be measured to be representative of the total number of target lesions (according to RECIST). Br J Radiol 2009;82:681-6.

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Original Article

Relation between inflammatory cytokine levels in serum and bronchoalveolar lavage fluid and gene polymorphism in young adult patients with bronchiectasis

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Aim: Bronchiectasis develops as a result of genetic and environmental factors and its etiopathogenesis is not still clear. Recent studies have revealed that inflammatory cytokines, which are formed as a result of chronic infection and inflammation, play a role in the pathogenesis of bronchiectasis. For this purpose, the level of inflammatory cytokines in bronchiectasis and the presence or absence of a genetic predisposition with the gene polymorphism of these cytokines was investigated.

Material and methods: A total of 60 patients, 40 study cases and 20 controls, which were monitored with the diagnosis of bronchiectasis were included in the study. In these individuals, cytokine levels [interleukin (IL)-6, IL-8, IL-10, and tumor necrosis factor (TNF)-α] in serum and bronchoalveolar lavage (BAL) fluid, along with the routine blood tests, were determined. Furthermore, the polymorphism in IL-6, IL-8, IL-10, and TNF-α cytokine genes and its frequency were studied in the obtained DNA by the automatic sequence analysis method and the results were compared.

Findings: It was found that in serum and BAL fluid of the patient group, the IL-8 level was high, whereas the IL-10 level was low ($P<0.05$). No significant difference was detected in the other cytokines ($P>0.05$). It was found that in cytokine gene polymorphisms IL-8 -251 A/T, IL-10 -592 A/C, and IL-10 -819 T/C genotypes are associated with increased risk of bronchiectasis. It was detected that the IL-8 -251 A/T genotype increased the risk of having the disease by 4.19 fold. (OR =4.19, 95% CI =1.24-14.17, P=0.021). The IL-10 -592 C/A genotype increased the risk of having the disease by 5.71 fold (OR = 5.71, 95% CI = 1.35-24.06, P=0.017) and the IL-10 -819 T/C genotype increased the risk of having the disease by 5.06 fold (OR =5.06, 95% CI =1.20-21.27, P=0.048). No significant correlation was found between the other polymorphisms and bronchiectasis.

Conclusions: The IL-8, IL-10 levels and the gene polymorphism of these cytokines differ. In addition to detecting higher levels of pro-inflammatory IL-8 and lower levels of anti-inflammatory IL-10, detection of gene polymorphism related to these two cytokines in bronchiectasis gives rise to the thought that cytokines may have role in a predisposition to bronchiectasis. However, as the number of patients is small, precise remarks could not be made on this subject. There is need for further studies include a larger number of patients.

Keywords: Bronchiectasis; inflammation; cytokine; DNA sequence analysis; gene polymorphism

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Introduction

While bronchiectasis was a disease of all ages before the use of antibiotics, today it is a disease that is most commonly seen in childhood. As a result of obstruction of the bronchi due to any hereditary or acquired cause, colonized microorganisms in that region and inflammation cause permanent destruction in the walls of the bronchi and as a result, bronchiectasis develops (1-3).

In studies that were performed to research cytokine gene polymorphism in chronic inflammatory diseases, it was seen that there is a correlation between cytokine levels and various cytokine gene variants (4). As the presence of cytokine gene polymorphism may affect the cytokine levels, we believe that such a polymorphism may be present in bronchiectasis in which there is chronic inflammation.

This study was designed to determine the levels of pro-inflammatory and anti-inflammatory cytokines, which are the inflammatory markers, in serum and bronchoalveolar lavage (BAL) fluid of patients with bronchiectasis and to investigate the presence or absence gene polymorphism of these cytokines or not.

Material and methods

This study was conducted in the GATA Haydarpasa Training Hospital, Chest Disease Service between June 2011 and June 2012 in a prospective and controlled manner. Approval was obtained from the hospital ethics committee.

Study population

Young adult patients who were admitted to the Chest Disease Service with the diagnosis of bronchiectasis during the study period constituted the study population. The individuals that did not meet the study criteria (the patients who had previously had tuberculosis, patients diagnosed with cystic fibrosis, the patients diagnosed with immune deficiency, patients and the controls below the age of 20 years, individuals that could not tolerate the bronchoscopy procedure, those that had contraindications for the procedure, and patients that the diagnosis of bronchiectasis could not be made), as well as the individuals that did not sign the written consent were not included the study. All patients with bronchiectasis were included in the study after at least two months of follow-up.

Sixty cases with bronchiectasis were included the study; however eight cases were excluded from the study because these cases were accepted as pseudobronchiectasis, as they possessed the criteria for lung infection and their bronchiectasis recovered on high resolution computed tomography during control. Six cases were excluded from the study as they did not accept the bronchoscopy procedure. Two cases were excluded from the study as they did not sign the informed consent form. Four cases were excluded from the study as the test results could not be found in the automatization system. A total of 40 patients with stable disease, who were found to be eligible for the study and who provided written informed consent, were included in the study.

Twenty individuals with the same age and gender in the study group, who had an indication for a bronchoscopy procedure (the cases in which the etiology of hemoptysis, dyspnea, and chronic cough were selected) and in which no diseases were detected in the laboratory examinations and from whom written informed consent was obtained, were accepted as the control group.

Obtaining study samples

After informing the patients that had no contraindication for bronchoscopy about the procedure, written informed consent was obtained. Following topical anesthesia, bronchoscopy was performed with a fiberoptic bronchoscopy device through the oral route to the study group of 40 individuals and to the control group of 20 individuals who had bronchoscopy indication. The samples of BAL fluid that were taken from the case and the control groups during the procedure and the serum samples of the cases that were taken during admission were stored at –80 ℃.

Cytokine levels

By using commercially available kits, the cytokine levels [interleukin (IL)-6, IL-8, IL-10, and tumor necrosis factor (TNF)-α] were studied in serum and BAL fluid samples that were taken from the case and the control groups and the levels were determined. (Assaypro trademark, Catalogue Number: EI1006-1, Missouri, United States of America). The ELISA method was used in the measurements of serum and BAL fluid cytokine levels.

DNA extraction and single nucleotide polymorphism

Using a NucleoSpin blood (Mavherey-Nagel) extraction kit, DNA was isolated from the leucocytes in the blood that were obtained for complete blood count during

the admission of patient and the control groups. The Sanger method was used for DNA sequence analysis in investigation of the single nucleotide polymorphisms (SNP) in gene regions that are responsible for cytokine secretion (5). The forward and reverse primers that were used to determine polymorphisms were achieved in commercially available lyophilized forms (Invitrogen Life Technologies, USA). Primers and sequences of IL-6, IL-8, IL-10 and TNF-α genes used in our study are given in *Table 1*. A total of eight polymorphisms, one at the -174 G/C promoter region of IL-6 gene, one at the -251 A/T, the -161 C/A promoter region of the IL-8 gene, one at the -1082 G/A, -819 C/T, the -592 C/A promoter region of the IL-10 gene, one at -308 G/A, and -238 G/A promoter region of the TNF-α gene were detected by SNP genotyping polymerase chain reaction (PCR) and automatic DNA sequence analysis. The genotypes of the IL-6 -174 G/C, IL-8 -251 A/T, IL-10 -592 C/A and TNF-α -308 G/A polymorphism by DNA sequence analysis are demonstrated in *Figure 1*.

Table 1 Primers and sequences of cytokine genes used in the study.

Primers	Sequences
IL-6 Forward primer (FWD)	5' AGCGCTAGCCTCAATGACGAC 3'
IL-6 Reverse primer (REV)	5' GCGGGTGGGGCTGATTGGAA 3'
IL-8 FWD	5' CTTGTTCTAACACCTGCCACTC 3'
IL-8 REV	5' GGCAAACCTGAGTCATCACA 3'
IL-10 FWD	5' ATCCAAGACAACACTACTAA 3'
IL-10 REV	5' TAAATATCCTCAAAGTTCC 3'
TNF-α FWD	5' TTCCTGCATCCTGTCTGGAA 3'
TNF-α REV	5' CAGCGGAAAACTTCCTTGGT 3'

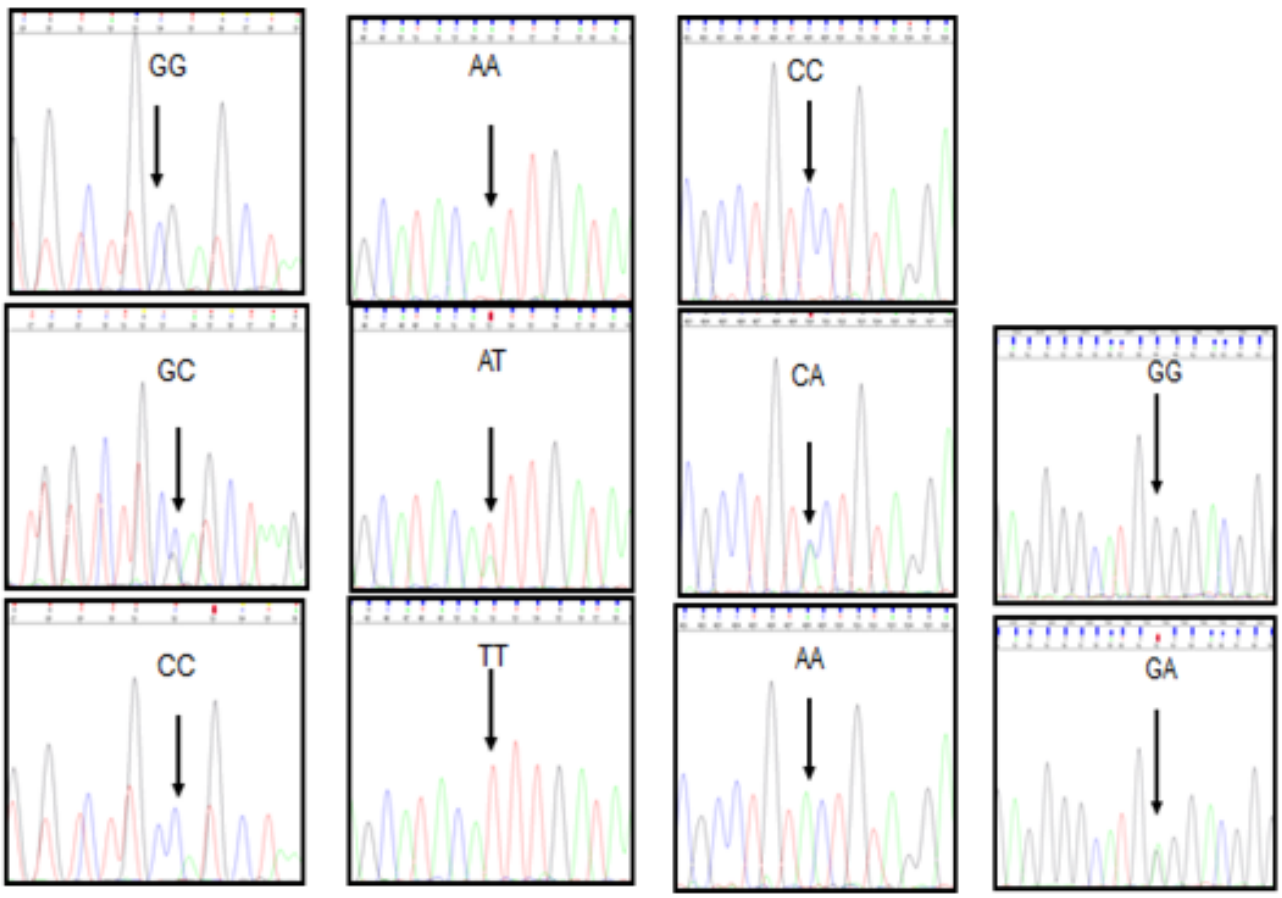

Figure 1 Demonstration of two or three different genotypes of the IL-6 -174 G/C, IL-8 -251 A/T, IL-10 -592 C/A and TNF-α -308 G/A polymorphism by sequence analysis of PCR product using a primer, respectively. PCR, polymerase chain reaction.

Table 2 Demographic characteristics of the study and the control groups

	Number [%]		P value
	Patients (n=40)	Control (n=20)	
Age (years)	23.40±4.32	21.60±1.96	0.082
Education			
Illiterate	2 [5]	2 [10]	0.159
Primary school	15 [37.5]	12 [60]	
High school	14 [35]	6 [30]	
University	9 [22.5]	–	
Income level			
Low	23 [57.5]	9 [45]	0.641
Medium	15 [37.5]	10 [50]	
High	2 [5]	1 [5]	
Smoking			
Smoker	13 [32.5]	2 [10]	0.058
Nonsmoker	27 [67.5]	18 [90]	

Table 3 The serum level of pro-inflammatory cytokines (IL-6, IL-8, and TNF-α) and anti-inflammatory cytokines (IL-10) in the patient and control groups

	Mean ± SD		P
	Bronchiectasis (n=40)	Control (n=20)	
Serum IL-6 (ng/mL)	0.1±0.03	0.1±0.01	0.944
Serum IL-8 (ng/mL)	1.71±1.05	0.5±0.09	<0.001
SerumIL-10 (ng/mL)	0.03±0.02	0.6±0.08	0.014
SerumTNF-α (ng/mL)	0.1±0.01	0.4±0.14	0.120

Table 4 The bronchoalveolar lavage (BAL) fluid level of pro-inflammatory cytokines (IL-6, IL-8, and TNF-α) and anti-inflammatory cytokine (IL-10) in the patient and control groups

	Mean ± SD		P
	Bronchiectasis (n=40)	Control (n=20)	
BAL IL-6 (ng/mL)	0.01±0.003	0.01±0.002	0.164
BAL IL-8 (ng/mL)	0.41±0.04	0.7±0.17	0.001
BAL IL-10 (ng/mL)	0.08±0.07	0.12±0.07	0.045
BAL TNF-α (ng/mL)	0.05±0.17	0.003±0.004	0.186

Statistics

SPSS 13.0 program was used for statistical analysis and the significance was evaluated at the level of P<0.05. During the evaluation of the study data, in addition to the descriptive statistical methods, independent samples *t*-test was used for normal distribution variables and the Mann-Whitney U-test was used for the variables that were not normally distributed. Logistic regression analysis was used for evaluation of gene polymorphism. The chi-square test was used in comparison of genotype and allele frequency ratios and odds ratio (relative risk) with a confidence interval of 95% was calculated to determine risk factors.

Results

This study was conducted on a total of 60 subjects who were comprised of 40 patients with clinically stable bronchiectasis and 20 control subjects. The study was conducted in a military hospital. The males were the dominant gender in the study due to the institutional characteristics of a military hospital. Therefore, all subjects included in the study and control groups were comprised of males. Of the cases, 12 were diagnosed in other centers before admission to the military hospital, and 29 patients were recently diagnosed with bronchiectasis.

The mean age of the study group was 23.04±4.62 and the mean age of the control group was 23.04±2.62. There was no statistical difference between the study and the control groups in terms of age (P=0.82). The demographic characteristics of the study and the control groups are presented in *Table 2*.

The serum and BAL fluid levels of pro-inflammatory cytokines (IL-6, IL-8, and TNF-α) and anti-inflammatory cytokine (IL-10) were determined in the case and control groups.

No statistically significant difference was detected between groups in terms of serum IL-6 and TNF-α levels (P=0.944 and P=0.120). The serum IL-8 level of the study group was higher than the control group and this difference was statistically significant (P=0.001). The serum IL-10 level of the study group was lower than the control group and this difference was statistically significant (P=0.014) (*Table 3*).

There was no statistically significant difference between groups in terms of IL-6 and TNF-α levels in BAL fluid (P=0.164 and P=0.186). The IL-8 level in the BAL fluid in the study group was higher than the control group (P=0.001) and the IL-10 level of the study group was lower (P=0.045) (*Table 4*).

Table 5 Comparison of the genotype and allele frequencies of polymorphisms in the IL-6 -174 G/C promoter region between groups

		Bronchiectasis, n [%]	Control, n [%]	Odds ratio (OR), (% 95 CI)	P
IL-6 -174 G/C polymorphism					
Genotype	C/C	6 [15]	4 [20]	Reference	–
	G/C	12 [30]	9 [45]	2.095 (0.45-9.62)	0.34
	G/G	22 [55]	7 [35]	0.88 (0.19-4.11)	0.88
Allele	C	24 [30]	17 [42.5]	Reference	–
	G	56 [70]	23 [57.5]	1.72 (0.78-3.79)	0.17

Table 6 Comparison of the genotype and allele frequencies of polymorphisms in the IL-8 -161 C/A and -251 A/T promoter region between groups

		Bronchiectasis, n [%]	Control n, [%]	Odds ratio (OR), (% 95 CI)	P
IL-8 -161 A/C Polymorphism					
Genotype	A/A	0	0	–	–
	A/C	14 [35]	9 [45]	Reference	–
	C/C	26 [65]	11 [55]	1.52 (0.51-4.54)	0.453
Allele	C	14 [17.5]	9 [22.5]	Reference	
	A	66 [82.5]	31 [77.5]	1.37 (0.53-3.50)	0.512
IL-8 -251 A/T Polymorphism					
Genotype	T/T	9 [22.5]	11 [55]	Reference	–
	T/A	24 [60]	7 [35]	4.19 (1.24-14.17)	0.021
	A/A	7 [17.5]	2 [10]	4.27 (0.71-25.91)	0.110
Allele	A	38 [47.5]	11 [27.5]	Reference	–
	T	42 [52.5]	29 [72.5]	2.38 (1.05-5.42)	0.038

Allele and genotype analysis of IL-6 -174 G/C, IL-8 -161 C/A, IL-8 -251 A/T, IL-10 -592 C/A, IL-10 -819 C/T, IL-10 -1082 G/A, and TNF-α -308 G/A, TNF-α 238 G/A polymorphism was conducted on 60 samples taken from the case and control groups.

When the allele and genotype frequency of the IL-6 -174 G/C polymorphism was examined, no significant difference was found between the groups in terms of G and C alleles, and CC, GC, and GG, genotype frequencies, and the disease risk (P=0.174, P=0.34, P=0.88, respectively) (*Table 5*).

When the allele and genotype frequency of the IL-8 -161 C/A polymorphism was examined, no significant difference was found between groups in terms of A and C allele frequencies and AC and CC genotypes and disease risk (P=0.512, P=0.453, respectively). When the allele and genotype frequency of IL-8 -251 A/T polymorphism was examined, a significant difference was found between two groups in terms of allele frequencies (P=0,038). The T allele increased the risk by 2.38 fold according to A and this risk was statistically significant (OR =2.38, 95% CI =1.05-5.42, P=0.038). The AA genotype increased the risk by 4.27 fold according to TT (OR =4.27; 95% CI =0.71-25.91, P=0.11), and a significant difference was detected between the two groups in terms of TA and TT genotypes (P=0.021). The TA genotype increased the risk by 4.19 fold according to TT (OR =4.19; 95% CI =1.24-14.17; P=0.021) and this risk was statistically significant (*Table 6*).

In the IL-10 -592 C/A polymorphism, there was no significant difference in the study group in terms of A and C allele frequencies and no significant difference between the two groups in terms of AA and CC genotypes and disease risk (P=0.503, P=0.345, respectively). There was a significant difference between the two groups in terms of AC genotype numbers, and the AC genotype frequency was significantly higher in the study group (P=0.017). The genotype AC increased the risk by 5.71 fold according to CC (OR =5.71, 95% CI =1.35-24.06, P=0.017) and this risk was statistically significant. In the IL-10 -819 T/C polymorphism, no

Table 7 Comparison of the genotype and allele frequencies of polymorphisms in the IL-10 -592 C/A, -819 T/C, and -1082 G/A promoter region between groups

		Bronchiectasis, n [%]	Control, n [%]	Odds ratio (OR), (% 95 CI)	P
IL-10 -592 A/C Polymorphism					
Genotype	C/C	14 [35]	12 [60]	Reference	–
	A/C	20 [50]	3 [15]	5.71 (1.35-24.06)	0.017
	A/A	6 [15]	5 [25]	1.03 (0.25-4.23)	0.345
Allele	A	32 [40]	27 [67.5]	Reference	–
	C	48 [60]	13 [32.5]	0.72 (0.32-1.60)	0.503
IL-10-819 T/C Polymorphism					
Genotype	C/C	15 [37.5]	12 [60]	Reference	–
	T/C	19 [47.5]	3 [15]	5.06 (1.20-21.27)	0.048
	T/T	6 [15]	5 [25]	0.96 (0.23-3.92)	0.345
Allele	C	49 [61.25]	26 [65]	Reference	–
	T	31 [38.75]	14 [35]	1.17 (0.53-2.58)	0.689
IL-10 -1082 G/A Polymorphism					
Genotype	A/A	23 [57.5]	8 [40]	Reference	–
	A/G	14 [35]	12 [60]	2.464 (0.81-7.51)	0.065
	G/G	3 [7.5]	0	–	–
Allele	A	60 [75]	28 [70]	Reference	–
	G	20 [25]	12 [30]	1.28 (0.55-2.99)	0.559

significant difference was detected between the two groups in terms of T and C allele frequencies and TT and CC genotypes and disease risk (P=0.689, P=0.345, respectively). The TC genotype frequency was significantly higher in the study group and this was statistically significant (P=0.048). The TC genotype increased the risk by 5.06 fold according to the CC genotype (OR =5.06; 95% CI =1.20-21.27, P=0.048) and this risk was statistically significant. In the IL-10 -1082 G/A polymorphism, there was no significant difference between the two groups in terms of A and G allele frequencies, and GA and AA genotypes and disease risk (P=0.559, P=0.065, respectively) (*Table 7*).

In the TNF-α -308 G/A polymorphism, no significant difference was detected between the groups in terms of A and G allele frequencies, and GA and GG genotypes and disease risks (P=0.655, P=0.806, respectively). In the TNF-α -238 G/A polymorphism, there was no significant difference between groups in terms of A, G allele frequencies, GA and GG genotypes and disease risk (P=0.374, P=0.509, respectively) (*Table 8*).

Discussion

In this study, the inflammatory cytokine levels, which are markers of inflammation, and the gene polymorphism of these cytokines, were investigated in cases with bronchiectasis. It was observed that the IL-8 and IL-10 levels in serum and BAL were statistically different in bronchiectasis, and gene polymorphism was related to these cytokines.

Olveira *et al*. reported a relationship between IL-6 and TNF-α and respiratory parameters in patients with bronchiectasis. In their study, serum levels of IL-6 were significantly higher in patients with bronchiectasis when compared to the levels in the control group. However, the TNF-α level was not different between the two groups (6). In the current study, although levels of IL-6, a pro-inflammatory cytokine, in serum and BAL was found to be higher in the study group; when compared to the control group a statistically significant difference was not detected. Also high levels of serum IL-8 in the patients and the high levels of IL-8 in BAL were parallel. This significance and correlation shows that the bronchial inflammation in patients who are not in the exacerbation period continues. This gives rise to the thought that if inflammation continues, the bronchial tissue injury will also continue at different localizations and with different intensities. When the serum and BAL fluid levels of TNF-α were examined, no statistically significant difference

Table 8 Comparison of the genotype and allele frequencies of polymorphisms in the TNF-α -308 G/A and -238 G/A promoter region between groups

		Bronchiectasis, n [%]	Control, n [%]	Odds ratio (OR), (% 95 CI)	P
TNF-α -308 G/A Polymorphism					
Genotype	A/A	0	0	–	–
	G/A	8 [20]	3 [15]	Reference	–
	G/G	32 [80]	17 [85]	1.41 (0.33–6.04)	0.806
Allele	A	8 [10]	3 [7.5]	Reference	–
	G	72 [90]	37 [92.5]	1.37 (0.34–5.47)	0.655
TNF-α -238 G/A Polymorphism					
Genotype	A/A	0	0	–	–
	G/A	3 [7.5]	1 [5]	Reference	
	G/G	37 [92.5]	19 [95]	1.54 (0.15–15.83)	0.509
Allele	A	3 [3.75]	1 [2.5]	Reference	–
	G	77 [96.25]	39 [97.5]	1.52 (0.15–15.09)	0.374

was found between the groups.

IL-8, which is chemo-attractive for neutrophils, can be found at high concentrations in induced sputum and BAL samples of patients with bronchiectasis and chronic obstructive lung disease (7). Guran *et al*. investigated the association between symptom scores, spirometry, high resolution computed tomography findings and inflammatory markers (TNF-α and IL-8 levels) in induced sputum in 27 children with stable non-cystic fibrosis bronchiectasis. They found that symptom scores correlated significantly with FEV1 and sputum IL-8 levels, and a significant correlation existed between HRCT severity scores and symptoms, FEV1, sputum IL-8 and TNF-α levels. In conclusion, the researchers concluded that the patients with bronchiectasis have an ongoing inflammatory process (8). TNF-α and IL-8 are strong chemo-attractive mediators and cause chemotaxis and activation of neutrophils. TNF-α is a strong paracrine and autocrine regulator that shows local effects in immunoinflammatory reactions at low concentrations. Studies show that TNF-α is the most important cytokine in acute inflammation and antitumor immunity. TNF-α regulates adhesion and chemotaxis by activating the neutrophils and endothelial cells (9,10).

Bergin *et al*. showed high levels of IL-8 in airway samples from patients with non-cystic fibrosis bronchiectasis. The finding of high IL-8 levels supports the use of appropriate anti-inflammatory therapies (11). Although the physiological importance of high IL-8 levels in the stable period of bronchiectasis cases is not known, it supports the fact that inflammation continues and the systemic cellular response is active in even stable periods. Thus, the thought of the importance and necessity of anti-inflammatory treatment in bronchiectasis emerges. The increased levels of various cytokines in plasma or serum of patients with bronchiectasis supports the thought that the local inflammatory response is connected to systemic circulation with these mediators. Thus, it can be assumed that the serum levels of inflammatory cytokines may be helpful in the detection of the severity and the progression of the disease. In the current study, while the serum levels of IL-6, IL-8, and TNF-α in the study group were higher than the control group, only the difference in the IL-8 levels was statistically significant. There was no statistically significant correlation between IL-6 and TNF-α levels and bronchiectasis. In one study, the serum levels of TNF-α were significantly higher in patients with bronchiectasis when compared to the control group. However, IL-8 levels were not different between the patients and healthy controls (12). In contrast, in the present study, the TNF-α serum levels were not higher in the patient group. However, serum levels of IL-8 were significantly higher in the patient group.

Angrill *et al*. performed BAL in 49 patients with stable bronchiectasis and nine control subjects. In this study, BAL levels of TNF- α, IL-6, IL-8 and IL-10 were measured. In comparison with the control group, BAL levels of TNF-α, IL-6 and IL-8 were significantly higher in patients with bronchiectasis. However, IL-10 was not different between patients with bronchiectasis and control subjects. In the

present study, patients with bronchiectasis exhibited a significant increase in the BAL levels of IL-8 and a significant decrease in the BAL levels of IL-10 (13).

IL-10 is an anti-inflammatory cytokine that inhibits the effect of pro-inflammatory cytokines such as IL-8 and TNF-α. When the IL-10 level is low, as its inhibitory effect disappears, the levels of IL-8 and TNF-α are expected to be high. Cytokine IL-10 prevents the cytokine synthesis and secretion from T lymphocytes and macrophages. IL-10 and the cytokines that are synthesized from other Th2 cells increase in chronic graft-versus-host disease. From this perspective, IL-10 can be used for the treatment of situations requiring the inhibition of cellular immunity. IL-10 concentrations decrease in induced sputum secretions in patients with COPD and bronchiectasis. These decreased levels of IL-10 can increase lung inflammation (14). Today IL-10 has taken its place in clinical studies that are being conducted on its usability in the treatment of other chronic inflammatory diseases (inflammatory bowel disease, rheumatoid arthritis, and psoriasis) in which steroid resistant patients were included.

The levels of IL-10 in serum and BAL fluid were significantly different between groups. Furthermore, as IL-10 inhibits TNF-α and IL-8 secretions from macrophages, in the current study, IL-10 levels decreased and as it decreased levels of IL-10 cannot inhibit IL-8 and TNF-α secretions, IL-8 and TNF-α levels in serum and BAL fluid increased. However, while the IL-8 levels were significantly higher in the patient group according to the control group, TNF-α levels were not high in the patient group when compared to the control group. If the IL-10 levels increased, the anti-inflammatory process would increase more and the inflammatory process would be limited before the inflammation destructs the bronchus. From this perspective, if a selective activator of IL-10 receptors can be found, the therapeutic potential of IL-10 in bronchiectasis will further increase.

It was shown that IL-6 and TNF-α gene polymorphisms are associated with the development, progression, and severity of some diseases such as arthritis, chronic inflammatory diseases, and diabetes (15-17). In the current study, no statistically significant difference was found in the IL-6 -174 G/C polymorphism and the TNF-α -308 G/A and -238 G/A polymorphism.

Polymorphisms of the two promoter regions that belong to IL-8 cytokine gene were examined. In the current study when the IL-8 -161 allele frequencies of the patient and the control groups were examined, no significant difference was found between the two groups in terms of allele frequencies. There was no significant difference between groups in terms of AC and CC genotypes. The CC genotype increased the disease risk by 1.52 fold according to the AC genotype. However, as this risk is not statistically significant, it is assumed that IL-8 -161 C/A polymorphism does not produce a genetic predisposition to bronchiectasis in terms of alleles and genotypes. However, when the -251 allele frequencies were examined, a significant difference was found between two groups in terms of allele frequencies. The T genotype increased the disease risk by 2.38 fold according to the A genotype and this risk was statistically significant. A significant difference was found between the groups in terms of AT and TT genotypes; TA genotype increased the disease risk by 4.19 fold according to the TT genotype and as this risk was statistically significant. It was surmised that the IL-8 -251 A/T polymorphism produces a genetic predisposition to bronchiectasis in terms of the T allele and TA genotype. Being a TA or AA genotype and possessing the A allele is an increased risk factor for bronchiectasis whereas possessing TT genotype and T allele is a protective factor.

When the literature is examined, most of the studies about IL-8 cover respiratory diseases such as asthma. In a study in which Campa *et al*. investigated the relationship between lung cancer and -251 A/T polymorphism, it was shown that the levels of IL-8 protein in respiratory tract epithelial cells of individuals with -251 A/T polymorphism who were diagnosed with lung cancer was higher it was associated with smoking addiction (18). In another study conducted by Arınır *et al*., a correlation between IL-8 -251 A/T polymorphism and chronic obstructive lung disease was seen. A positive correlation with the severity of asthma was found in individuals with this polymorphism (19). Additionally, the current study showed that in respiratory tract diseases that are associated with inflammation, the IL-8 -251 A/T polymorphism may be an increased risk factor in the pathogenesis of the disease. In this case, the presence of IL-8 -251 A/T polymorphism shows it may produce a genetic predisposition to disease. However, to claim this, it is necessary to perform this study in larger scaled groups by increasing the number of patients.

In the current study when the IL-10 -592 allele frequencies in patient and control groups were examined, there was no significant difference between the two groups in terms of allele frequencies. No significant difference was detected between the groups in terms of AA and CC genotypes; however, a statistically significant difference was

found between AC genotype numbers, and AC genotype frequency was significantly higher in the study group compared to the control group. AC genotype increased the disease risk by 5.71 fold according to the CC genotype and this risk was statistically significant, thus, it was surmised that the IL-10 -592 C/A polymorphism produces a genetic predisposition to bronchiectasis. When the IL-10 -819 allele frequencies were examined, no significant difference was detected between the two groups in terms of allele frequencies and there was no significant difference between the groups in terms of -819 TT and CC genotypes. However, a statistically significant difference was detected between the groups in terms of TC genotype frequency and TC genotype frequency was higher in the study group compared to the control group. The TC genotype increased the disease risk by 5.06 fold according to the CC genotype and as this risk was statistically significant, thus it was surmised that the IL-10 -819 T/C polymorphism produces a genetic predisposition to bronchiectasis. On the other hand, when the -1082 allele frequencies were examined, there was no significant difference between the two groups in terms of allele frequencies. As no significant difference was found between the groups in terms of GG, AG, and AA genotypes, it was surmised that the IL-10 -1082 G/A polymorphism does not produce a genetic predisposition to bronchiectasis in terms of alleles and genotypes. IL-10 levels are affected by the polymorphism in the promoter region of the gene. Polymorphism is seen at points 1082, 819, and 592 nucleotide reversed from the beginning site of transcription. The IL-1082 A allele caused a decrease in IL-10 level and caused the inflammation to progress in a more severe form (20,21).

One of the limitations of the present study was the predominance of the male gender. Therefore, the researchers were unable to generalize the current results to the female gender. Another limitation of the study was the small number of patients due to the single-center study design. Further studies are warranted with a larger number of patients.

In conclusion, when examining the inflammatory and anti-inflammatory cytokines in bronchiectasis with chronic infection and inflammation, it was revealed that IL-8, an inflammatory cytokine, is high in both serum and BAL and IL-10, an anti-inflammatory cytokine is low in serum and BAL. The treatment method of blocking pro-inflammatory cytokines is used in the treatment of various diseases (such as inflammatory bowel disease, psoriasis, and ankylosing spondylitis). Also in bronchiectasis, the inflammation can be limited just before the inflammation destructs the bronchus by blocking the tissue destruction effect of inflammatory cytokines. Furthermore, the process can be terminated against the inflammation without causing injury, by the activation of the receptors in which IL-10 is effective. The detection of gene polymorphisms related to cytokines in genetic analysis may provide the development of gene therapies in the future for individuals who are thought to have a genetic predisposition.

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References

1. Pasteur MC, Bilton D, Hill AT, et al. British Thoracic Society guideline for non-CF bronchiectasis. Thorax 2010;65 Suppl 1:i1-58.
2. Barker AF, Ahmed SY. Bronchiectasis. In: Fishman AP, Elias JA, Fishman JA, et al. eds. Fishman's Pulmonary Diseases and Disorders. 4th ed. New York, McGraw-Hill, 2008:2183-92.
3. Martínez García MA. Bronchiectasis: still an orphan disease? Arch Bronconeumol 2005;41:407-9.
4. Bidwell J, Keen L, Gallagher G, et al. Cytokine gene polymorphism in human disease: on-line databases. Genes Immun 1999;1:3-19.
5. Sanger F, Coulson AR. A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. J Mol Biol 1975;94:441-8.
6. Olveira G, Olveira C, Gaspar I, et al. Fat-free mass depletion and inflammation in patients with bronchiectasis. J Acad Nutr Diet 2012;112:1999-2006.
7. Beeh KM, Beier J, Kornmann O, et al. Long-term repeatability of induced sputum cells and inflammatory markers in stable, moderately severe COPD. Chest 2003;123:778-83.
8. Guran T, Ersu R, Karadag B, et al. Association between inflammatory markers in induced sputum and clinical characteristics in children with non-cystic fibrosis bronchiectasis. Pediatr Pulmonol 2007;42:362-9.
9. Takabayashi T, Vannier E, Clark BD, et al. A new biologic role for C3a and C3a desArg: regulation of TNF-alpha and IL-1 beta synthesis. J Immunol 1996;156:3455-60.
10. Toews GB. Cytokines and the lung. Eur Respir J Suppl 2001;34:3s-17s.
11. Bergin DA, Hurley K, Mehta A, et al. Airway inflammatory markers in individuals with cystic fibrosis and non-cystic

fibrosis bronchiectasis. J Inflamm Res 2013;6:1-11.
12. Martínez-García MA, Perpiñá-Tordera M, Román-Sánchez P, et al. The association between bronchiectasis, systemic inflammation, and tumor necrosis factor alpha. Arch Bronconeumol 2008;44:8-14.
13. Angrill J, Agustí C, De Celis R, et al. Bronchial inflammation and colonization in patients with clinically stable bronchiectasis. Am J Respir Crit Care Med 2001;164:1628-32.
14. Takanashi S, Hasegawa Y, Kanehira Y, et al. Interleukin-10 level in sputum is reduced in bronchial asthma, COPD and in smokers. Eur Respir J 1999;14:309-14.
15. Fishman D, Faulds G, Jeffery R, et al. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. J Clin Invest 1998;102:1369-76.
16. Kroeger KM, Carville KS, Abraham LJ. The -308 tumor necrosis factor-alpha promoter polymorphism effects transcription. Mol Immunol 1997;34:391-9.
17. Cuenca J, Pérez CA, Aguirre AJ, et al. Genetic polymorphism at position-308 in the promoter region of the tumor necrosis factor (TNF): implications of its allelic distribution on susceptibility or resistance to diseases in the Chilean population. Biol Res 2001;34:237-41.
18. Campa D, Hung RJ, Mates D, et al. Lack of association between -251 T>A polymorphism of IL8 and lung cancer risk. Cancer Epidemiol Biomarkers Prev 2005;14:2457-8.
19. Arinir U, Klein W, Rohde G, et al. Polymorphisms in the interleukin-8 gene in patients with chronic obstructive pulmonary disease. Electrophoresis 2005;26:2888-91.
20. Turner DM, Williams DM, Sankaran D, et al. An investigation of polymorphism in the interleukin-10 gene promoter. Eur J Immunogenet 1997;24:1-8.
21. Ma SL, Tang NL, Lam LC, et al. The association between promoter polymorphism of the interleukin-10 gene and Alzheimer's disease. Neurobiol Aging 2005;26:1005-10.

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Original Article

Association between *RUNX3* promoter methylation and non-small cell lung cancer: a meta-analysis

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Background: Runt-related transcription factor 3 (*RUNX3*) is a known regulator in the transforming growth factor (*TGF*)-β signaling pathway, which promoter methylation playing a crucial role in diverse neoplasias. However, the relationship between *RUNX3* promoter methylation and non-small cell lung cancer (NSCLC) remains to be clarified.

Methods: We searched Pubmed, Embase, Cochrane Central, and Chinese Biological Medicine database, for articles published in English or Chinese until March 7, 2014. Our main analyses were focused on the association between *RUNX3* promoter methylation and risk of NSCLC by meta-analysis methods. If heterogeneity was observed, we used random effects model to calculate the overall odds ratios, otherwise fixed effects model was used. Subgroup analyses and meta-regression analyses were employed to detect the sources of the heterogeneity. Sensitivity analysis was performed to evaluate the stability of our studies. A funnel plot and Egger's test were conducted to investigate any potential publication bias.

Results: A total of 1,368 samples from 13 literatures were involved in this meta-analysis. The pooled odds ratio (OR) of *RUNX3* methylation in NSCLC specimens compared to non-cancer controls was 6.70 [95% confidence interval (CI): 4.64-9.67]. In the analysis of specimen-types subgroup, the summary OR was 5.79 (95% CI: 3.97-8.46) for tissue specimen subgroup, and that was 45.64 (95% CI: 5.89-353.72) for serum specimen subgroup. The ORs for the age ≤60 years, 60-65 years and >65 years subgroup were 5.19 (95% CI: 3.27-8.24), 9.45 (95% CI: 2.45-36.45) and 13.23 (95% CI: 5.59-31.28) respectively. The result of meta-regression indicated that age was fundamental source of heterogeneity (coefficient =0.61, P=0.046, adjusted R^2 =100%). No publication bias was detected. In cancer specimens, the *RUNX3* methylation was associated with histological type of the NSCLC, but no significant differences were found for *RUNX3* methylation in relation to gender, smoking history, tumor TNM stage or tumor differentiation level.

Conclusions: This meta-analysis of pooled data provides additional evidence to support a strong association between methylation of the *RUNX3* promoter and NSCLC. *RUNX3* methylation was increasing with age.

Keywords: Non-small cell lung cancer (NSCLC); runt-related transcription factor 3 (*RUNX3*); promoter; methylation; meta-analysis

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Background

Lung cancer is one of the most common malignancy and a leading cause of cancer-related deaths worldwide (1). Non-small cell lung cancer (NSCLC) accounts for 80% of all primary lung cancers, which comprises adenocarcinoma, squamous cell carcinoma and large cell carcinoma (2). Current knowledge regarding epigenetic changes play an integral role in the transformation, promotion and progression of cancer (3,4). DNA methylation is one of the most common forms of epigenetic modification. The

abnormal hypermethylation patterns of promoter site in various tumor suppressor genes (*TSGs*) are a pivotal mechanism in a wide variety of malignancies including lung cancer (5,6).

In humans 1p36.1 chromosomal loci, where *RUNX3* is located at this locus, observed to undergo frequent deletion could induce pulmonary carcinogenesis (7,8). *RUNX3* is a known regulator in the transforming growth factor (*TGF*)-*β* signaling pathway, which has recently been reported as a candidate tumor suppressor (9-11). Decreased *RUNX3* expression or deletion are mainly due to methylation or allelic loss, that results in the limited function of Smad proteins and the promotion of *TGF-β* signaling, which leads to tumor development (12). Previous studies have demonstrated *RUNX3* promoter methylation playing a crucial role in neoplasias, including colorectal (13), gastric (14,15), lung (16), bladder (17), breast (18,19) oral (20), and liver cancers (21), either using cell lines, or primary cancer tissues. However, the relationship between *RUNX3* promoter methylation and NSCLC remains to be clarified.

Although this association has been investigated in separate studies, the results are somewhat contradictory (22,23), possibly due to small sample size and underpowered in a single study. Therefore, we performed a meta-analysis using all available related studies to assess the association of *RUNX3* promoter methylation and NSCLC.

Methods

Search strategies and selection criteria

We searched Pubmed, Embase, Cochrane Central, and Chinese Biological Medicine database, for articles published in English or Chinese. We identified the publications using the text words (*RUNX3* or *PEBP2αC* or *CBFA3* or *AML2*) AND (lung or pulmonic) AND (cancer or neoplasm). The search updated on March 7, 2014. In addition, we also reviewed the reference from retrieved papers and relevant review articles. We only recruited data from fully published papers, not meeting or conference abstracts.

Study selection

Two investigators (Yali Liang and Lianping He) first independently screened the titles and abstracts to identify relevant articles. A second screening was based on full-text articles to further see whether they had met the inclusion criteria. Discrepancies were resolved by consensus.

Studies were included if they meet the following criteria: (I) the specimens from peripheral serum or surgically respected primary tumor tissue (not cell line or sputum); (II) the exposure of interest was *RUNX3* promoter methylation; (III) the outcome of interest was NSCLC; (IV) odds ratio (OR) with corresponding 95% confidence intervals (CIs) (or data to calculate them) were published.

Quality assessment

Two investigators independently assessed methodological quality of eligible studies with the Newcastle-Ottawa scale (NOS). The quality scale consists of three parameters: selection, comparability, and exposure assessment. The quality score ranges from 0 to 9. Studies with a score equal to or higher than 4 were considered "high-quality", whereas those scored less than 4 were considered "low-quality".

Data extraction

In order to control the bias and improve the reliability, the investigator followed a standardized data-collection form to extract all data. The following information was collected from each study: first author, year of publication, country of the study objects, specimen origin, number of cases and controls, the methylation status of *RUNX3* promoter in cancer and control samples, correlation between methylation and clinicopathological characteristics in NSCLC.

Statistical analysis

Our main analyses were focused on the association between *RUNX3* promoter methylation and risk of NSCLC. The effect measures of interest were ORs and corresponding 95% CI for case-control study.

Heterogeneity test for pooled ORs was performed by I^2 statistic (statistically significant level at $I^2 \geq 50\%$) (24). If heterogeneity was observed, we used random effects model (DerSimonian-Laird method) to calculate the overall odds ratios, otherwise fixed effects model (Mantel-Haenszel) was used. Subgroup analysis was performed according to specimen types (lung tissue or peripheral serum), and age categories (≤60 years; between 60 and 65 years; >65 years). If the heterogeneity was strong, meta-regression analyses were employed to analyze the sources of the heterogeneity. Sensitivity analysis was performed by deleting one study in each turn to evaluate the stability

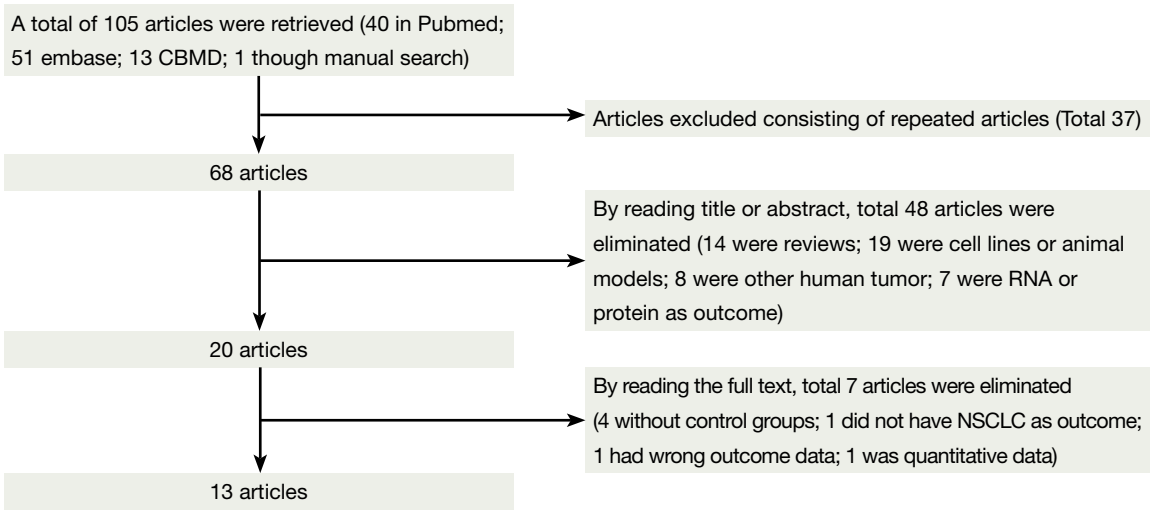

Figure 1 Flow diagram: publications documenting the association between runt-related transcription factor 3 (*RUNX3*) promoter methylation and non-small cell lung cancer (NSCLC).

of the final results. A funnel plot and Egger's test were conducted to investigate any potential publication bias. We also assessed the correlation between methylation status and clinicopathological characteristics (gender, smoking history, tumor stage, differentiation and histopathology) in NSCLC; histopathological tumor type includes adenocarcinoma, squamous cell carcinoma, and other histological type (large cell carcinoma and mixed histologies carcinoma).

The statistical analyses were performed with Stata12.0 software and review manager 4.2, two-sided P values less than 0.05 were considered statistically significant.

Results

Study selection and characteristics

One hundred and four potentially relevant studies were identified by the electronic search strategy, and 1 study was obtained by manually searching all references cited in the original studies. We justified eligible studies by further screening of their titles, abstracts and full texts. As a result, we retrieved 21 potentially relevant articles. Eight studies were excluded, because one studies did not exactly define as NSCLC (25); one study measured *RUNX3* methylation status by the RT-PCR (22); and one study data there had errors (26); and four studies did not establish control groups (27-30). Finally, the remaining 13 studies included in our study (*Figure 1*) (23,31-42) were included in our meta-analysis. All of them were case-control studies, and two papers were written in Chinese. The main characteristics of the reviewed studies are showed in *Table 1*. The total sample size was 1,368 (759 cases and 609 controls).

RUNX3 promoter methylation and risk of NSCLC

Among these 13 studies, substantial heterogeneity was not obvious (I^2 =47.4%). Hence, fixed effects model (Mantel-Haenszel) was used to calculate the pooled OR and 95% CIs (*Figure 2*). Overall, the pooled OR for *RUNX3* methylation in cancer specimens compared with normal specimens was 6.70 (95% CI: 4.64-9.67).

Subgroup analysis and meta-regression

Subgroup analysis was performed according to specimen types (tissue or peripheral serum) and age categories (≤60 years; between 60 and 65 years; >65 years). In the analysis of specimen-types subgroup, the summary OR was 5.79 (95% CI: 3.97-8.46) for tissue specimen subgroup, and that was 45.64 (95% CI: 5.89-353.72) for serum specimen subgroup. There was no evidences of heterogeneity in different specimen types subgroup (I^2 =43.9%; I^2 =0%). The OR for the age ≤60 years subgroup was 5.19 (95% CI: 3.27-8.24), that for the 60-65 years group was 9.45 (95% CI: 2.45-36.45) and that for the >65 years subgroup was 13.23 (95% CI: 5.59-31.28). Heterogeneity was not observed within different age categories subgroup (I^2 =39.8%; I^2 =43.8%;

Table 1 Characteristics of studies in this meta-analysis

Author (Ref)	Mean/median age (years) [range]	M+/M–		Specimen types	Methods	Methylation site	RUNX3 expression	Quality score
		Patients	Controls					
Hou DR *et al.* (34) 2009, China	55 [38-64]	30/32	11/51	Tissue	MSP	Promoter hypermethylation	Negative	6
Zhang Y *et al.* (31) 2011, China	59 [35-80]	18/60	8/70	Tissue	MSP	Cpg islands	Not reported	5
Yu GP *et al.* (32) 2012, China	57 [38-72]	26/32	10/48	Tissue	MSP	Promoter	Not reported	7
Lu DG *et al.* (33) 2011, China	59.6 [42-75]	25/37	0/46	Serum*	MSP	Promoter hypermethylation	Not reported	5
Yanagawa N *et al.* (35) 2003, Japan	67.3 [39-86]	15/60	2/73	Tissue	MSP	Promoter hypermethylation	Not reported	6
Suzuki M *et al.* (36) 2005, Japan	65	25/92	0/51	Tissue	MSP	Cpg islands	Not reported	4
Yanagawa N *et al.* (37) 2007, Japan	68.1 [39-86]	25/76	3/98	Tissue	MSP	Promoter hypermethylation	Not reported	8
Yoshino M *et al.* (38) 2009, Japan	63.2 [44-90]	9/35	2/30	Tissue	MSP	Cpg islands	Not reported	8
Li QL *et al.* (39) 2004, Korea	Not reported	6/19	0/25	Tissue	MSP	Promoter	Not reported	7
Chung JH *et al.* (40) 2011, Korea	59.2 [34-85]	29/61	0/20	Tissue	q-MSP	Cpg islands	Not reported	8
Tan SH *et al.* (23) 2007, Singapore	Not reported	11/9	0/10	Serum*	MSP	Promoter hypermethylation	Not reported	4
Omar MF *et al.* (41) 2012, Singapore	Not reported	3/6	3/2	Tissue	MSP	Promoter hypermethylation	Positive	4
Licchesi JD *et al.* (42) 2008, USA	69.6 [48-80]	17/1	13/33	Tissue	MSP	Promoter hypermethylationhy	Negative	6

M+, the number of tissues with methylation; M–, the number of tissues with unmethylation. *, peripheral serum.

I^2 =0%), as showed in *Figures 3* and *4*.

Heterogeneity was borderline among these 13 studies (I^2 =47.4%). Therefore, we performed further analyses to detect the sources of the heterogeneity using the meta-regression method with restricted maximum likelihood modification. The result of meta-regression indicated that the trend in ORs was correlated with age, which accounted for the heterogeneity (coefficient =0.61, P=0.046, adjusted R^2 =100%). However, the other factor (specimen type) could not explain the heterogeneity. The results are showed in *Table 2*.

Sensitivity analysis and publication bias

We conducted sensitivity analysis to assess the stability of the overall effects by deleting a single study, the overall ORs did not substantially changed, with a range from 6.00 (95% CI: 4.13-8.73) to 7.84 (95% CI: 5.21-11.79).

The funnel plot and Egger's test were performed to estimate publication bias (*Figures 5*,*6*), there was no evidence of publication bias with regard to *RUNX3* methylation in relation to NSCLC risk (Egger's test: t=2.12, P=0.058).

Correlation between methylation of RUNX3 promoter and clinicopathological characters

Table 3 showed the correlation between methylation of *RUNX3* promoter and clinicopathological characters. A portion of these 13 studies provided the relationship of *RUNX3* methylation and clinicopathological

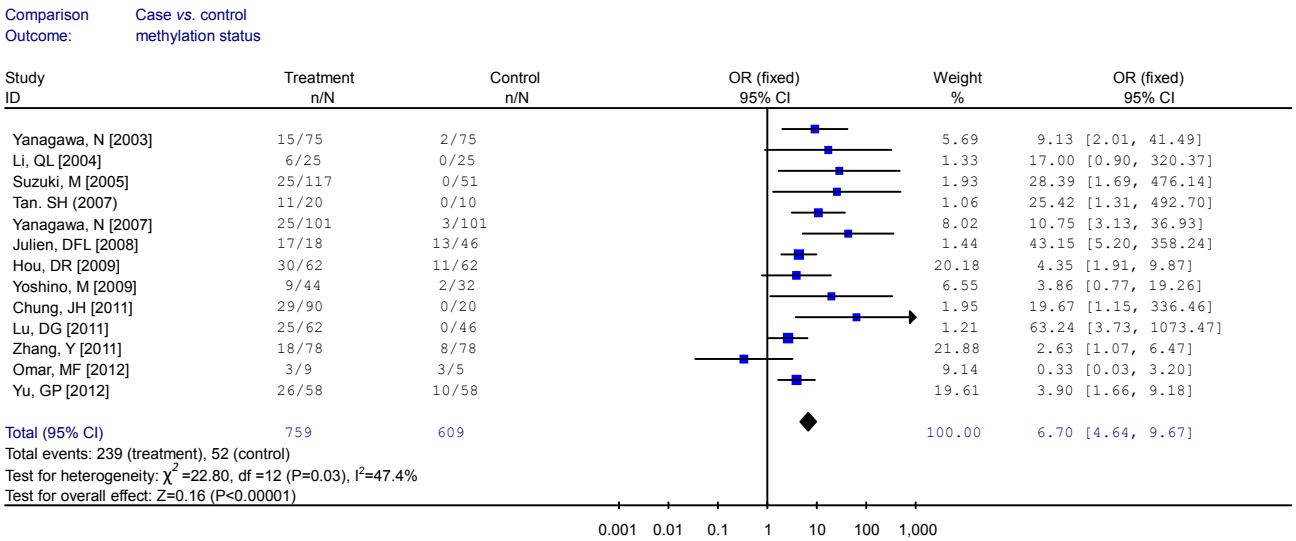

Figure 2 Forest plot of runt-related transcription factor 3 (*RUNX3*) promoter methylation in cancer specimens *vs*. non-cancer controls.

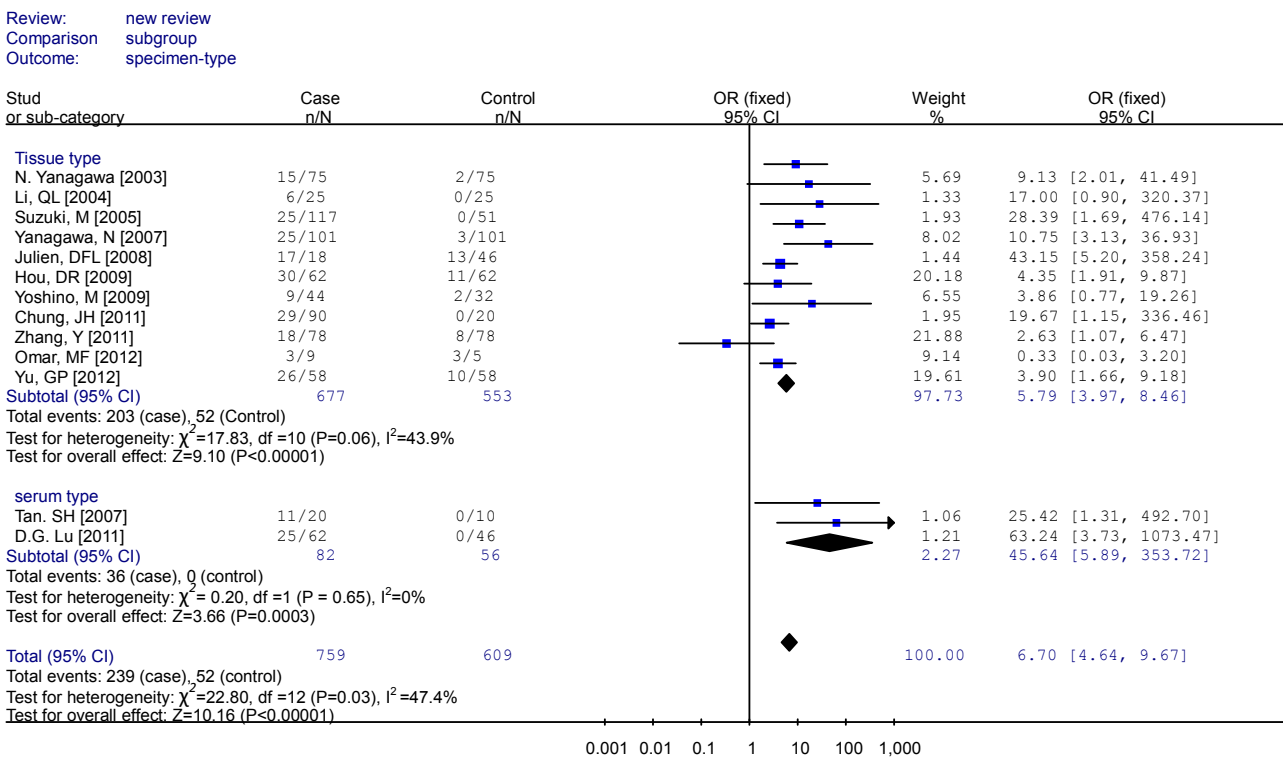

Figure 3 Forest plot of different specimen types subgroups analysis.

characteristics in their cancer specimens. The overall results demonstrated that *RUNX3* methylation was less frequent in adenocarcinoma compared with squamous cell carcinoma (OR =2.25, 95% CI: 1.48-3.42), but the frequencies was similar in adenocarcinoma and other histological type (OR =0.49, 95% CI: 0.22-1.10). Highly and moderately differentiated cancer specimens also had a lower methylation than poor differentiation (OR =0.39, 95% CI: 0.06-2.36).

There were no significant differences in *RUNX3*

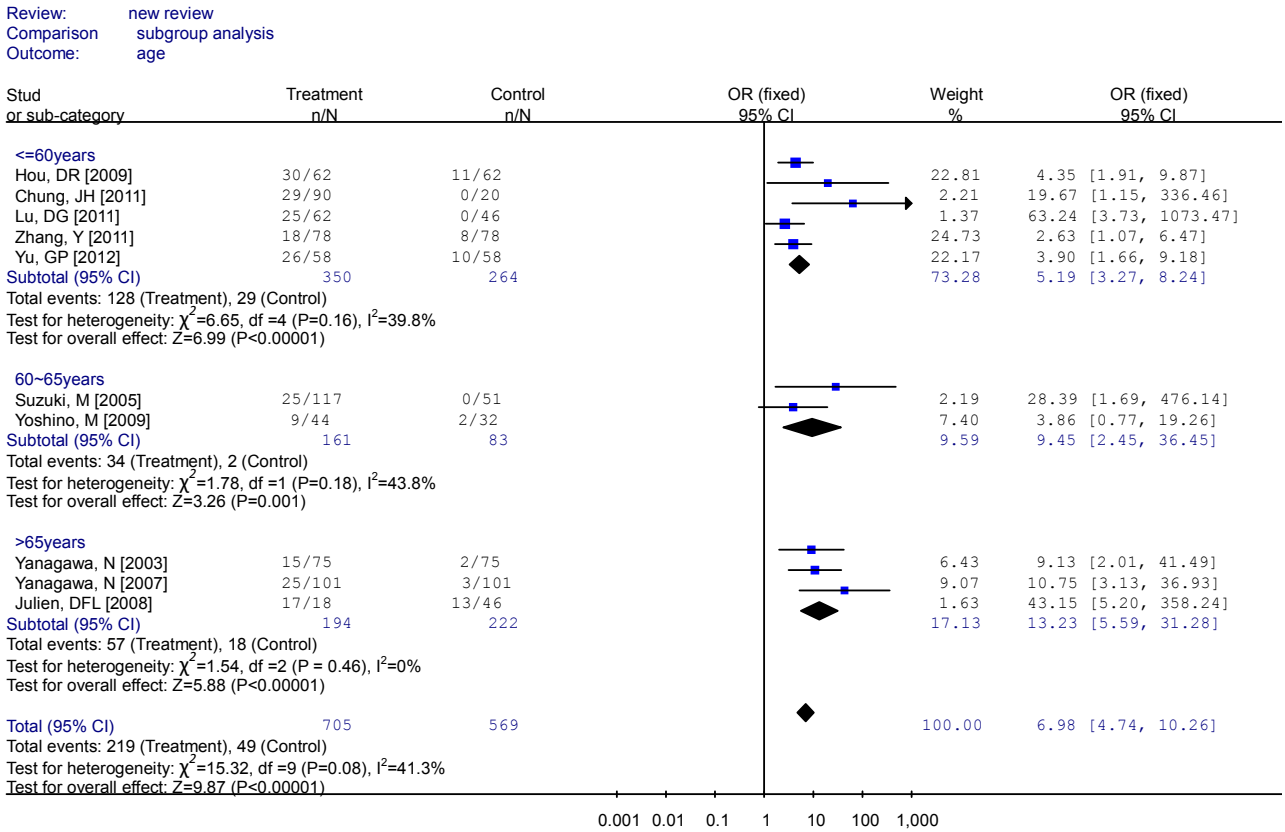

Figure 4 Forest plot of different age categories subgroups analysis.

Table 2 The results of meta-regression

Sources	Coefficient (95% CI)	t	P	Adjusted R^2 (%)
Speciman type	–2.82 (–6.29-0.64)	–1.93	0.096	58.78
Age	0.61 (0.01-1.20)	2.42	0.046	100.00

CI, confidence interval.

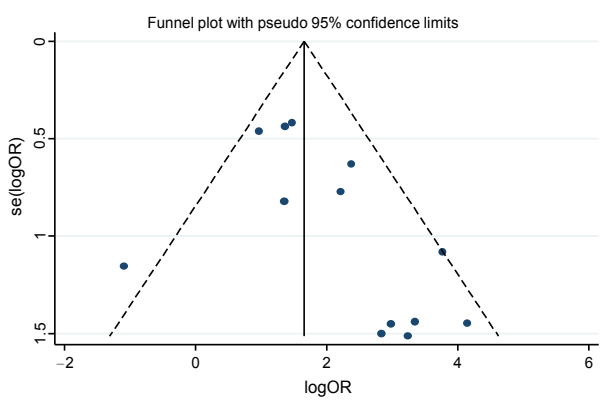

Figure 5 Funnel plot of runt-related transcription factor 3 (*RUNX3*) promoter methylation in cancer specimens *vs.* non-cancer controls.

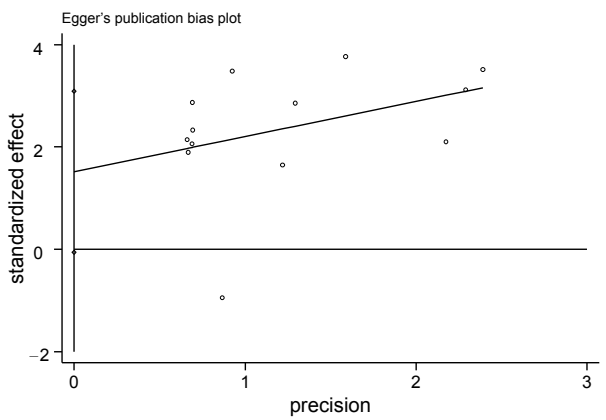

Figure 6 Egger's publication bias plot of runt-related transcription factor 3 (*RUNX3*) promoter methylation in cancer specimens *vs.* non-cancer controls.

Table 3 Correlation between methylation of *RUNX3* promoter and clinicopathological characters in NSCLC

NO. study	Characters		M+/M–	OR (95% CI)	I^2 (%)	P (Egger' test)
6	Gender	Male	73/156	0.80 (0.51-1.26)[b]	0.8	0.054
		Female	57/116			
5	Smoking	Yes	52/133	0.64 (0.38-1.07)[b]	25.2	0.438
		No	53/102			
9	Pathological type	ACC/SCC	102/201; 44/180	2.25 (1.48-3.42)[b]	34.3	0.135
5		ACC/other type	46/124; 18/18	0.49 (0.22-1.10)[b]	0	0.595
5		SCC/other type	21/106; 18/9	0.10 (0.04-0.29)[b]	0	0.068
3	Differentiation	H/M	40/68	0.39 (0.06-2.36)[a]	86.4	0.276
		P	41/33	0.47 (0.26-0.85)[b]		
6	TNM stage	I-II	58/151	0.79 (0.24-2.62)[a]	78.3	0.953
		III-IV	69/105			

M+, the number of tissues with methylation; M–, the number of tissues with unmethylation; other type, large-cell carcinoma and adenosquamous cell carcinoma. H/M, highly and moderately differentiated; P, poor differentiation. [a], by random effect model (DerSimonian-Laird method); [b], by fixed effects model (Mantel-Haenszel). Abbreviations: *RUNX3*, runt-related transcription factor 3; SCC, squamous cell carcinoma; ACC, adenocarcinoma; NSCLC, non-small cell lung cancer; OR, odds ratio; CI, confidence interval.

methylation status in cancer sample in relation to gender, smoking and tumor stage. The results are showed in *Figures 7-12*.

Discussion

Our meta-analysis focuses on relationship between *RUNX3* promoter methylation and the risk of NSCLC. The overall OR for methylation status in cancer versus normal specimens was 6.70 (95% CI: 4.64-9.67) by a fixed effects model on pooled data from 13 studies. In the specimen types-specific subgroup analysis, results showed: the OR in the tissue sample subgroup was 5.79 (95% CI: 3.97-8.46), that in serum samples subgroup was 45.64 (95% CI: 5.89-353.72), which further confirmed *RUNX3* methylation was a potential risk factor for NSCLC. Among peripheral blood of non-cancer objects, the methylation of *RUNX3* was absent, and so the methylation of *RUNX3* could be regarded as cancer-specific phenomenon. Therefore, in terms of clinical application, the detection *RUNX3* methylation of peripheral blood may be useful as a diagnostic approach. The trend of association between *RUNX3* methylation and NSCLC was correlated with age. The OR was 5.19 (95% CI: 3.27-8.24) for the age ≤60 years subgroup, 9.45 (95% CI: 2.45-36.45) for the group of 60-65 years, and 13.23 (95% CI: 5.59-31.28) for the >65 years subgroup. The coefficient for age was calculated to be 0.61 by meta-regression analysis, indicating that the tendency for *RUNX3* methylation increased with age. DNA methylation, genomic imprinting, and histone modifications were examples of epigenetic factors known to undergo change in the aging and malignant counterparts (43). *RUNX3* exhibited altered DNA methylation patterns in aging, displaying sometimes tissue- and cell type-specific features with consequent different functional outcomes (44). Some studies reported *RUNX3* methylation occurring preferentially in older malignant tumor patients (45). These results suggested that *RUNX3* methylation related with individual age in malignancies. We found that the ORs for *RUNX3* methylation increased from 5.19 in the younger age group, through 9.45, to 13.33 in the oldest age group. The coefficient for age was calculated to be 0.61 by meta-regression analysis, indicating that the tendency for *RUNX3* methylation increased with advancing age. The results suggested *RUNX3* methylation may be preferentially occurs in older NSCLC patients.

Although no publication bias was detected using Egger's test, the funnel plot showed one of the studies exceeded the 95% confidence limits. We performed sensitivity analysis in each to estimate the robustness of our results by deleting one study. The overall ORs were slightly changed from 6.00

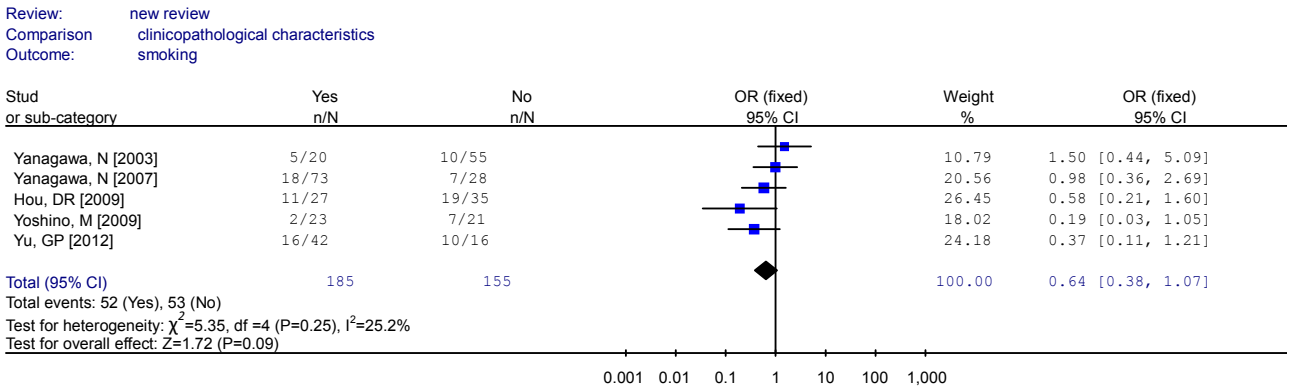

Figure 7 Forest plot of smoking in non-small cell lung cancer (NSCLC).

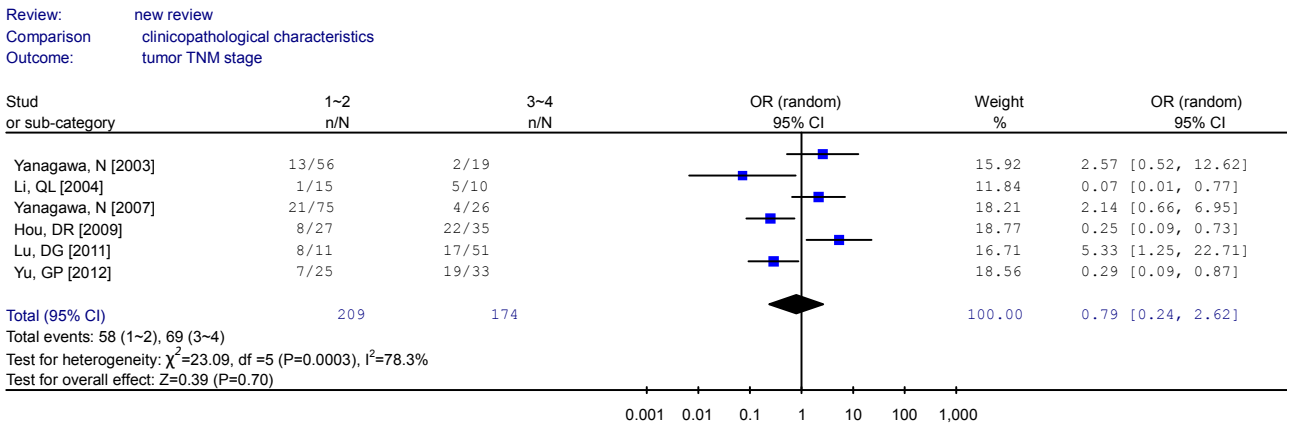

Figure 8 Forest plot of tumor stage in non-small cell lung cancer (NSCLC).

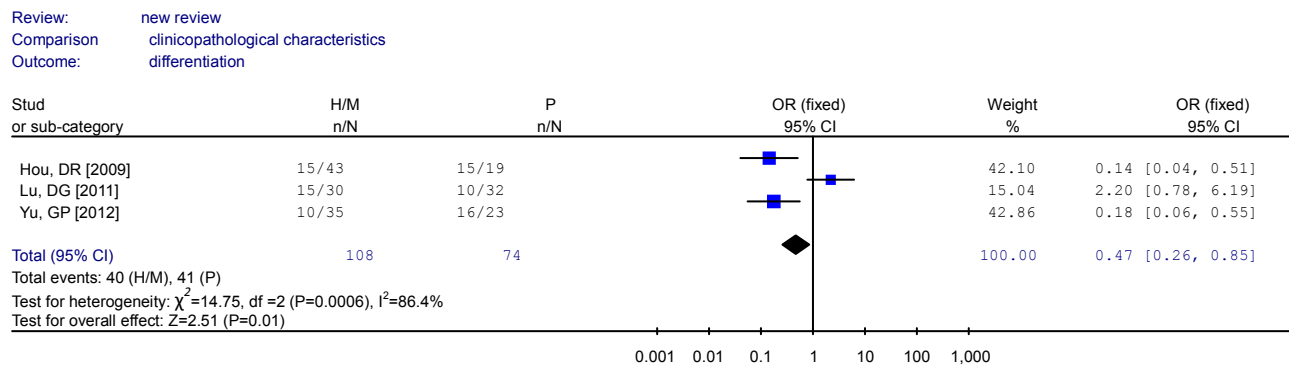

Figure 9 Forest plot of differentiation in non-small cell lung cancer (NSCLC).

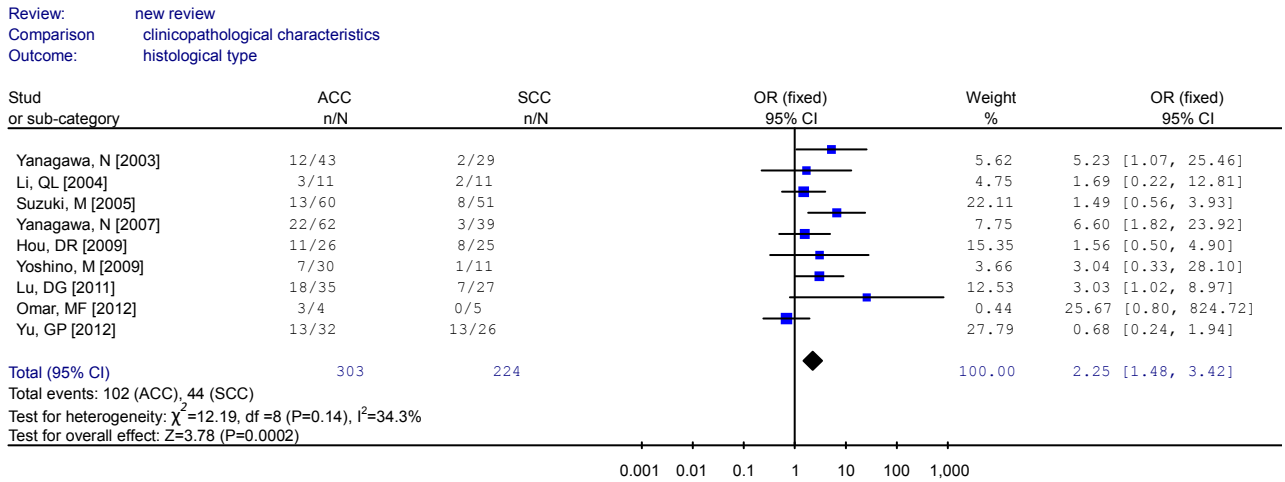

Figure 10 Forest plot of different histological type (ACC/SCC) in non-small cell lung cancer (NSCLC). ACC, adenocarcinoma; SCC, squamous cell carcinoma.

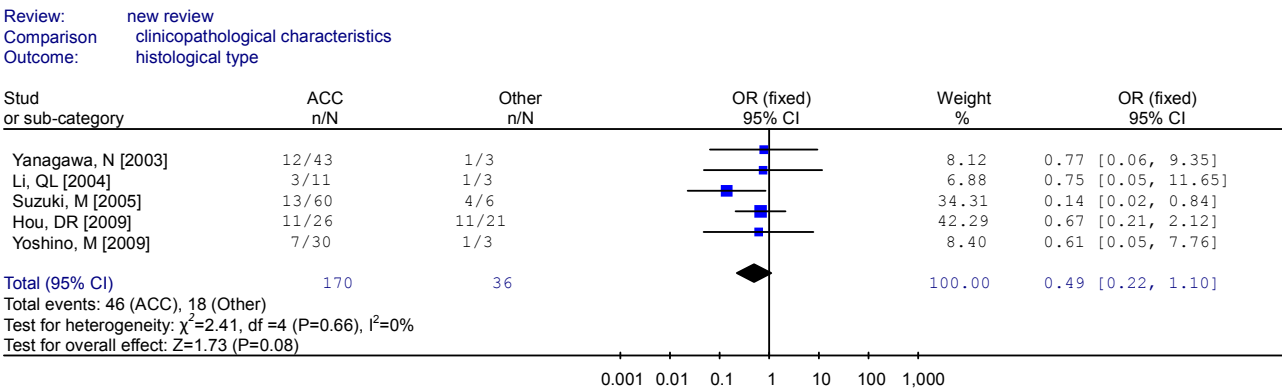

Figure 11 Forest plot of different histological type (ACC/Other) in non-small cell lung cancer (NSCLC). ACC, adenocarcinoma.

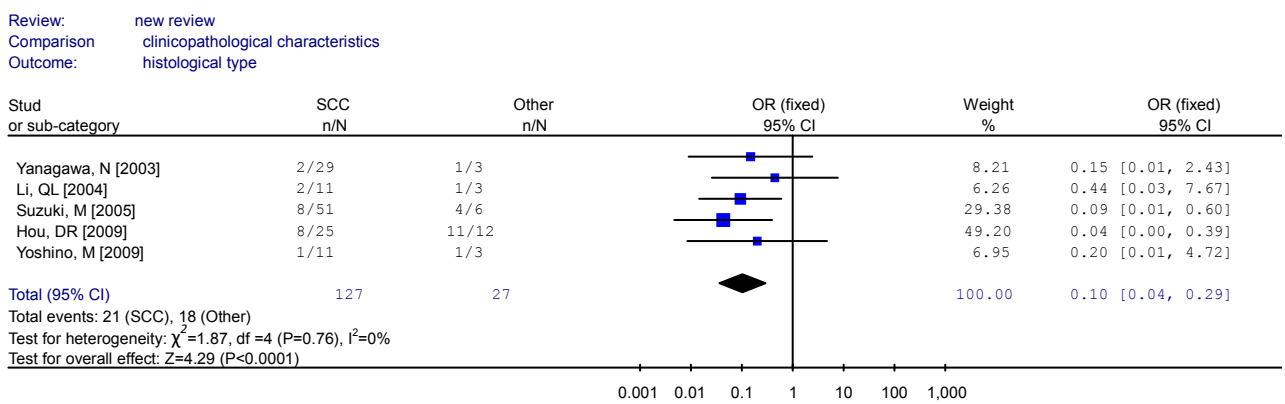

Figure 12 Forest plot of different histological type (SCC/Other) in non-small cell lung cancer (NSCLC). SCC, squamous cell carcinoma.

(95% CI: 4.13-8.73) to 7.84 (95% CI: 5.21-11.79), which were still significant. To produce a more robust estimation, we performed sensitivity analysis by deleting one study in each turn. The overall ORs were slightly changed from 6.00 (95% CI: 4.13-8.73) to 7.84 (95% CI: 5.21-11.79), which were still significant, indicating a strong association between *RUNX3* promoter methylation and NSCLC.

There were no significant differences in *RUNX3* methylation in cancer tissues in relation to gender, smoking history, or tumor TNM stage.

The aggregated results found that *RUNX3* methylation was less frequent in squamous cell carcinoma compared with adenocarcinoma and other histological type , suggesting that inactivation of *RUNX3* might play a less significant role in the pathogenesis of squamous cell carcinoma, as those previous studies suggested (30).

Analysis of the pooled data also showed that undifferentiated NSCLC had a higher frequency of promoter methylation than well-differentiated, which was significant in the fixed model (OR =0.47, CI: 0.26-0.85) but non-significant in the random effects model. This phenomenon may be associated with the smaller number of studies analyzed. But the results also indicated that *RUNX3* promoter methylation may be related to poor prognosis.

Some limitations of this meta-analysis should be addressed. First, Although we searched literature as completely as possible, the results calculated in our meta-analysis maybe exist bias as we only collected full published papers and articles published in English or Chinese. Second, our results were based on unadjusted, whereas a more precise analysis should be conducted if adjustment estimates were available.

Conclusions

Despite these limitations, this meta-analysis provides additional evidence to support a strong association between methylation of the *RUNX3* promoter and NSCLC. The tendency of association for *RUNX3* methylation increased with age. The *RUNX3* methylation was also associated with histological type of the NSCLC. However, it is necessary to conduct large sample studies using standardized and well-matched controls.

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Disclosure: The authors declare no conflict of interest.

References

1. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2009. CA Cancer J Clin 2009;59:225-49.
2. Stinchcombe TE, Socinski MA. Current treatments for advanced stage non-small cell lung cancer. Proc Am Thorac Soc 2009;6:233-41.
3. Esteller M, Corn PG, Baylin SB, et al. A gene hypermethylation profile of human cancer. Cancer Res 2001;61:3225-9.
4. Jones PA, Baylin SB. The fundamental role of epigenetic events in cancer. Nat Rev Genet 2002;3:415-28.
5. Risch A, Plass C. Lung cancer epigenetics and genetics. Int J Cancer 2008;123:1-7.
6. Shames DS, Girard L, Gao B, A genome-wide screen for promoter methylation in lung cancer identifies novel methylation markers for multiple malignancies. PLoS Med 2006;3:e486.
7. Herzog CR, Wang Y, You M. Allelic loss of distal chromosome 4 in mouse lung tumors localize a putative tumor suppressor gene to a region homologous with human chromosome 1p36. Oncogene 1995;11:1811-5.
8. Nomoto S, Haruki N, Tatematsu Y, et al. Frequent allelic imbalance suggests involvement of a tumor suppressor gene at 1p36 in the pathogenesis of human lung cancers. Genes Chromosomes Cancer 2000;28:342-6.
9. Hanai J, Chen LF, Kanno T, et al. Interaction and functional cooperation of PEBP2/CBF with Smads. Synergistic induction of the immunoglobulin germline Calpha promoter. J Biol Chem 1999;274:31577-82.
10. Lee JM, Kwon HJ, Bae SC, et al. Lung tissue regeneration after induced injury in Runx3 KO mice. Cell Tissue Res 2010;341:465-70.
11. Lee KS, Lee YS, Lee JM, et al. Runx3 is required for the differentiation of lung epithelial cells and suppression of lung cancer. Oncogene 2010;29:3349-61.
12. Bae SC, Choi JK. Tumor suppressor activity of RUNX3. Oncogene 2004;23:4336-40.
13. Subramaniam MM, Chan JY, Soong R, et al. RUNX3 inactivation in colorectal polyps arising through different pathways of colonic carcinogenesis. Am J Gastroenterol

2009;104:426-36.
14. Chen W, Gao N, Shen Y, et al. Hypermethylation downregulates Runx3 gene expression and its restoration suppresses gastric epithelial cell growth by inducing p27 and caspase3 in human gastric cancer. J Gastroenterol Hepatol 2010;25:823-31.
15. Homma N, Tamura G, Honda T, et al. Spreading of methylation within RUNX3 CpG island in gastric cancer. Cancer Sci 2006;97:51-6.
16. Yanada M, Yaoi T, Shimada J, et al. Frequent hemizygous deletion at 1p36 and hypermethylation downregulate RUNX3 expression in human lung cancer cell lines. Oncol Rep 2005;14:817-22.
17. Jeong P, Min BD, Ha YS, et al. RUNX3 methylation in normal surrounding urothelium of patients with non-muscle-invasive bladder cancer: potential role in the prediction of tumor progression. Eur J Surg Oncol 2012;38:1095-100.
18. Lau QC, Raja E, Salto-Tellez M, et al. RUNX3 is frequently inactivated by dual mechanisms of protein mislocalization and promoter hypermethylation in breast cancer. Cancer Res 2006;66:6512-20.
19. Subramaniam MM, Chan JY, Soong R, et al. RUNX3 inactivation by frequent promoter hypermethylation and protein mislocalization constitute an early event in breast cancer progression. Breast Cancer Res Treat 2009;113:113-21.
20. de Freitas Cordeiro-Silva M, Stur E, Agostini LP, et al. Promoter hypermethylation in primary squamous cell carcinoma of the oral cavity and oropharynx: a study of a Brazilian cohort. Mol Biol Rep 2012;39:10111-9.
21. Park WS, Cho YG, Kim CJ, et al. Hypermethylation of the RUNX3 gene in hepatocellular carcinoma. Exp Mol Med 2005;37:276-81.
22. Jin M, Kawakami K, Fukui Y, et al. Different histological types of non-small cell lung cancer have distinct folate and DNA methylation levels. Cancer Sci 2009;100:2325-30.
23. Tan SH, Ida H, Lau QC, et al. Detection of promoter hypermethylation in serum samples of cancer patients by methylation-specific polymerase chain reaction for tumour suppressor genes including RUNX3. Oncol Rep 2007;18:1225-30.
24. Labeau SO, Van de Vyver K, Brusselaers N, et al. Prevention of ventilator-associated pneumonia with oral antiseptics: a systematic review and meta-analysis. Lancet Infect Dis 2011;11:845-54.
25. Kim TY, Lee HJ, Hwang KS, et al. Methylation of RUNX3 in various types of human cancers and premalignant stages of gastric carcinoma. Lab Invest 2004;84:479-84.
26. Castro M, Grau L, Puerta P, et al. Multiplexed methylation profiles of tumor suppressor genes and clinical outcome in lung cancer. J Transl Med 2010;8:86.
27. Suzuki M, Wada H, Yoshino M, et al. Molecular characterization of chronic obstructive pulmonary disease-related non-small cell lung cancer through aberrant methylation and alterations of EGFR signaling. Ann Surg Oncol 2010;17:878-88.
28. Yanagawa N, Tamura G, Oizumi H, et al. Inverse correlation between EGFR mutation and FHIT, RASSF1A and RUNX3 methylation in lung adenocarcinoma: relation with smoking status. Anticancer Res 2011;31:1211-4.
29. Tang Y, Wu F, Hu C. RUNX3 promoter hypermethylation and prognosis of early surgically resected non-small cell lung cancers. Zhong Nan Da Xue Xue Bao Yi Xue Ban 2011;36:650-4.
30. Sato K, Tomizawa Y, Iijima H, et al. Epigenetic inactivation of the RUNX3 gene in lung cancer. Oncol Rep 2006;15:129-35.
31. Zhang Y, Wang R, Song H, et al. Methylation of multiple genes as a candidate biomarker in non-small cell lung cancer. Cancer Lett 2011;303:21-8.
32. Yu GP, Ji Y, Chen GQ, et al. Application of RUNX3 gene promoter methylation in the diagnosis of non-small cell lung cancer. Oncol Lett 2012;3:159-162.
33. Lu DG, Ji XQ, Liu W. Significance of RUNX3 Hypermethylation in Serum DNA of Non-small Cell Lung Cancer Patients. Cancer Research on Prevention and Treatment 2011;38:671-4.
34. Hou DR, Wang HZ. Analysis of promoter hypermethylation of Runx3 gene in non-small cell lung carcinoma. Progress in Modern Biomedicine 2009;9:3692-5.
35. Yanagawa N, Tamura G, Oizumi H, et al. Promoter hypermethylation of tumor suppressor and tumor-related genes in non-small cell lung cancers. Cancer Sci 2003;94:589-92.
36. Suzuki M, Shigematsu H, Shames DS, et al. DNA methylation-associated inactivation of TGFbeta-related genes DRM/Gremlin, RUNX3, and HPP1 in human cancers. Br J Cancer 2005;93:1029-37.
37. Yanagawa N, Tamura G, Oizumi H, et al. Promoter hypermethylation of RASSF1A and RUNX3 genes as an independent prognostic prediction marker in surgically resected non-small cell lung cancers. Lung Cancer 2007;58:131-8.
38. Yoshino M, Suzuki M, Tian L, et al. Promoter

hypermethylation of the p16 and Wif-1 genes as an independent prognostic marker in stage IA non-small cell lung cancers. Int J Oncol 2009;35:1201-9.
39. Li QL, Kim HR, Kim WJ, et al. Transcriptional silencing of the RUNX3 gene by CpG hypermethylation is associated with lung cancer. Biochem Biophys Res Commun 2004;314:223-8.
40. Chung JH, Lee HJ, Kim BH, et al. DNA methylation profile during multistage progression of pulmonary adenocarcinomas. Virchows Arch 2011;459:201-11.
41. Omar MF, Ito K, Nga ME, et al. RUNX3 downregulation in human lung adenocarcinoma is independent of p53, EGFR or KRAS status. Pathol Oncol Res 2012;18:783-92.
42. Licchesi JD, Westra WH, Hooker CM, et al. Epigenetic alteration of Wnt pathway antagonists in progressive glandular neoplasia of the lung. Carcinogenesis 2008;29:895-904.
43. Damaschke NA, Yang B, Bhusari S, et al. Epigenetic susceptibility factors for prostate cancer with aging. Prostate 2013;73:1721-30.
44. D'Aquila P, Rose G, Bellizzi D, et al. Epigenetics and aging. Maturitas 2013;74:130-6.
45. Waki T, Tamura G, Sato M, et al. Age-related methylation of tumor suppressor and tumor-related genes: an analysis of autopsy samples. Oncogene 2003;22:4128-33.

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Original Article

High resolution computed tomography findings in smear-negative pulmonary tuberculosis patients according to their culture status

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Objective: The aim of this study was to assess the clinical features and high resolution computed tomography (HRCT) findings in smear-negative pulmonary tuberculosis (PTB) and to evaluate the correlation between these parameters and the culture results.

Methods: We retrospectively studied 78 active smear-negative PTB patients. They were divided into two groups according to their culture results. The HRCT findings and clinical features at the beginning of the antituberculosis treatment were reviewed.

Results: The mean age was 22.48±3.18 years. Micronodules (87%), large nodules (63%) and centrilobular nodules (62%) were the most common HRCT findings. HRCT findings were observed in the right upper (72%), left upper (56%), right lower (32%), and left lower lobes (29%). Cough (37%) and chest pain (32%) were the most frequent symptoms at presentation.

Conclusions: There were no significant differences in the HRCT findings and clinical features between the two groups. Thus, in cases of smear-negative and culture-negative PTB, the patient with compatible clinical and radiological features should be considered for tuberculosis treatment.

Keywords: Smear; culture; negative; tuberculosis (TB); high resolution computed tomography (HRCT)

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Introduction

Tuberculosis (TB) is one the most common causes of death from an infectious disease worldwide, after Human Immunodeficiency Virus (HIV) infection, with an estimated 8.7 million new cases of TB in 2011 (1). Turkey is categorized as a low endemicity TB region according to the World Health Organization (2). Nearly one-half to one-third of the pulmonary tuberculosis (PTB) cases are smear-negative, and approximately 1.9 million new PTB cases were found to be smear-negative in 2011 (1). Smear negativity in PTB is a common clinical problem leading to serious delays in the establishment of a diagnosis. A significant proportion of the patients treated for smear-negative PTB never have bacteriological confirmation, even with improved culture methods. Therefore, most cases of smear-negative PTB are diagnosed on the basis of the clinical presentation, radiological findings and other laboratory tests. It is an important decision whether to treat a patient when the microbiological data does not support the clinical suspicion. Thus, we decided to examine the clinical features and high resolution computed tomography (HRCT) findings of smear-negative PTB patients at the beginning of antituberculosis treatment and to compare the findings of smear-negative, culture-positive PTB patients with smear-negative and culture-negative PTB patients.

Materials and methods

This was a retrospective cohort study conducted at the

Pulmonary Department of Gulhane Military Medical Academy Haydarpasa Training Hospital. It was approved by the Ethics Review Board of our institution. Data of patients who were diagnosed with smear-negative PTB between January 2007 and December 2010 were reviewed. All the patients had at least three negative sputum smear reports for acid-fast bacillus (AFB) and/or two negative gastric lavage and/or bronchoalveolar lavage AFB reports. Mycobacterial cultures of all the specimens were performed on both Löwenstein-Jensen solid medium and in the BACTEC MGIT 960 liquid culture system (Becton, Dickinson Microbiology System, Sparks, Shannon, Ireland). A Gram stain and microorganism identification was performed in all the samples according to standard protocols to exclude pulmonary infections other than PTB. Initially all of the patients were treated with a single course of broad-spectrum antibiotics (excluding antituberculosis drugs and fluoroquinolones) in order to exclude pulmonary infections. Antituberculosis treatment was started if the radiologic findings and clinical parameters were consistent with active PTB. HRCTs were performed at the beginning of the treatment and at the third month of the treatment for follow-up. The patients who had negative cultures had a thorough follow-up clinical and radiological evaluation at the third month of therapy to determine whether there had been a response that could be attributed to the antituberculosis treatment.

The treatment was stopped in the patients who did not have radiological and clinical improvement by the third month. In these cases the radiological lesions were considered to be inactive or sequelae.

All the patients completed the six months course of antituberculosis therapy. No other etiology was defined. The patients were divided into two groups according to their culture results: smear-negative culture-positive and smear-negative culture-negative. All the patients were newly diagnosed smear-negative PTB cases without a previous history of TB. The standard 6-month antituberculosis treatment consisting of a 2-month initial phase of rifampicin, isoniazid, pyrazinamide and ethambutol, and a 4-month maintenance phase of rifampicin and isoniazid was given to the patients via directly observed treatment (DOTS).

Two radiologists, who were blinded to the culture and clinical results, evaluated the initial HRCT and interpreted the findings. If there was any disagreement between them, their final consensus report was recorded. The HRCT images were analyzed retrospectively for the morphology, number and lobar distribution of pulmonary lesions. The HRCT was performed using the Aquilion One dynamic volume CT (Toshiba, Japan) scanner, according to the Hi Rez Chest HCT (0.5 mm) imaging protocol: 0.5 s rotation time, pitch; fast, 120 kV, sure exposure standard mA, 0.5 mm ×64 scan slice thickness. The descriptive terms used to interpret the HRCT findings are as follows (3): micronodule (discrete, small, round, focal opacity less than 3 mm), large nodule (rounded or irregular opacity, well or poorly defined, measuring 3 mm to 3 cm in diameter), centrilobular nodule, tree-in-bud pattern, cavity, ground glass opacity, calcified nodule, consolidation, mass (>3 cm), interlobular septal thickening, bronchial wall thickening, bronchiectasis, emphysema, mediastinal lymphadenopathy (LAP, >10 mm), bulla, hilar LAP, calcified LAP and pleural thickening.

Number Cruncher Statistical Systems software (NCSS, East Kaysville, Utah, USA) was used for the statistical analysis in this study. The data are expressed as the means ± standard deviation (SD) for continuous data such as age. An independent *t*-test was used to compare differences between the groups for the continuous data. Pearson's chi-square test and Fisher's exact test were used to compare the distribution of the categorical data between the groups. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. A P value <0.05 was considered statistically significant.

Results

There were a total of 85 patients. Seven patients were excluded from the study as they did not have radiological and clinical improvement after three months of the treatment, leaving 78 patients. There were 50 culture-positive patients (Group 1) and 28 culture-negative patients (Group 2). The patients (n=7) who had both a positive AFB culture and a histopathological diagnosis (caseation granulomatous inflammation) with surgical biopsy or transbronchial lung biopsy were also included in Group 1. No patients in our study population were HIV positive.

The mean patient-age was 22.48±3.18 years (range, 20-36 years). All the patients were male because the study was performed in a military hospital. There was no statistically significant difference in the mean age between the two groups (P=0.793).

Cough was the most frequent symptom, being present in 37% of the patients, followed by chest pain in 32%, expectoration in 18%, hemoptysis in 10%, fever in 9% and weight loss in 8% of the patients. There were no statistically significant differences in symptoms between the two groups

Table 1 Comparison of clinical and laboratory parameters

	N [%]		P	OR (95% CI)
	Group I [50]	Group II [28]		
Clinical parameters				
Mean age (years)	22.41±3.39	22.61±2.83	0.793	
Cough	17 [34]	12 [43]	0.437	0.69 (0.27-1.78)
Chest pain	16 [32]	9 [32]	0.990	0.99 (0.37-2.68)
Expectoration	10 [20]	4 [14]	0.528	1.50 (0.42-5.32)
Hemoptysis	5 [10]	3 [11]	0.921	1.44 (0.26-7.98)
Fever	5 [10]	2 [7]	0.672	0.93 (0.20-4.20)
Weight loss	4 [8]	2 [7]	0.892	1.13 (0.19-6.60)
Laboratory parameters				
WBC (billion cells/L)	8.22±2.61	7.85±2.47	0.548	
ESR (mm/hr)	14.72±18.17	12.48±17.99	0.606	

Data presented as no. [%], Mean +/– SD. OR, odds ratio; CI, confidence interval; WBC, white blood cell; ESR, erythrocyte sedimentation rate.

(P>0.05).

The clinical and laboratory data are presented in *Table 1*. There were no statistically significant differences in the laboratory parameters between the two groups (P>0.05). The most common HRCT findings were micronodules (87%), large nodules (63%), and centrilobular nodules (62%). The radiological data are presented in *Table 2*. Again, there were no significant differences in the radiological findings between the groups. The localization of the radiological lesions was also evaluated. Radiological lesions were observed in the right (72%) and left (56%) upper lobes most frequently (*Table 3*). There were no significant differences in the distribution of lesions between the two groups (P>0.05).

Discussion

The early diagnosis of PTB is critical for TB control. Patients with active smear-negative PTB are also capable of transmitting the infection (4). However, the diagnosis of a patient with smear-negative PTB is difficult and often delayed. In medical practice, only 68% of PTB are culture-confirmed, but the clinical features and HRCT findings of smear-negative and culture-negative PTB are not well known. Presently the most important criteria for establishing a presumptive diagnosis of smear negative PTB are based on clinical findings, radiographic signs, risk factors or a combination of these. In our study, we investigated the use of the clinical features and HRCT findings to differentiate smear-negative culture-positive PTB from smear-negative and culture-negative disease.

Cough was the most common symptom reported in both groups. Other symptoms were chest pain, expectoration, hemoptysis, fever and weight loss; Nakashi *et al.* also reported that cough was seen in 83% of patients as the most common symptom in smear-negative PTB (5). Chest pain is a symptom for PTB and is related to pleural involvement. This symptom was the second most common complaint in both groups. Interestingly, Tozkoparan *et al.* found chest pain to be the most common symptom (38%) for smear-negative PTB and emphasized the significantly high existence of chest pain in active smear-negative PTB (6). Other related symptoms in our patients were expectoration, hemoptysis, fever and weight loss. Our results showed that smear-negative culture-positive PTB cannot be discriminated from smear-negative and culture-negative PTB based on clinical symptoms and laboratory parameters.

The chest radiograph as a tool for the diagnosis of active PTB is sensitive but limited by poor specificity (7). HRCT may identify a finding of PTB not seen on a chest radiograph (8). HRCT is usually recommended when the radiographic findings are normal or inconclusive, and PTB is suspected clinically, for the confirmation of diagnosis and determination of activity. Investigators have tried to design HRCT diagnostic criteria to rank the risk of PTB in patients with suspected PTB (5), and others have created an active PTB prediction model based on HRCT findings to differentiate smear-positive active PTB from other

Table 2 Patient HRCT findings

HRCT findings	N [%]			P	OR (95% CI)
	All [78]	Group 1 [50]	Group 2 [28]		
Micronodules	68 [87]	43 [86]	25 [89]	0.677	0.73 (0.18-3.11)
Large nodules	49 [63]	33 [66]	16 [57]	0.437	1.45 (0.56-3.76)
Centrilobular nodules	48 [62]	33 [66]	15 [54]	0.279	1.68 (0.65-4.33)
TIB pattern	32 [41]	24 [48]	8 [29]	0.094	2.30 (0.85-6.21)
BWT	28 [36]	18 [36]	10 [36]	0.980	1.01 (0.38-2.65)
Bronchiectasis	25 [32]	14 [28]	11 [39]	0.306	0.60 (0.22-1.59)
Mediastinal LAP	23 [29]	14 [28]	9 [32]	0.700	0.82 (0.30-2.24)
Consolidation	22 [28]	15 [30]	7 [25]	0.638	1.28 (0.45-3.66)
Calcified nodule	22 [28]	11 [22]	11 [39]	0.104	0.43 (0.15-1.19)
Ground glass opacity	20 [26]	13 [26]	7 [25]	0.923	1.05 (0.37-3.05)
Cavity	20 [26]	11 [22]	9 [32]	0.325	0.59 (0.22-1.68)
Hilar LAP	16 [21]	11 [22]	5 [18]	0.664	1.30 (0.40-4.20)
Pleural thickening	16 [21]	9 [18]	7 [25]	0.463	0.66 (0.21-2.02)
Calcified LAP	12 [15]	7 [14]	5 [18]	0.651	0.74 (0.21-2.62)
IST	10 [13]	8 [16]	2 [7]	0.262	2.47 (0.49-12.57)
Mass	9 [12]	7 [14]	2 [7]	0.363	2.11 (0.41-10.97)
Emphysema	5 [6]	4 [8]	1 [4]	0.444	2.34 (0.25-22.1)
Bulla	3 [4]	2 [4]	1 [4]	0.925	1.12 (0.90-12.9)

Data presented as no. [%]. HRCT, High resolution computerized tomography; OR, odds ratio; CI, confidence interval; LAP, lymphadenopathy; TIB, tree-in-bud; IST, interlobular septal thickening; BWT, bronchial wall thickening.

Table 3 Anatomic distribution of involved lobes with radiologic lesions

	N [%]		P	OR (95% CI)
	Group 1 [50]	Group 2 [28]		
Right upper lobe	35 [70]	21 [75]	0.638	0.78 (0.27-2.21)
Left upper lobe	30 [60]	14 [50]	0.393	1.50 (0.59-3.81)
Right lower lobe	17 [34]	8 [28]	0.622	1.29 (0.47-3.52)
Left lower lobe	17 [34]	6 [21]	0.243	1.89 (0.64-5.54)
Right middle lobe	9 [18]	7 [25]	0.463	0.66 (0.21-2.01)

Data presented as no. [%]. OR, odds ratio; CI, confidence interval.

pulmonary infections (9). There are several studies about HRCT findings of smear-negative PTB and the role of HRCT in predicting the activity of PTB (6,8,10), but no previous studies have revealed the relationship between the HRCT findings and the AFB culture results. We found that micronodules, large nodules and centrilobular nodules were the most common HRCT findings in our study in both groups. The HRCT findings of smear-negative PTB were compared with previous studies in *Table 4*.

Micronodules were the most common HRCT finding of smear-negative PTB in our study. The four most common HRCT findings are presented in *Figure 1*. HRCT enables the creation of a differential diagnosis of diffuse micronodular lung disease by showing the distribution of micronodules in and around the secondary pulmonary lobule (13,14). HRCT findings of centrilobular nodules or a predominant distribution of the micronodules in the secondary lobule may help to distinguish bronchogenic spread from miliary PTB (15). We found that the presence of micronodules tended to be higher in Group 2, although

Table 4 Comparison of HRCT findings with previous studies

HRCT findings	Our study (n:78)	Nakanishi *et al.* (5) (n:47)	Lee JJ *et al.* (11) (n:52)	Yeh JJ *et al.* (9) (n:84)	Hatipoglu *et al.* (10) (n:32)	Tozkoparan *et al.* (6) (n:52)	Lai FM *et al.* (12) (n:14)
Micronodules	87	–	100	–	–	71[c]	–
Large nodules	63	85	88[a]	–	69[b]	–	–
CNs	62	86	–	51.2	91	73	93
TIB pattern	41	45	87	53.6	71	–	57
BWT	36	–	–	82.1	44	–	–
Bronchiectasis	32	21	29	–	56	63	64
Mediastinal LAP	29	–	–	–	–	15	64
Consolidation	28	9	73	85.7	44	54	–
Calcified nodule	28	–	–	–	–	37	–
Cavity	26	26	73	60.7	50	33	64
GGO	26	–	6	88.1	38	10	–
Pleural Thickening	21	–	54	–	63	–	36
IST	13	49	–	65.5	34	–	71
Mass	12	15	42	–	–	–	–
Emphysema	6	–	–	–	44	6	–
Bulla	4	–	2	–	–	–	–

Data presented as %. HRCT, high resolution computed tomography; CNs, centrilobular nodules; TIB, tree-in-bud; IST, interlobular septal thickening; BWT, bronchial wall thickening; GGO, ground glass opacity. [a], Large nodules are defined as nodules in the study; [b], Large nodules and micronodules are defined as nodules in the study; [c], Micronodules and large nodules are defined as nodules in the study.

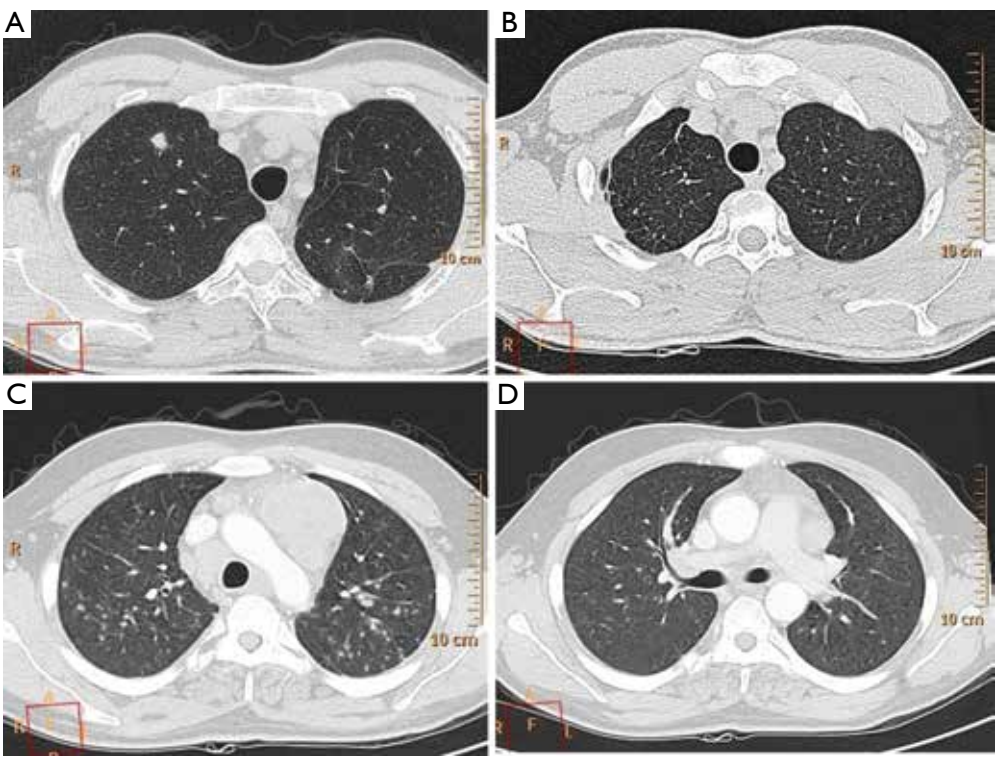

Figure 1 Some HRCT patterns. (A) Large nodule; (B) Micronodule; (C) Tree-in-bud pattern; (D) Centrilobular nodule.

there was no statistically significant difference between the groups. Micronodules are known as acute inflammatory lesions and are one of the radiological lesions showing active PTB (11). Lee *et al.* found that micronodules were present in all of the patients with newly diagnosed and bacteriogically proven PTB and were the most common HRCT finding seen in active PTB, as in our study (11). Micronodules are most often seen in the acute early stages of PTB and consist of solid caseous material within or around the terminal or respiratory bronchioles (16). Matsuoka *et al.* reported that computed tomography findings do not reliably discriminate between smear-negative patients and those with very few AFB excreting smear-positive patients, and the frequency of micronodules did not significantly differ among the four groups of smear-negative and smear- positive PTB (17). We suggest that micronodules appear to be a useful radiologic HRCT finding for both smear negative culture-positive and smear-negative culture-negative PTB.

Centrilobular lesions on HRCT are highly sensitive and specific for active PTB (12). The presence of centrilobular nodules tended to be higher in Group 1 in our study, but there was no statistically significant difference between the two groups. Tozkoparan *et al.* showed that centrilobular nodules were the most common HRCT finding and were significantly more common in active smear-negative PTB (6). Hatipoglu *et al.* also found that centrilobular lesions or branching linear structures, with a tree-in-bud appearance and macronodules were most commonly seen in cases of active PTB (10). If there is a clinical suspicion, an HRCT can be used to show centrilobular lesions, which are the most common findings of early bronchogenic spread (10). Yeh *et al.* reported that the absence of centrilobular nodules (51.2%) is one of the five variables that are independent risk factors predictive of smear-positive, active PTB (9). Kosaka *et al.* determined that the frequency of centrilobular opacities did not differ between smear-negative and smear-positive PTB (18). We found large nodules in 63% of the patients with smear-negative PTB. Nakanishi *et al.* found that large nodules were seen in 85% of the patients with smear-negative culture-positive PTB, and large nodules were significantly associated with an increase in the risk for PTB (5). The presence of large nodules in Group 1 tended to be greater in our study, but there was no significant difference between the two groups.

We found a tree-in-bud pattern in 48% of the patients with smear-negative culture-positive PTB and in 29% of the patients with smear-negative culture-negative PTB. Tree-in-bud patterns on CT were first used by Im *et al.* for the endobronchial spread of PTB (16). The tree-in-bud pattern is thought to be a reliable criterion for active disease, but not pathognomonic for active PTB. Raniga *et al.* reported that the tree-in-bud pattern, suggestive of endobronchial spread, and hence active disease, was the most common and characteristic of the findings on HRCT (8). Although the smear-negative culture-positive group more frequently tended to have the tree-in-bud pattern, there was no significant difference in the tree-in-bud pattern between the two groups.

The radiologic findings of PTB occur in the apical or posterior segment of the upper lobes in the majority of adult patients and in the superior segment of the lower lobes in others (19). In our study, the HRCT findings were observed mainly in the right and left upper lobes. There was no significant difference in the lobar predominance (upper lobe *vs.* middle or lower lobes) between the groups. Okutan *et al.* also reported similar localizations on HRCT in those patients with smear-negative culture-negative PTB requiring a histopathological examination (20).

The main limitation in this study was the lack of HIV positive patients. Smear-negative PTB is more common among HIV positive patients (21). The HRCT findings of HIV positive patients with smear-negative PTB may be different or atypical (21,22). Other medical conditions may be misdiagnosed as smear-negative PTB and may complicate the clinical diagnosis of PTB in HIV positive patients (23). Reader experience is also another limitation in our study.

Conclusions

Significant differences were not observed in the HRCT findings and clinical features of smear-negative culture-positive and smear-negative culture-negative PTB patients. We conclude that it is not possible to predict those patients whose culture specimens will ultimately prove to be positive among those patients with smear-negative PTB according to their clinical features and HRCT findings. Additionally, the HRCT and clinical features were suboptimal for distinguishing smear-negative culture-positive PTB from smear-negative culture-negative PTB in this study. Thus, in cases of smear-negative and culture-negative PTB, the patient with compatible clinical and radiological features should be considered for tuberculosis treatment.

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References

1. 2012 WHO. Global Tuberculosis Report 2012. Available online: http://www.who.int/tb/publications/global_report/gtbr12_main.pdf
2. Erdem H, Akova M. Leading infectious diseases problems in Turkey. Clin Microbiol Infect 2012;18:1056-67.
3. Hansell DM, Bankier AA, MacMahon H, et al. Fleischner Society: glossary of terms for thoracic imaging. Radiology 2008;246:697-722.
4. Tostmann A, Kik SV, Kalisvaart NA, et al. Tuberculosis transmission by patients with smear-negative pulmonary tuberculosis in a large cohort in the Netherlands. Clin Infect Dis 2008;47:1135-42.
5. Nakanishi M, Demura Y, Ameshima S, et al. Utility of high-resolution computed tomography for predicting risk of sputum smear-negative pulmonary tuberculosis. Eur J Radiol 2010;73:545-50.
6. Tozkoparan E, Deniz O, Ciftci F, et al. The roles of HRCT and clinical parameters in assessing activity of suspected smear negative pulmonary tuberculosis. Arch Med Res 2005;36:166-70.
7. Wang YH, Lin AS, Lai YF, et al. The high value of high-resolution computed tomography in predicting the activity of pulmonary tuberculosis. Int J Tuberc Lung Dis 2003;7:563-8.
8. Raniga S, Parikh N, Arora A, et al. Is HRCT Reliable In Detecting Disease Activity In Pulmonary Tuberculosis? Indian J Radiol Imaging 2006; 16:221-8.
9. Yeh JJ, Yu JK, Teng WB, et al. High-resolution CT for identify patients with smear-positive, active pulmonary tuberculosis. Eur J Radiol 2012;81:195-201.
10. Hatipoğlu ON, Osma E, Manisali M, et al. High resolution computed tomographic findings in pulmonary tuberculosis. Thorax 1996;51:397-402.
11. Lee JJ, Chong PY, Lin CB, et al. High resolution chest CT in patients with pulmonary tuberculosis: characteristic findings before and after antituberculous therapy. Eur J Radiol 2008;67:100-4.
12. Lai FM, Liam CK, Paramsothy M, et al. The role of 67 gallium scintigraphy and high resolution computed tomography as predictors of disease activity in sputum smear-negative pulmonary tuberculosis. Int J Tuberc Lung Dis 1997;1:563-9.
13. Lee KS, Kim TS, Han J, et al. Diffuse micronodular lung disease: HRCT and pathologic findings. J Comput Assist Tomogr 1999;23:99-106.
14. Fujita J, Higa F, Tateyama M. Radiological findings of mycobacterial diseases. J Infect Chemother 2007;13:8-17.
15. Fujita J, Bandoh S, Kubo A, et al. HRCT shows variations in appearance in disseminated tuberculosis in adults. Int J Tuberc Lung Dis 2006;10:222-6.
16. Im JG, Itoh H, Shim YS, et al. Pulmonary tuberculosis: CT findings--early active disease and sequential change with antituberculous therapy. Radiology 1993;186:653-60.
17. Matsuoka S, Uchiyama K, Shima H, et al. Relationship between CT findings of pulmonary tuberculosis and the number of acid-fast bacilli on sputum smears. Clin Imaging 2004;28:119-23.
18. Kosaka N, Sakai T, Uematsu H, et al. Specific high-resolution computed tomography findings associated with sputum smear-positive pulmonary tuberculosis. J Comput Assist Tomogr 2005;29:801-4.
19. Jeong YJ, Lee KS. Pulmonary tuberculosis: up-to-date imaging and management. AJR Am J Roentgenol. 2008;191:834-44.
20. Okutan O, Tas D, Demirer E, et al. The clinical and radiological features of patients with smear and culture-negative pulmonary tuberculosis requiring histopathological examination. Turk Gogus Kalp Damar Cerrahısı Dergısı-Turkısh Journal of Thoracıc And Cardıovascular Surgery 2012;20:572-6.
21. Siddiqi K, Lambert ML, Walley J. Clinical diagnosis of smear-negative pulmonary tuberculosis in low-income countries: the current evidence. Lancet Infect Dis 2003;3:288-96.
22. Laissy JP, Cadi M, Boudiaf ZE, et al. Pulmonary tuberculosis: computed tomography and high-resolution computed tomography patterns in patients who are either HIV-negative or HIV-seropositive. J Thorac Imaging 1998;13:58-64.
23. Colebunders R, Bastian I. A review of the diagnosis and treatment of smear-negative pulmonary tuberculosis. Int J Tuberc Lung Dis 2000;4:97-107.

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Original Article

The performance and limitation of T-SPOT.TB for the diagnosis of TB in a high prevalence setting

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Background: Tuberculosis (TB) diagnosis remains difficulty. The previous reports have shown that the T-SPOT.TB assay may be a more promising diagnostic tool for TB, however, it needs a further study to evaluate the diagnostic value of T-SPOT.TB for the specific populations in a high prevalence setting.

Methods: In this present study, we conducted stratified and comparable analyses to explore the clinical value and the limitation of T-SPOT.TB assay in TB diagnosis in a high TB prevalence setting, Southern China. A total of 413 subjects including 163 pulmonary TB (PTB), 39 extrapulmonary TB (EPTB), 106 non-TB pulmonary diseases (NTBPDs), 20 medical staff and 85 healthy controls were included in the study.

Results: According to T-SPOT.TB, there had a high incidence of latent TB infection (LTBI) in general population in Southern China, especially in the NTBPDS and medical staff. The T-SPOT.TB had a high performance in the diagnosis of active TB (ATB) in a lower risk of TB infection population such as the general population, however, the T-SPOT.TB for the diagnosis of ATB in the high risk of TB infection populations involving close contacts such as the patients with pulmonary diseases (PD) or medical staff isn't reliable due to the interference by LTBI. Under this condition, the value of rule-out of the assay was seemed to be better than that of rule-in. We believed that the T-SPOT.TB is suitable for screening both the EPTB and the ATB combined with diabetes mellitus (DM). However, we found that the sensitivity of T-SPOT.TB in sputum smear-negative population wasn't as high as that in smear-positive population.

Conclusions: The T-SPOT.TB testing results should be interpreted with caution combined with subject's characteristics in a high prevalence setting.

Keywords: T-SPOT.TB; diagnostic tool; tuberculosis (TB); extrapulmonary TB (EPTB)

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Introduction

Tuberculosis (TB) poses a great global threaten to human health. According to the report of the World Health Organization, in 2011, there were an estimated 8.7 million new cases of TB [13% co-infected with human immunoddficiency virus (HIV)] and 1.4 million people died from TB (1). The difficulty in diagnosis hampers the progress of prevention and control for TB. As a classical immunoassay, the tuberculin skin test (TST) has been widely used in the diagnosis of TB for a long time, but the method was found in a poor specificity and accuracy,

mainly due to the cross-reactions of tuberculin purified protein derivatives (PPD) with that induced by BCG (2-4). Recently, the T-SPOT.TB assay has shown to be higher performance for diagnosis of TB. The T-SPOT.TB assay is a simplified enzyme-linked immunospot (ELISPOT) method, which is designed for the detection of effector T cells that respond to stimulation by specific antigens [early secreted antigenic target 6 kDa (ESAT-6) and culture filtrate protein 10 kDa (CFP10)] for *Mycobacterium tuberculosis* (MTB) (3,5-9). ESAT-6 and CFP10 only present in MTB but absent in bacille Calmette-Guerin (BCG) strains, which ensure that the T-SPOT.TB assay could be a high specificity theoretically. The previous reports have shown that the T-SPOT.TB assay may be a more accurate indicator of the presence of LTBI and active TB (ATB), however, most of these studies occurred in the low prevalence settings (10-14). Therefore, it is significant to evaluate the diagnostic value of T-SPOT.TB for the specific populations in a high prevalence setting. In this present study, we conducted stratified and comparable analyses to explore the clinical value and the limitation of T-SPOT.TB assay for TB diagnosis in a high TB prevalence setting, China, one of the 22 high-burden countries (1).

Study populations and methods

Ethical statement

All participants were treated in accordance with the Declaration of Helsinki on the participation of human subjects in medical research. Written informed consent was obtained from each of subjects and the study was approved by the Ethics Committee of Shanghai Pulmonary Hospital.

Study population

A total of 413 subjects were included in the study. The subjects were divided into four groups including ATB group, non-TB pulmonary diseases (NTBPDs) group, medical staff group and healthy controls group. Both the ATB group and the NTBPDs group were randomly selected from the inpatients attending Shanghai Pulmonary Hospital between January 2005 and December 2008, including 202 cases of ATB patients [163 cases of pulmonary TB (PTB) plus 39 cases of extrapulmonary TB (EPTB)] and 106 cases of NTBPDs patients, respectively. In addition, 20 medical staff from Department of Tuberculosis, Shanghai Pulmonary Hospital and 85 healthy volunteers were also enrolled in this study. All subjects were shown to be negative by HIV antibody testing. Amongst ATB group, there were found in 30 cases of patients combined with diabetes mellitus (DM). However, there had no drop-out subjects in this study. The general characteristics included in this study are shown in *Table 1*.

A definite diagnosis of ATB, PTB, and EPTB was made according to the criteria by Chinese Antituberculosis Association, mainly relying on signs and symptoms, an X-ray, computer tomography (CT) and identifying MTB in a clinical sample (sputum or a tissue biopsy). In detail, the definite diagnose of ATB was made on as following: (I) 'culture/biopsy-confirmed ATB' relied on the positive culture or smear of MTB from sputum or biopsy specimen; (II) 'clinical ATB' lack of bacterial/pathological evidence was based on clinical manifest or radiographic responses to anti-TB treatment. NTBLDs are the patients who had no past history of TB, including pneumonia, bronchitis, lung carcinoma and other lung diseases. The diagnostic criteria for smear negative PTB is as follows: (I) had typical clinical symptoms of PTB and identical chest X-ray manifestations; (II) recieved a positive effect with anti-TB treatment; (III) excluded other non-tuberculous PD; (IV) presented positive serum test; (V) had a positive result by PCR for the detection of sputum sample; (VI) the pathology of lung tissue confirmed TB; (VII) detected a positive mycobacterium in bronchoalveolar lavage fluid; (VIII) a tuberculous-like lesion was confirmed in the bronchial and lung tissue by pathology examination. It was confirmed if there had three clauses in former six clauses or one of 7th and 8th clauses. The diagnosis of NTBLDs was made on the bacteriology, clinical manifest and other checks. NTBPDs and DM were independently diagnosed by professional clinicians based on the relevant medicine evidence. Besides, the laboratory staff was blinded to the clinical diagnosis of the subjects when they performed the tests in this present study.

T-SPOT.TB assay

T-SPOT.TB assay was performed following the instructions of the assay kit (Oxford Immunote Ltd., Edinburgh, UK). The procedure is described as following. Peripheral blood mononuclear cells (PBMCs) is separated from a whole blood sample and washed to remove any sources of background interfering signal. Four wells including a nil control, panel A (ESAT-6), panel B (CFP10), and a positive control containing phytohaemagglutinin is required for each sample. The PBMCs is incubated with the antigens and the secreted cytokine by sensitized T cell is captured by specific antibodies

Table 1 General characteristic of the participants in this study

	ATB	NTBPDs	Medical staff	Healthy controls
Total number	202	106	20	85
Median age [range, IQR]	34 [10-87, 21-42]	38 [12-85, 22-45]	30.5 [22-60, 27-36]	28.5 [18-48, 26-35.5]
Male/female	110/92	60/46	10/10	46/39
Presence of TB history	13 (6.4%)	0	0	0
Received drug therapy	5 (2.48%)	0	0	0
Combined diabetes	30	0	0	0
HIV test				
Positive	0	0	0	0
Negative	202	106	20	85
Classification				
Active PTB	163	0	0	0
Active EPTB	39	0	0	0
Pneumonia	0	43 (40.57%)	0	0
Bronchitis	0	32 (30.19%)	0	0
Lung cancer	0	21 (19.81%)	0	0
Other PD	0	10 (9.43%)	0	0
Sputum smear				
Positive	93	0	–	–
Negative	109	106	–	–

TB, tuberculosis; PTB, pulmonary TB; HIV, human immunoddficiency virus; ATB, active tuberculosis; NTBPDs, non-tuberculosis pulmonary diseases; EPTB, extrapulmonary tuberculosis; PD, pulmonary diseases; IQR, interquartile-range.

Table 2 The testing results of T-SPOT.TB in different subgroups

	ATB[#] (N=202)	NTBPDs[#] (N=106)	Medical staff[#] (N=20)	Healthy controls (N=85)
Positive (%)	173 (85.64)	36 (33.96)	6 (30.00)	7 (8.24)
Negative (%)	29 (14.36)	70 (66.04)	14 (70.00)	78 (91.76)

[#], P<0.05, compared with healthy controls; ATB, active tuberculosis; NTBPDs, non-tuberculosis pulmonary diseases.

on the membrane, and then the cells and other unwanted materials are removed by washing. Finally, the cytokine was detected by a chromogenic spot assay. The evaluation of the testing result is interpreted by spot count, according to the following algorithm: (I) the test result is 'Positive' if (panel A minus nil control) and/or (panel B minus nil control) ≥6 spots; and (II) the test result is 'Negative' if both (panel A minus nil control) and (panel B minus nil control) ≤5 spots. This includes values less than zero.

Statistical analysis

Difference in categorical variables was evaluated using chi-square Test. The statistical analyses were performed with the SPSS 13.0 (SPSS Inc., Chicago, Illinois, USA). A P value of less than 0.05 was considered significant.

Results

Infection risk of TB in the PTB, NTBPDs, medical staff and healthy controls

According to the testing results of T-SPOT.TB, the positive rates in the ATB, NTBPDs, medical staff, and healthy controls were 85.64%, 33.96%, 30.00% and 8.24%, respectively. The T-SPOT positive incidence in NTBPDs and medical staff was significantly higher than that in the healthy controls (*Table 2*).

Table 3 Performance of T-SPOT.TB for the diagnosis of active tuberculosis when compared with the healthy controls

T-SPOT	ATB N=202	Controls N=85	Performance (95% CI)				
			Sen (%)	Spe (%)	+LR	–LR	DOR
Positive	173	7	85.64 (80.14-89.81)	91.76 (83.96-95.95)	10.40 (5.10-21.20)	0.16 (0.11-0.22)	66.47 (27.92-158.28)
Negative	29	78					

ATB, active tuberculosis; Sen, sensitivity; Spe, specificity; +LR, positive likelihood ratio; –LR, negative likelihood ratio; DOR, diagnostic odds ratio.

Table 4 Performance of T-SPOT.TB for the diagnosis of active tuberculosis when compared with the non-tuberculosis pulmonary diseases

T-SPOT	ATB (N=202)	NTBPDs (N=106)	Performance (95% CI)				
			Sen (%)	Spe (%)	+LR	–LR	DOR
Positive	173	36	85.64 (80.14-89.81)	66.04 (56.6-74.35)	2.52 (1.92-3.31)	0.22 (0.15-0.31)	11.6 (6.61-20.36)
Negative	29	70					

ATB, active tuberculosis; NTBPDs, non-tuberculosis pulmonary diseases; Sen, sensitivity; Spe, specificity; +LR, positive likelihood ratio; –LR, negative likelihood ratio; DOR, diagnostic odds ratio.

Table 5 Comparison of the sensitivities of T-SPOT.TB between active PTB and extrapulmonary TB

T-SPOT	APTB (N=163)	EPTB (N=39)	P
Positive	137	36	
Negative	26	3	>0.05
Sensitivity (%, 95% CI)	84.05 (77.65-88.88)	92.31 (79.68-97.35)	

TB, tuberculosis; PTB, pulmonary TB; EPTB, extrapulmonary TB; APTB, active pulmonary tuberculosis.

The performance of T-SPOT.TB for the diagnosis of ATB

Compared with the healthy controls, the sensitivity, specificity, likelihood ratio positive (+LR), likelihood ratio negative (–LR), and diagnostic odd ratio (DOR) of T-SPOT.TB for the diagnosis of ATB were 85.64%, 91.76%, 10.40%, 0.16%, and 66.47%, respectively (*Table 3*). These results were shown to be a higher performance in the diagnosis of ATB in a healthy population, but not perfect.

When compared with the NTBPDs, the sensitivity, specificity, +LR and –LR, and DOR of T-SPOT.TB for the diagnosis of ATB were 85.64%, 66.04%, 2.52%, 0.22%, and 11.6%, respectively (*Table 4*). The specificity, +LR, and DOR were significantly lower than those in *Table 3* (P<0.05), indicating that T-SPOT.TB had a lower performance in the diagnosis of ATB in the NTBPDs population.

Subgroup analyses of the sensitivities of T-SPOT.TB in the diagnosis of ATB

Both APTB group and EPTB group were shown to have high sensitivities. No significant difference was found in the sensitivities of T-SPOT.TB between two groups (*Table 5*). Subgroup analysis revealed that the sensitivity of T-SPOT.TB in the diagnosis of ATB wasn't subjected to diabetes (*Table 6*). According to the testing results stratified by sputum smear microscopy, the sensitivity in sputum smear-positive group was significantly higher than that in smear-negative group (*Table 7*).

Discussion

As a promising approach for the diagnosis of ATB and latent TB infection (LTBI), it is inspiring of the clinical application of the interferon-gamma release assays (IGRAs) including QuantiFERON TB Gold (QFT-G; Cellestis, Ltd., Carnegie, Australia) and T-SOPT.TB. However, for T-SOPT.TB, there still need to accumulate clinical evidences in different settings and populations to validate its potential values and limitations. In this study, we investigated the TB infection in the populations including

Table 6 Comparison of the sensitivities of T-SPOT.TB in the diagnosis of active TB with or without diabetes

T-SPOT	Diabetes (N=30)	Non-diabetes (N=172)	P
Positive	29	144	
Negative	1	28	>0.05
Sensitivity (%, 95% CI)	96.67 (83.33-99.41)	83.72 (77.48-88.49)	

TB, tuberculosis.

Table 7 The testing results of T-SPOT.TB for ATB stratified by sputum smear microscopy

T-SPOT	Smear positive (N=93)	Smear negative (N=109)	P
Positive	87	86	
Negative	6	23	<0.05
Sensitivity (%, 95% CI)	93.55 (86.63-97.01)	78.90 (70.32-85.51)	

ATB, active tuberculosis.

ATB, NTBPDs, medical staff, and healthy controls by T-SOPT.TB assay.

According to the results of T-SPOT.TB, the positive rates in the NTBPDs, medical staff, and healthy controls were 33.96%, 30.00% and 8.24%, respectively. This demonstrates that the healthy controls in Southern China may be a high incidence of LTBI. Surprisingly, there were found in more than 30% suspected LTBI in the NTBPDs and medical staff. We postulates that the increasing expose factor in medical settings may play an important role in this outcome, which suggests that it is urgent and important to improve the personal safety protection in the hospital setting of high prevalence area.

When compared with a low risk of TB infection population (the healthy controls), the sensitivity, specificity, +LR, –LR, and DOR of T-SPOT.TB for the diagnosis of ATB were 85.64%, 91.76%, 10.40%, 0.16%, and 66.47%, respectively. It suggests that the T-SPOT.TB had a higher performance in the diagnosis of ATB in a lower risk of TB infection population. In contrast, the previous studies in low prevalence setting revealed that the T-SPOT.TB had a very high specificity (90-100%) (11,13,15).

However, when compared with a high risk of TB infection population (the NTBPDs controls), the sensitivity, specificity, +LR, –LR, and DOR of T-SPOT.TB for the diagnosis of ATB were 85.64%, 66.04%, 2.52%, 0.22%, and 11.6%, respectively. The specificity, +LR, and DOR were lower, indicating T-SPOT.TB had a lower performance (especially for specificity) in the diagnosis of ATB in a high risk of TB infection population. Therefore, we believed that the performance of T-SPOT.TB for the diagnosis of ATB in a high risk of TB infection population isn't as good as that in a low risk of TB infection population, and that the high incidences of LTBI interfere in the diagnosis of ATB. So, it need be cautious for interpreting the testing results of T-SPOT.TB for the diagnosis of ATB in the high risk of TB infection populations such as the patients with PD or medical staff or close contacts. Under this condition, the value of rule-out is seemed to be better than that of rule-in.

According to a meta-analysis in 2011 (16), the specificity of the IGRAs for the diagnosis of LTBI, IGRAs varied 98-100%. IGRAs positivity was clearly associated with exposure to contagious TB cases. In conclusion, IGRAs may have a relative advantage over the TST in detecting LTBI and allow the exclusion of TB infection with higher reliability. Another meta-analysis including a combined sample size of 26,680 participants revealed that IGRAs didn't have high accuracy for the prediction of ATB, although use of IGRAs in some populations might reduce the number of people considered for preventive treatment (17). However, because that these meta-analysis didn't conduct subgroup analyses of specific populations, which is different from our findings. Our study revealed that IGRAs may have the diagnostic value of rule-out in a high risk of TB infection population; however, our study also demonstrated that IGRAs for the diagnosis of ATB in a low risk of TB infection population had high accuracy and DOR. However, according to an updated meta-analysis of the diagnostic accuracy of IGRAs for TB disease (18), the overall sensitivity, specificity, +LR, –LR and DOR of IGRAs were 0.85 (95% CI: 0.84-0.86), 0.84 (95% CI: 0.83-0.85), 7.82 (95% CI: 6.01-10.19), 0.17 (95% CI: 0.14-0.21), and 59.27 (95% CI: 40.19-

87.42), respectively. For ten studies evaluating T-SPOT TB in China, the combined sensitivity, specificity, +LR, –LR and DOR were 0.88 (95% CI: 0.86-0.91), 0.89 (95% CI: 0.86-0.92), 8.86 (95% CI: 5.42-14.46), 0.13 (95% CI: 0.10-0.17), and 88.15 (95% CI: 41.76-186.07), respectively. Compared with this meta-analysis, the specificity, +LR and DOR of IGRAs for detecting ATB in a high risk of TB infection population in this study is significantly lower. However, there had no difference in the sensitivities between two studies.

In this study, we also conducted the subgroup analyses of the sensitivities of T-SPOT.TB for the diagnosis of ATB according to PTB and EPTB, with/without DM, and sputum smear-positive and smear-negative. We found that both active PTB group and EPTB group were shown to have the higher sensitivities. No significant difference was found in the sensitivity between two groups. Given that the diagnosis of EPTB is very difficult at present, it is valuable that the saving time assay achieves such a high sensitivity. In this study, there were 30 cases of ATB patients with DM. In order to clarify whether the alternation of immune status induced by DM interferes in the T-SPOT.TB sensitivity or not, we performed a subgroup analysis for the DM group and NDM group. The results revealed that the sensitivity of T-SPOT.TB for the diagnosis of ATB wasn't subjected to diabetes, which indicates that T-SPOT.TB is also suitable for screening of ATB with DM.

We found that the T-SPOT.TB sensitivity for diagnosis of ATB in sputum smear-positive group was significantly higher than that in smear-negative group. We postulate that TB bacilli numbers is likely to affect the T-SPOT.TB. The previous study showed that IFN-gamma-producing RD1-specific T cells, as measured in the T-SPOT.TB assay, may be directly related to bacterial load in patients undergoing treatment for PTB (19). The study included in 491 smear-negative children from two hospitals in Cape Town, South Africa revealed that in a high-burden setting, the T-SPOT.TB did not have added value beyond clinical data and conventional tests for diagnosis of TB disease in smear-negative children (20). However, it needs a further study to confirm.

It must be pointed out this study had some limitations. The sample numbers of EPTB, DM, and medical staff is small, which may result in low validations of evidence. Additionally, due to the lack of the "gold standard" of LTBI diagnosis (21,22), we couldn't evaluate the complete performance of T-SPOT.TB for the diagnosis of LTBI.

According to T-SPOT.TB, there has a high incidence of LTBI in the general population in Southern China, especially in the NTBPDs and medical staff. The T-SPOT.TB had a higher performance in the diagnosis of ATB in a low risk of TB infection population, but the T-SPOT.TB for diagnosis of ATB in the high risk of TB infection populations involving close contacts such as the patients with PD or medical staff isn't reliable due to the interference by LTBI. T-SPOT.TB is suitable for screening the PTB, EPTB and the ATB combined with DM. However, the T-SPOT.TB sensitivity in sputum smear-negative population isn't as high as that in smear-positive population. Therefore, we believe that the T-SPOT.TB testing results should be interpreted with caution combined with subject's characteristics in a high prevalence setting.

Conclusions

The T-SPOT.TB assay for the diagnosis of TB has some limitations in a high prevalence setting and its clinical significance should be interpreted with caution combined with subject's characteristics.

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References

1. World Health Organization. Country profiles. Global tuberculosis report 2012.
2. Ramos JM, Robledano C, Masiá M, et al. Contribution of interferon gamma release assays testing to the diagnosis of latent tuberculosis infection in HIV-infected patients: a comparison of QuantiFERON-TB Gold In Tube, T-SPOT.TB and tuberculin skin test. BMC Infect Dis 2012;12:169.
3. Zhou L, Shen S, He M, et al. T-SPOT.TB in the diagnosis of tuberculous peritonitis. Zhong Nan Da Xue Xue Bao Yi Xue Ban 2013;38:526-31.
4. Lee YM, Park KH, Kim SM, et al. Risk factors for false-

negative results of T-SPOT.TB and tuberculin skin test in extrapulmonary tuberculosis. Infection 2013;41:1089-95.
5. Lalvani A, Nagvenkar P, Udwadia Z, et al. Enumeration of T cells specific for RD1-encoded antigens suggests a high prevalence of latent Mycobacterium tuberculosis infection in healthy urban Indians. J Infect Dis 2001;183:469-77.
6. Pathan AA, Wilkinson KA, Klenerman P, et al. Direct ex vivo analysis of antigen-specific IFN-gamma-secreting CD4 T cells in Mycobacterium tuberculosis-infected individuals: associations with clinical disease state and effect of treatment. J Immunol 2001;167:5217-25.
7. Lalvani A, Pathan AA, Durkan H, et al. Enhanced contact tracing and spatial tracking of Mycobacterium tuberculosis infection by enumeration of antigen-specific T cells. Lancet 2001;357:2017-21.
8. Lalvani A, Pathan AA, McShane H, et al. Rapid detection of Mycobacterium tuberculosis infection by enumeration of antigen-specific T cells. Am J Respir Crit Care Med 2001;163:824-8.
9. Zhao J, Wang Y, Wang H, et al. Low agreement between the T-SPOT®.TB assay and the tuberculin skin test among college students in China. Int J Tuberc Lung Dis 2011;15:134-6.
10. Sultan B, Benn P, Mahungu T, et al. Comparison of two interferon-gamma release assays (QuantiFERON-TB Gold In-Tube and T-SPOT.TB) in testing for latent tuberculosis infection among HIV-infected adults. Int J STD AIDS 2013;24:775-9.
11. Talbot EA, Harland D, Wieland-Alter W, et al. Specificity of the tuberculin skin test and the T-SPOT.TB assay among students in a low-tuberculosis incidence setting. J Am Coll Health 2012;60:94-6.
12. Bienek DR, Chang CK. Evaluation of an interferon-gamma release assay, T-SPOT.TB, in a population with a low prevalence of tuberculosis. Int J Tuberc Lung Dis 2009;13:1416-21.
13. Barsegian V, Mathias KD, Wrighton-Smith P, et al. Prevalence of latent tuberculosis infection in German radiologists. J Hosp Infect 2008;69:69-76.
14. Cheallaigh CN, Fitzgerald I, Grace J, et al. Interferon gamma release assays for the diagnosis of latent TB infection in HIV-infected individuals in a low TB burden country. PLoS One 2013;8:e53330.
15. Adams LV, Waddell RD, Von Reyn CF. T-SPOT. TB Test(R) results in adults with Mycobacterium avium complex pulmonary disease. Scand J Infect Dis 2008;40:196-203.
16. Diel R, Goletti D, Ferrara G, et al. Interferon-γ release assays for the diagnosis of latent Mycobacterium tuberculosis infection: a systematic review and meta-analysis. Eur Respir J 2011;37:88-99.
17. Rangaka MX, Wilkinson KA, Glynn JR, et al. Predictive value of interferon-γ release assays for incident active tuberculosis: a systematic review and meta-analysis. Lancet Infect Dis 2012;12:45-55.
18. Dai Y, Feng Y, Xu R, et al. Evaluation of interferon-gamma release assays for the diagnosis of tuberculosis: an updated meta-analysis. Eur J Clin Microbiol Infect Dis 2012;31:3127-37.
19. Ribeiro S, Dooley K, Hackman J, et al. T-SPOT.TB responses during treatment of pulmonary tuberculosis. BMC Infect Dis 2009;9:23.
20. Ling DI, Nicol MP, Pai M, et al. Incremental value of T-SPOT.TB for diagnosis of active pulmonary tuberculosis in children in a high-burden setting: a multivariable analysis. Thorax 2013;68:860-6.
21. Lagrange PH, Simonney N, Herrmann JL. New immunological tests in the diagnosis of tuberculosis. Rev Mal Respir 2007;24:453-72.
22. Moczko J, Słomko Z, Breborowicz G. Mathematical foundations for biophysical methods in fetal monitoring. II. Cardiotocography. Ginekol Pol 1989;60:343-9.

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Original Article

Vaspin and lipocalin-2 levels in severe obsructive sleep apnea

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Background: Vaspin and lipocalin-2 are less-known recent members of adipocytokine family. There are ongoing studies investigating the role of vaspin ve lipocalin-2 in metabolic syndrome (MS). Obstructive sleep apnea syndrome (OSAS) is independently associated with an increased prevalence of MS. We aimed to measure the levels of vaspin and lipocalin-2 which are secreted from adipocytes in patients with severe OSAS and examine the relationship between these two adipocytokines and OSAS.

Methods: The study consisted of two groups: severe OSAS patients with an apnea-hypopnea index (AHI) of >30/h (OSAS group, 34 subjects) and age-matched healthy volunteers with a AHI <5/h (control group, 25 subjects) Serum levels of vaspin and lipocalin-2 in these two groups were compared.

Results: Serum levels of vaspin were significantly lower in OSAS group; patients with severe OSAS compared with control group; healthy volunteers (OSAS group: 0.69±0.5 *vs.* control group: 1.24±1.13; P=0.034). The difference between the two groups in terms of serum levels of lipocalin-2 has not reached statistical significance (OSAS group: 61.6±18.2 *vs.* control group: 68.5±20.1; P=0.17).

Conclusions: We found that serum vaspin levels were significantly lower in patients with severe OSAS compared with healthy controls. Lipocalin-2 levels were similar. The decrease in serum vaspin levels in severe OSAS patients may be important in diagnosis and follow-up of these patients.

Keywords: Obstructive sleep apnea syndrome (OSAS); vaspin; lipocalin-2

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Background

Obstructive sleep apnea syndrome (OSAS) is a disorder characterized by repetitive obstructions of the upper airway with a prevalance of 2-4% (1-3). Repetitive pauses of airflow cause a reduction in oxygen saturation (4). OSAS is usually associated with obesity, increased cardiovascular disease, hypertension, dyslipidemia and diabetes mellitus (5-7).

Adipocytes secrete numerous molecules called adipocytokines which are suspected to have a role in the pathogenesis of metabolic syndrome (MS) (8). Vaspin and lipocalin-2 are the two members of this adipocytokine family. A serine protease inhibitor, vaspin is an insulin-sensitizing adipocytokine. An increase in serum vaspin levels was suggested to be a compensatory response to antagonize the activity of the proteases expressed in insulin resistance and obesity. In another words, a high vaspin level has a defensive effect against insulin resistance (9).

Lipocalin-2 was reported to be associated with obesity and insulin resistance in humans and rats (10). Similarly, an association between lipocalin-2 and MS, dyslipidemia, hyperinsulinemia and hyperglycemia was also reported. Lipocalin-2 levels were found higher in patients with coronary heart disease (11).

Studies on vaspin and lipocalin-2 pointed out the importance of these molecules for a better understanding

of MS and its components. Recently, Kim *et al*. suggested that the presence of OSAS even in nonobese individuals was significantly associated with impaired glucose metabolism, which can be responsible for future risk for diabetes and cardiovascular disease (12). So, we wondered how vaspin and lipocalin-2 levels, which are closely related with glucose metabolism alter in patients with OSAS who have an increased glucose intolerance and obesity incidence. Therefore, in this study we aimed to measure the levels of vaspin and lipocalin-2 which are secreted from adipocytes in patients with severe OSAS and examine the relationship between these two adipocytokines and OSAS.

Materials and methods

Study group

Patients were selected from polysomnography (PSG) studies at Bezmialem Foundation University Sleep Laboratory in a duration of nine months. Patients whose ages were between 25 and 65 with recently diagnosed severe OSAS with an apnea-hypopnea index (AHI) >30/h and healthy volunteers with an AHI <5/h were included into our study. Patients with diabetes mellitus, malignancy, chronic renal disease, chronic liver disease, psychiatric disorders, uncontrolled hypertension, coronary artery and cerebrovascular disease, patients with sleep disorders other than OSAS such as upper airway resistance syndrome, periodic leg movement syndrome, or narcolepsy and pregnants were excluded from the study. All volunteers underwent a thorough pysical examination and their height, weight, waist circumference and neck circumference were recorded. Weight and height were measured to the nearest kilogram and centimeter, respectively, and body mass index (BMI) was calculated [BMI = weight/(height)^2]. Neck circumference was measured at the cricothyroid level, waist circumference in the middle between the 12th rib, and the iliac crest by a measure tape. Fasting glucose level, urea, creatinine, triglycerides, total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), fasting serum insulin level, HbA1c (glycated hemoglobin), complete blood count, free T3, free T4 and TSH were analyzed in all subjects. Serum of subjects was stored at –80 ℃ in order to measure vaspin; lipocalin-2 levels. A written consent from all subjects and the approval of the ethics committee of the Foundation University of Bezmialem were obtained.

Polysomnography (PSG)

A Compumedics E 3142 PSG device was used (Compumedics Inc., Melbourne, Australia). PSG findings were evaluated based on the guidelines published by American Academy of Sleep Medicine (AASM) in 2007, and the diagnosis of OSAS was confirmed (13). The average number of episodes of AHIwas calculated. Apnea was defined as complete cessation of airflow ≥10 s. Hypopnea was defined as a reduction of more than 50% of three respiratory signals, airflow signal or either respiratory or abdominal signals of respiratory inductance plethysmography, with an associated decrease of ≥3% in oxygen saturation or an arousal. OSAS was defined as an AHI ≥5/h with associated symptoms (sleep attacks or excessive daytime sleepiness), unsatisfying sleep, fatigue or insomnia, or heavy snoring and/or breathing pauses reported by the subject's partner or an AHI ≥15/h regardless of associated symptoms. OSAS was defined as an AHI of ≥5/h plus clinical symptoms. Patients with an AHI of ≥30/h were included in OSAS group (patients with severe OSAS, n=34) and subjects with an AHI of <5/h were included in control group (normal controls, n=25). The PSG data was scored by three investigators.

Blood assay

Serum of all subjects was obtained from the venous blood samples taken in tubes with gel seperator between 08:00-09:00 hours in the morning after an average of 12 hours of fasting by centrifugation 3,600 rev/min for 10 minutes. The homeostasis model assessment insulin resistance index (HOMA-IR), as a measure of insulin sensitivity, was calculated as fasting insulin concentration (μu/mL) × fasting glucose concentration (mmol/L)/22.5 (14).

Serum of the subjects for the measurement of vaspin and lipocalin-2 was transferred into eppendorf tubes and stored at –80 ℃ until the day of the analysis. On the study day, vaspin ve lipocalin-2 levels were measured from the samples reached room temperature using a commercial enzyme immunoassay kit (Biovendor, Modrice, Czech Republic) according to the manufacturer's instructions in an analyzer brand named Thermo Scientific Multiskan FC (USA). Samples were measured in duplicate, and the average was used in the data analysis.

Statistical analysis

Statistical Package for Social Sciences (SPSS) for Windows

Table 1 Comparison of anthropometric and biochemical characteristics of two groups

	Group A: OSAS patients (n=34) Mean ± SD	Group B: normal controls (n=25) Mean ± SD	P	Type of distribution
Age (years)	44.82±9.03	42.4±7.81	0.286	ND
BMI (kg/m^2)	31.44±3.97	29.88±6.77	0.310	ND
Waist circumference (cm)	96.29 ±5.88	90.96±9.76	0.020	ND
Neck circumference (cm)	40.03±4.65	37.80±4.29	0.065	ND
Vaspin (ng/mL)	0.690±0.499	1.237±1.134	0.034	ND
Lipocalin-2 (ng/mL)	61.56±18.21	68.53±20.10	0.170	ND
Insulin (mU/L)	16.99±8.41	12.39±8.66	0.045	ND
HOMA-IR	4.10±2.16	3.05±2.98	0.128	ND
HbA1c (%)	5.53±0.33	5.37±0.33	0.063	ND
Fasting glucose (mg/dL)	96.06±10.21	92.52±10.07	0.191	ND
Total cholesterol (mg/dL)	206.85±40.36	200.8±34.6	0.550	ND
Triglyceride (mg/dL)	120.3±45.8	105.5±21.1	0.139	ND
LDL-C (mg/dL)	141.9±34.7	129.7±34.6	0.188	ND
HDL-C (mg/dL)	46.24±8.66	49.84±6.94	0.092	ND
AHI	50.31±21.76	2.32±1.26	0.001	NND
Apne index	24.42±19.12	1.05±0.60	0.001	NND
Desaturation index	4.56±2.38	3.04±0.53	0.001	ND
Low saturation	77.82±11.82	90.68±3.40	0.001	NND
REM AHI	25.59±22.70	5.81±4.70	0.001	NND
Central apnea	1.21±1.60	0.43±0.28	0.035	NND
ESS score	11.41±6.15	9.36±6.72	0.172	ND

Mean ± SD, mean ± standard deviation; OSAS, obstructive sleep apnea syndrome; BMI, body mass index; HOMA-IR, homeostasis model assesment insuline resistance; HbA1c, glycated hemoglobin; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; AHI, apnea hypopnea index; REM AHI, rapid eye movements apnea hypopnea index; ESS, epworth sleepiness score.

20.0 software was used to perform the statistical analysis of the data. The continuous variables were expressed as mean ± standard deviation. One sample Kolmogrov-Sminov test was done to see if the continuous independent variables were normally distributed. Normally distributed independent continuous variales were compared student *t*-test whereas non-normally distributed independent continuous variables were compared with Mann-Whitney U test between two groups. Chi-square test was used for categorical variables. Bivariate correlation analyses were done by Spearman's test. A P value<0.05 was considered statistically significant.

Results

A total of 59 participants (34 from severe OSAS group of whom 8 were women; 25 from healthy volunteers of whom 10 were women) completed the study. There was no statistical difference between OSAS group (patients with severe OSAS group; AHI =49.4±23.1 events/h of sleep) and control group (Healthy volunteers; AHI =2.32±1.26 events/h of sleep) by means of sex, age, BMI, (P=0.175, P=0.286, P=0.31, respectively) (*Table 1*). There was no difference between OSAS group and control group in terms of lipocalin-2 levels while vaspin levels were found to be lower in OSAS group (P=0.34) (*Table 1*). Fasting serum insulin levels in OSAS group were significantly higher (P=0.045). There was no difference between the two groups in terms of fasting glucose, triglycerides, total cholesterol, LDL-C, HDL-C, HbA1c and HOMA-IR (*Table 1*).

The correlation between vaspin and lipocalin-2 levels

Table 2 Correlations between vaspin and anthropometric, biochemical and sleep characteristics of OSAS patients

	r	P value
Age	0.253	0.149
BMI	0.225	0.201
Waist circumference	0.257	0.143
Lipocalin-2	–0.289	0.097
Insulin	–0.010	0.940
HOMA-IR	–0.043	0.747
HbA1c	–0.006	0.962
Fasting glucose	–0.135	0.309
Total cholesterol	–0.036	0.841
Triglyceride	–0.153	0.388
LDL-C	–0.131	0.460
HDL-C	0.047	0.790
AHI	0.155	0.381
Apneaindex	–0.236	0.179
Desaturationindex	–0.027	0.880
Minimum SpO_2	–0.024	0.889
REM AHI	–0.185	0.327
Central apnea index	–0.437	0.042
ESS	–0.129	0.468

OSAS, obstructive sleep apnea syndrome; BMI, body mass index; HOMA-IR, homeostasis model assessment insulin resistance HbA1c, glycated hemoglobin; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; AHI, apne hipopne index; REM AHI, rapid eye movements apnea hypopnea index; ESS, epworth sleepiness score.

Table 3 Correlations between lipocalin-2 and anthropometric, biochemical and sleep characteristics of OSAS patients

	r	P value
Age	0.015	0.931
BMI	0.112	0.529
Waist circumference	0.158	0,373
Vaspin	–0.289	0.097
Insulin	0.115	0.517
HOMA-IR	–0.059	0.659
HbA1c	0.084	0.526
Fasting glucose	0.091	0.494
Total cholesterol	–0.027	0.880
Triglyceride	0.485	0.004
LDL-C	–0.023	0.895
HDL-C	–0.220	0.211
AHI	–0.075	0.674
Apnea index	0.267	0.167
Desaturation index	0.175	0.322
Minimum SpO_2	0.136	0.443
REM AHI	0.191	0.311
Central apnea index	–0.156	0.487
ESS	–0.073	0.680

OSAS, obstructive sleep apnea syndrome; BMI, body mass index; HOMA-IR, homeostasis model assessment insulin resistance HbA1c, glycated hemoglobin; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; AHI, apne hipopne index; REM AHI, rapid eye movements apnea hypopnea index; ESS, epworth sleepiness score.

and anthropometric and biochemical characteristics of the patients with severe OSAS was also evaluated. A significant negative correlation between vaspin levels and central apnea index was found. A positive correlation was also found between serum levels of vaspin and lipocalin-2 but this correlation has not reached statistical significance. Finally, a significant positive correlation was found between triglyceride levels and lipocalin-2 levels in patients with severe OSAS (*Tables 2,3*). PSG results of each group were presented in *Table 1*.

Discussion

Vaspin and lipocalin-2 are recently identified members of adipocytokine family. In this study we measured serum levels of vaspin and lipocalin-2 in patients with OSAS which was found to be closely associated with obesity and insulin resistance. We also aimed to investigate the relationship between these adipocytokines and sleep and metabolic characteristics. Study groups included into our study did not differ in terms of sex, age, BMI, HOMA-IR, HbA1c, and neck circumference. Only waist cimcumference was significantly higher in OSAS group compared with healthy volunteers. We postulated that a linear association might exist between severity of OSAS and serum vaspin and lipocalin-2 levels so we included severe patients with AHI ≥30 events /h of sleep expecting more prominent results.

We failed to find a research paper analyzing vaspin and lipocalin-2 in patients with OSAS. Relatively few studies with other adipocytokines exist. In this study we report a

significantly lower vaspin level in OSAS patients.

Previous studies showed a correlation between high serum vaspin level and obesity and high insulin resistance (15,16). However, this correlation between high serum vaspin level and obesity and high insulin resistance deteriorated in patients with uncontrolled type 2 diabetes and it was shown that serum vaspin levels decreased in these patients (15). Byung-Joon Ko *et al*. evaluated prepubertal 168 boys *vs*. 178 girls and found that serum vaspin levels increased significantly in subjects with obesity and high HOMA-IR (16). In our study groups were similar in terms of obesity and HOMA-IR leading a better assesment of effects of OSAS on vaspin levels.

Wang *et al*. compared 192 patients with OSAS and 144 healthy controls in terms of serum levels of omentin-1 which is an important member of the adipokine family and found significantly lower levels of omentin-1 in patients with OSAS (17). Recurrent episodes of hypoxia in OSAS lead to the release of various adhesion molecules and levels of inflammatory markers such as TNF, IL-6, CRP increases (18). Previously, it was shown that omentin-1 levels decreased in proinflammatory states (19). The finding that omentin-1 levels decreased in OSAS patients may be attributed to the secretion of these proinflammatory markers in OSAS. Similarly, recurrent episodes of hypoxia and increased oxidative stress may have led to a lower serum vaspin level in our study.

Trakada *et al*. compared severe OSAS patients with healthy controls in terms of serum visfatin levels, an another member of the adipocytokine family and found no difference among two groups. They also found a higher HOMA-IR and insulin levels in severe OSAS patients compared with healthy controls (20). Similarly, Makino *et al*. compared 230 patients with mild, moderate and severe OSAS patients in terms of levels of an another adipocytokine, adiponectin and insulin resistance. Adiponectin was shown to have an insulin resistance lowering effect and was found to be decreased in patients with obesity and high insulin resistance (21). While they found increased HOMA-IR levels in severe OSAS patients, they found no difference in terms of adiponectin levels among these three groups (22). In these studies, the compared groups differed in terms of insulin resistance which may have confounded the effects of OSAS on these markers. In our study, HOMA-IR levels were similar in both two groups, severe OSAS group and healthy controls group.

In our study a significant negative correlation between vaspin levels and central apnea index was found. If this finding is supported by larger studies, vaspin may reveal itself as a valuable marker in suspecting a high central apnea index.

There was no significant difference among patients with OSAS and healthy controls in terms of serum levels of lipocalin-2 in our study. No study investigated lipocalin-2 levels in OSAS before but there are studies showing higher lipocalin-2 levels with higher BMI and insulin resistance and lower levels of lipocalin-2 in patients under treatment reducing insulin resistance such as thiazolidinediones (23,24). This difference may be attributed to different underlying pathophysiological mechanisms leading to obesity, insulin resistance and OSAS. Our finding of an inverse relationship between lipocalin-2 and triglycerides is coherent with the findings of previous studies.

The limitation of our study included small sample size, exclusion of patients with mild and moderate OSAS, lack of measurement of serum vaspin and lipocalin-2 levels after continuous positive airway pressure (CPAP) treatment.

Conclusions

In conclusion, we found that serum vaspin levels were lower in severe OSAS patients compared to healthy controls and lipocalin-2 levels did not differ among these groups. The decrease in serum vaspin levels in severe OSAS patients may be important in diagnosis and follow-up of these patients. A study investigating the change in vaspin levels after CPAP treatment may yield important results. Further studies with a larger sample are needed to confirm our results.

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References

1. Bixler EO, Vgontzas AN, Ten Have T, et al. Effects of age on sleep apnea in men: I. Prevalence and severity. Am J Respir Crit Care Med 1998;157:144-8.
2. Bixler EO, Vgontzas AN, Lin HM, et al. Prevalence of sleep-disordered breathing in women: effects of gender. Am J Respir Crit Care Med 2001;163:608-13.
3. Young T, Peppard PE, Gottlieb DJ. Epidemiology of obstructive sleep apnea: a population health perspective. Am J Respir Crit Care Med 2002;165:1217-39.
4. Deegan PC, McNicholas WT. Pathophysiology of obstructive sleep apnoea. Eur Respir J 1995;8:1161-78.

5. Grunstein R, Wilcox I, Yang TS, et al. Snoring and sleep apnoea in men: association with central obesity and hypertension. Int J Obes Relat Metab Disord 1993;17:533-40.
6. Lattimore JD, Celermajer DS, Wilcox I. Obstructive sleep apnea and cardiovascular disease. J Am Coll Cardiol 2003;41:1429-37.
7. Coughlin SR, Mawdsley L, Mugarza JA, et al. Obstructive sleep apnoea is independently associated with an increased prevalence of metabolic syndrome. Eur Heart J 2004;25:735-41.
8. Matsuzawa Y, Funahashi T, Kihara S, et al. Adiponectin and metabolic syndrome. Arterioscler Thromb Vasc Biol 2004;24:29-33.
9. Hida K, Wada J, Eguchi J, et al. Visceral adipose tissue-derived serine protease inhibitor: a unique insulin-sensitizing adipocytokine in obesity. Proc Natl Acad Sci U S A 2005;102:10610-5.
10. Wang Y, Lam KS, Kraegen EW, et al. Lipocalin-2 is an inflammatory marker closely associated with obesity, insulin resistance, and hyperglycemia in humans. Clin Chem 2007;53:34-41.
11. Choi KM, Lee JS, Kim EJ, et al. Implication of lipocalin-2 and visfatin levels in patients with coronary heart disease. Eur J Endocrinol 2008;158:203-7.
12. Kim NH, Cho NH, Yun CH, et al. Association of obstructive sleep apnea and glucose metabolism in subjects with or without obesity. Diabetes Care 2013;36:3909-15.
13. Iber C, Ancoli IS, Chesson AL, et al. The AASM Manual for the Scoring of Sleep and Associated Events: Rules, Terminology, and Technical Specifications. American Academy of Sleep Medicine, Westchester, 2007.
14. Bonora E, Targher G, Alberiche M. Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. Diabetes Care 2000;23:57-63.
15. Youn BS, Klöting N, Kratzsch J, et al. Serum vaspin concentrations in human obesity and type 2 diabetes. Diabetes 2008;57:372-7.
16. Ko BJ, Lee M, Park HS, et al. Elevated vaspin and leptin levels are associated with obesity in prepubertal Korean children. Endocr J 2013;60:609-16.
17. Wang Q, Feng X, Zhou C, et al. Decreased levels of serum omentin-1 in patients with obstructive sleep apnoea syndrome. Ann Clin Biochem 2013;50:230-5.
18. Yokoe T, Minoguchi K, Matsuo H, et al. Elevated levels of C-reactive protein and interleukin-6 in patients with obstructive sleep apnea syndrome are decreased by nasal continuous positive airway pressure. Circulation 2003;107:1129-34.
19. Shibata R, Ouchi N, Kikuchi R, et al. Circulating omentin is associated with coronary artery disease in men. Atherosclerosis 2011;219:811-4.
20. Trakada G, Steiropoulos P, Nena E, et al. Plasma visfatin levels in severe obstructive sleep apnea-hypopnea syndrome. Sleep Breath 2009;13:349-55.
21. Kawano J, Arora R. The role of adiponectin in obesity, diabetes, and cardiovascular disease. J Cardiometab Syndr 2009;4:44-9.
22. Makino S, Handa H, Suzukawa K, et al. Obstructive sleep apnoea syndrome, plasma adiponectin levels, and insulin resistance. Clin Endocrinol (Oxf) 2006;64:12-9.
23. Wang Y, Lam KS, Kraegen EW, et al. Lipocalin-2 is an inflammatory marker closely associated with obesity, insulin resistance, and hyperglycemia in humans. Clin Chem 2007;53:34-41.
24. Yan QW, Yang Q, Mody N, et al. The adipokine lipocalin 2 is regulated by obesity and promotes insulin resistance. Diabetes 2007;56:2533-40.

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Original Article

Comparative study of video-assisted thoracic surgery versus open thymectomy for thymoma in one single center

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Background: Due to the popularity of video-assisted thoracic surgery (VATS) techniques in clinical, thymoma patients via VATS thymectomy are increasing rapidly. However, compared with open thymectomy, the potential superiorities and defects of VATS thymectomy remain controversial.

Methods: A number of 129 patients who underwent thymectomy of early stage thymoma (Masaoka stage I and stage II) in one single center from January 2007 to September 2013 were selected in this retrospective study. Of those patients, 38 thymoma patients underwent VATS thymectomy (VATS group) and 91 underwent open thymectomy (open group) via either transsternal [44] or transthoracic approach [47] in the same period. The postoperative variables, which included postoperative hospital length of stay (LOS), the intensive care unit (ICU) LOS, the entire resection ratio, the number of thoracic drainage tubes, the quantity of output and duration of drainage, were analyzed. Meanwhile, the operation time and blood loss were considered as intraoperative variables.

Results: All thymoma patients in the analysis included 19 thymoma patients with myasthenia gravis, among which five patients via VATS thymectomy and 14 patients via open thymectomy respectively. There was no death or morbidity due to the surgical procedures perioperatively. The ICU LOS, operation time, entire resection ratio, and the number of chest tubes were not significantly different in two groups. The postoperative hospital LOS of VATS thymectomy was shorter than that of open thymectomy (5.26 versus 8.32 days, P<0.001). The blood loss of VATS thymectomy was less than open thymectomy (114.74 versus 194.51 mL, P=0.002). Postoperatively, the quantity of chest tubes output in VATS group was less than that in open thymectomy group (617.86 versus 850.08 mL, P=0.007) and duration of drainage in VATS group was shorter than that in open thymectomy group (3.87 versus 5.22 days, P<0.001).

Conclusions: VATS thymectomy is a safe and practicable treatment for early-stage thymoma patients. Thymoma according with Masaoka staging I-II without evident invading seems to be performed through VATS approach appropriately, which has shorter postoperative hospital LOS, less blood loss and less restrictions to activities, hence patients will recover sooner.

Keywords: Thymoma; thymectomy; video-assisted thoracic surgery (VATS); open surgery

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Introduction

Thymoma is one of the most common mediastinal tumors, which accounts for about 47% of anterior mediastinal tumors (1,2). It seems to be benign tumor but recurs and metastasizes easily, which leads to treatment with challenges. As it is well known, thymectomy was considered as the only curative treatment for resectable thymoma patients (3). Excellent 5- and 10-year-survival rates are noted for completely

resected early stage thymomas (4). Moreover, several findings had indicated that the thymus was involved in myasthenia gravis pathogenesis (5) and the most common indication for video-assisted thoracic surgery (VATS) thymectomy was the treatment of myasthenia gravis (6).

In 1992, Landreneau *et al*. (7) introduced the first thymoma patient with VATS thymectomy and after that, the number of thymoma patients with VATS thymectomy has rapidly increased. However, arguments on this technique always exist. Compared with open thymectomy, the potential superiorities and disadvantages of VATS thymectomy remained controversial. Since 2007, VATS thymectomy has been introduced and conducted in our hospital. From our experience of VATS thymectomy from January 2007 to September 2013, we tried to compare the outcomes of VATS thymectomy to that of open thymectomy including sternotomy and thoracotomy in a single institute retrospectively so as to confirm if patients underwent VATS thymectomy can benefit from this minimal invasive surgery.

Methods

There were 137 consecutive patients who underwent thymectomy diagnosed with early stage thymoma (Masaoka stage I and stage II) in the Cancer Institute & Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College from January 2007 to September 2013. Eight of those thymoma patients who received radiotherapy preoperatively were excluded. Consequently, one hundred and twenty-nine thymoma patients were reviewed retrospectively. We included two groups in our study: one group included 38 patients via VATS thymectomy, and the other group included 91 patients via open thymectomy including either sternotomy or thoracotomy. Computed tomography (CT) was considered the standard diagnostic modality for thymomas (8). All eligible thymoma patients were diagnosed as mediastinal tumor or mass by CT preoperatively and were given clear diagnosis as thymoma by pathology postoperatively, who were restricted to Muller-Hermelink type A, type B (B1/B2/B3) and type AB as well as Massaoka stage I-II. All collected clinical data were analyzed by SPSS 17.0. *T*-tests were performed for all continuous variables, and *Pearson* chi-square test or *Fisher's* exact test were performed for all categoric variables. P values ≤0.05 were considered significant.

Preoperatively, pulmonary function tests and electrocardiogram were necessary for every patient. None of them received needle biopsies. The symptoms of thymoma patients mainly stated include chest pain, tachypnea and cough. Nonetheless, most of thymoma patients who were in hospital were discovered by health physical examination and did not have any symptoms. Nineteen thymoma patients with myasthenia gravis mainly complained of ptosis or weakness but did not require special treatment.

All patients were managed in the thoracic surgery wards or in the intensive care unit (ICU), postoperatively. The symptoms of twelve thymoma patients with myasthenia gravis were improved or disappeared after thymectomy. We did not remove the chest tubes until the quantity of drainage was less than 200 mL routinely. Obviously, the completeness of resection had to be aimed because it was an important prognostic factor for local control. The whole connective membrane or fat tissue covering the lesion was used to confirm thymoma, which was resected completely by pathologists in our study.

Surgical techniques

VATS thymectomy

The procedures resembled what Jurado *et al*. (9) described previously. VATS thymectomy was performed from one side. The patients were placed in the supine position with arms extended, and both one shoulder and ipsilateral backside were raised by slipping pads below. Generally, we made two ports for working and one port for viewing in the intercostal spaces in detail according to the location and the size of the lesion by CT. Single-lung ventilation with a double lumen endotracheal tube was necessary for VATS thymectomy. Carbon dioxide insufflation was not required. The dissection was begun with inferior thymic poles mobilized carefully and thoroughly along the pericardium and anteriorly along the retrosternum. The perimediastinal fat tissue and thymus were swept by endoscopic ultrasonic scalpel as well as both pleural spaces had to be opened so that the mass was resected en bloc. All anterior mediastinal tissue was swept cephalad. The innominate vein was located at the junction with the superior vena cava, and dissection continued until the thymic vein was located; this was then doubly clipped. The dissection was completed by dissection along the left pleura which was performed bluntly to avoid injury to the left phrenic nerve. The resected specimen was then placed in an endoscopic pouch. If the mass was too large, we could enlarge one port appropriately or extract the huge mass piece by piece carefully in the endoscopic pouch to avoid potential intrathoracic spreading. One or two drainage tubes were placed unilaterally.

Transsternal thymectomy

As a common approach of open thoracic surgery, transsternal thymectomy was first described by Blalock *et al*. (10) in 1941, which was adopted by many thoracic surgeons for resectable thymoma. Sternum was incised by an electric motor saw. Bilateral pleural spaces were opened and vein extending from the innominate vein was divided. anteriormediastinal, tissue including the pericardial fat tissue and the removal intact of all poles of thymus was exenterated. Single-lung ventilation was not required. One or two drainage tubes were placed necessarily.

Transthoracic thymectomy

Whether transthoracic thymectomy performed on the left or the right side was based on the location of the thymoma. The patient was positioned on the left or the right lateral supine position that resembled VATS approach, and left or right anterolateral thoracotomy incision was carried out via the 4th intercostal space. Single-lung ventilation was essential as well. Both lobe of thymus were removed by opening bilateral mediastinal pleura. Meanwhile, pericardial fat tissue was swept as much as possible. One or two drainage tubes would be enough in the unilateral thorax.

Results

A number of 129 selected thymectomies were performed in the Cancer Institute & Hospital of Chinese Academy of Medical Sciences from January 2007 to September 2013. Of those, 38 thymoma patients were performed via VATS thymectomy, none of whom transformed to thoracotomy. A number of 91 thymoma patients via open thymectomy including 44 patients via transsternal thymectomy and 47 ones via transthoracic thymectomy. Moreover, 19 thymoma patients with myasthenia gravis, included 5 patients of the ones via VATS thymectomy and 14 patients of the others via open thymectomy respectively. Tumor size was not significantly different between VATS group and open surgery group. Subsequently, it was significantly different between VATS group and sternotomy group (5.4 versus 6.5 cm, P=0.013) as well as between VATS group and thoracotomy group (5.4 versus 7.2 cm, P<0.001). The rest of the data analysed showed that gender, age, BMI, pathological classification, staging, percentage of patients with myasthenia gravis, percentage of patients with complications including hypertension, diabetes mellitus and coronary heart disease as well as percent of main symptoms including tachypnea and cough between VATS thymectomy and open thymectomy were not significantly different. Percentage of chest pain was significantly different between VATS thymectomy and open thymectomy (9 versus 23, P=0.036), taking a selection bias into consideration. Relevant subject characteristics are summarized in *Table 1*.

There was no death or morbidity due to the surgical procedures perioperatively. The ICU length of stay (LOS), operation time, entire resection ratio, and the number of chest tubes were not significantly different in two groups. The postoperative hospital LOS of VATS thymectomy was shorter than that of open thymectomy (5.26 versus 8.32 days, P<0.001). The blood loss of VATS thymectomy was less than open thymectomy (114.74 versus 194.51 mL, P=0.002). Postoperatively, the quantity of chest tubes output in VATS group was less than that in open thymectomy group (617.86 versus 850.08 mL, P=0.007) and duration of drainage in VATS group was shorter than that in open thymectomy group (3.87 versus 5.22 days, P<0.001). Furthermore, compared with patients through transsternal approach, the ones who underwent VATS thymectomy had shorter postoperative hospital LOS (5.26 versus 9.16 days, P<0.001), less operation time (127.63 versus 155.46 min, P=0.024), less blood loss (114.74 versus 214.77 mL, P<0.001), smaller number of chest tubes (1.11 versus 1.41, P=0,001), less quantity of chest tubes output (617.68 versus 806.48 mL, P=0.026) and shorter duration of drainage (3.87 versus 5.68 days, P<0.001). In addition, patients in VATS group had longer operation time (127.84 versus 95.64 min, P=0.002) than that of patients via transthoracic thymectomy while shorter postoperative hospital LOS (5.26 versus 7.53 days, P<0.001), less quantity of chest tubes output (619.68 versus 890.89 mL, P=0.022), shorter duration of drainage (3.78 versus 4.79 days, P=0.009) remained in VATS group. Relevant results are indicated in *Tables 2-4*.

Discussion

Over the past few decades, advances in techniques have extended the application of VATS approach in thoracic surgery, especially thymectomy. In the past, many reports of VATS thymectomy were small-size retrospective researches, with limitation inevitably. In spite of controversies still existing in long-term curative effect compared with open thymectomy, VATS thymetomy is safe and feasible, and yet had short-term benefits in certain aspects. According

Table 1 Patients parameters

Variables	VATS (n=38)	Open (n=91)	P#
Gender (female)	19 (50.00%)	46 (50.55%)	1.000
Age [years]	50.50 [25-72]	49.63 [15-76]	0.921
BMI* [range]	24.97 [18-34]	25.13 [18-34]	0.444
Symptoms (%)			
Chest pain	9 (23.68)	23 (25.27)	0.036
Tachypnea	4 (10.53)	12 (13.19)	0.777
Cough	7 (18.42)	8 (8.79)	0.123
Myasthenia gravis (%)	5 (13.16)	14 (15.38)	0.745
Hypertension (%)	5 (13.16)	11 (12.09)	0.867
Diabetes mellitus (%)	2 (5.26)	0 (0)	0.085
Coronary heart disease (%)	1 (2.63)	1 (1.10)	0.504
Tumor size (cm)	5.4 (2.5-13.0)	6.8 (2.5-13.0)	0.671
Muller-Hermelink classification (%)			0.673
A	2 (5.26)	7 (7.69)	
B1	7 (18.42)	19 (20.88)	
B2	11 (28.95)	16 (17.58)	
B3	2 (5.26)	9 (9.90)	
AB	16 (42.11)	40 (43.96)	
Massaoka staging (%)			0.345
I	17 (44.74)	49 (53.85)	
II	21 (55.26)	42 (46.15)	

*, BMI, body mass index; #, gender, symptoms, myasthenia gravis, hypertension and Massaoka staging were analyzed by *Pearson* chi-square test. Diabetes mellitus, coronary heart disease and Muller-Hermelink classification were analyzed by *Fisher's* exact test. The rest of variables were done by *t*-test. P≤0.05 was regarded as statistic significance.

Table 2 Comparative peioperative parameters

Variables	VATS (n=38)	Open (n=91)	P#
Postoperative hospital LOS* [days]	5.26 [2-13]	8.32 [4-25]	<0.001
ICU* LOS [hours]	4.26 [0-90]	9.49 [0-168]	0.635
Operation time [minutes]	127.63 [30-300]	124.56 [55-270]	0.773
Blood loss [mL]	114.73 [20-600]	194.51 [50-1,500]	0.002
Completeness of covering membrane [%]	36 [95]	80 [88]	0.343
Number of chest tubes	1.11 [1-2]	1.21 [1-2]	0.121
Quantity of chest tube output [mL]	617.68 [100-1,580]	850.08 [100-2,720]	0.007
Duration of drainage [days]	3.87 [2-10]	5.22 [2-10]	<0.001

*, LOS, length of stay; ICU, intensive care unit; #, all perioperative variables were analyzed by *t*-test except the completeness of covering membrane that was done by *Fisher's* exact test. P≤0.05 was regarded as statistic significance.

Table 3 Comparisions of outcomes between VATS thymectomy and transsternal thymectomy in short term (n=82)

Variables	VATS (n=38)	Sternotomy (n=44)	P#
Gender (female)	19 (50.00%)	20 (45.45%)	0.681
Age [years]	50.05 [25-72]	47.02 [15-64]	0.149
BMI* [range]	24.97 [18-34]	25.66 [18-34]	0.393
Myasthenia gravis [%]	5 (13.16)	10 (22.73)	0.264
Tumor size (cm)	5.4 (2.5-13.0)	6.5 (2.5-13.0)	0.013
Muller-Hermelink classification (%)			0.267
A	2 (5.26)	0 (0)	
B1	7 (18.42)	9 (20.83)	
B2	11 (28.95)	11 (25.00)	
B3	2 (5.26)	8 (20.83)	
AB	16 (42.11)	16 (33.33)	
Massaoka staging (%)			0.948
I	17 (44.74)	20 (45.45)	
II	21 (55.26)	24 (54.55)	
Postoperative hospital LOS* [days]	5.26 [2-13]	9.16 [5-25]	<0.001
ICU* LOS [hours]	4.26 [0-90]	9.27 [0-168]	0.343
Operation time [minutes]	127.63 [30-300]	155.46 [70-270]	0.024
Blood loss [mL]	114.74 [20-600]	214.77 [100-600]	<0.001
Completeness of covering membrane (%)	36 (94.74)	37 (84.09)	0.117
Number of chest tubes	1.11 [1-2]	1.41 [1-2]	0.001
Quantity of chest tube output [mL]	617.68 [100-1,580]	806.48 [290-1,950]	0.026
Duration of drainage [days]	3.87 [2-10]	5.68 [3-10]	<0.001

*, LOS, length of stay; ICU, intensive care unit; BMI, body mass index; #, gender, symptoms, myasthenia gravis, hypertension and Massaoka staging were analyzed by *Pearson* chi-square test. Diabetes mellitus, coronary heart disease and Muller-Hermelink classification were analyzed by *Fisher's* exact test. The rest of variables of patients characteristics were analyzed by *t*-test. All perioperative variables were analyzed by *t*-test except the completeness of covering membrane that was done by *Fisher's* exact test. P≤0.05 was regarded as statistic significance.

to the results above, the gender distribution of thymoma is approximately equal, although it was reported that thymoma was slightly more common in women in older age (11). VATS thymectomy had reached shorter postoperative hospital LOS, less blood loss, less chest output and shorter duration of drainage. These results supported Liu *et al.* (12) and Pennathur *et al.* (13) report previously. We also made further contrast between VATS thymectomy and transsternal thymectomy or transthoracic thymectomy respectively. The outcomes indicated that patients who underwent VATS thymectomy had shorter postoperative hospital LOS, less operation time, less blood loss, less quantity of drainage, smaller number of chest tubes and shorter duration of drainage than that of the ones via transsternal approach. Certain results were similar to Meyer's (14). Patients in VATS group had longer operation time, but shorter postoperative hospital LOS, less quantity of drainage, and shorter duration of drainage than that of patients via thoracotomy. As the heavy wound led to postoperative pain more seriously in the open group, especially by transsternal approach, more postoperative care were required by patients and it delayed their return to normal daily life and work, compared to VATS group. Less incisions, clearer visual field and more accurate dissection, which lead to less blood loss during VATS thymectomy could contribute to recovery and postoperative hospital LOS decreased to less than one week. Without the activity restrictions associated with open thymectomy, patients via VATS approach would return the productive life sooner.

We compared VATS thymectomy with both sternotomy and transthoracic thymectomy comprehensively, especially

Table 4 Comparisions of outcomes between VATS thymectomy and transthoracic thymectomy in short term (n=85)

Variables	VATS (n=38)	Thoracotomy (n=47)	P#
Gender (female)	19 (50.00%)	26 (55.32%)	0.625
Age [years]	50.50 [25-72]	50.06 [21-76]	0.502
BMI* [range]	24.97 [18-34]	24.64 [18-32]	0.667
Myasthenia gravis (%)	5 (13.16)	4 (8.51)	0.505
Tumor size (cm)	5.4 (2.5-13)	7.2 (3.0-13.0)	<0.001
Muller-Hermelink classification (%)			0.148
A	2 (5.26)	7 (14.89)	
B1	6 (15.79)	10 (21.28)	
B2	11 (28.95)	5 (10.64)	
B3	2 (5.26)	1 (2.13)	
AB	16 (42.11)	24 (51.06)	
Massaoka staging (%)			0.119
I	17 (44.74)	29 (61.70)	
II	21 (55.26)	18 (38.30)	
Postoperative hospital LOS* [days]	5.26 [2-13]	7.53 [4-13]	<0.001
ICU* LOS [hours]	4.26 [0-90]	9.70 [0-96]	0.239
Operation time [minutes]	127.84 [30-300]	95.64 [55-160]	0.002
Blood loss [mL]	114.74 [20-600]	175.53 [50-1500]	0.123
Completeness of covering membrane (%)	36 (94.74)	43 (91.49)	0.687
Number of chest tubes	1.11 [1-2]	1.02 [1-2]	0.131
Quantity of chest tube output [mL]	619.68 [100-1,580]	890.89 [100-2,720]	0.022
Duration of drainage [days]	3.78 [2-10]	4.79 [2-9]	0.009

*, LOS, length of stay; ICU, intensive care unit; BMI, body mass index; #, gender, symptoms, myasthenia gravis, hypertension and Massaoka staging were analyzed by *Pearson* chi-square test. Diabetes mellitus, coronary heart disease and Muller-Hermelink classification were analyzed by *Fisher's* exact test. The rest of variables of patients characteristics were analyzed by *t*-test. All perioperative variables were analyzed by *t*-test except the completeness of covering membrane that was done by *Fisher's* exact test. P≤0.05 was regarded as statistic significance.

interiorly. In our 129 thymectomies, there was no death or morbidity due to the surgical procedures perioperatively. Previous reports showed that most of serious complications including vascular injury, chylothorax, nervus phrenicus damage, long-term air leakage and diaphragm injury mainly happened during VATS thymectomy processs. The overall morbidity was about 5.1-10% (3,15-20). Actually, these problems happened during open thymectomy process as well.

Although Blalok *et al*. (10) had reported in 1941 that thymectomy should be widely managed in treatment of myasthenia gravis, debates remained on the role of surgery in the treatment of myasthenia gravis. The conditions of thymoma patients with myasthenia gravis in our study were not severe preoperatively, and symptoms of 19 thymoma patients with myasthenia gravis had been improved or disappeared after thymectomy. The remission rate of myasthenia gravis was 100% in both VATS group and open surgery group. Gronseth *et al*. (21) summarized relevant papers and made a conclusion that the curative effect of thymectomy was better than chemotherapy for myasthenia gravis, and the overall remission rate was 70%. Zahid *et al*. (22) supported that surgical management of MG was becoming increasingly recognised as an effective treatment option. Combined with our results, we believed that thymectomy was an effective therapy for myasthenia gravis patients, especially through VATS approach.

For VATS thymectomy, one major concern is that whether the completeness of resection are comparable similar to open

thymectomy. From our experience, we mobilized the poles of thymus and eliminate peri-mediastinal fat as much as possible while achieving a complete removal of the thymic mass. Unilateral VATS approach may certainly limit our ability to resect contralateral tissue accurately. In fact, 36 thymic masses resected (accounting for 95%) in VATS thymectomy group confirmed by pathology had complete covering membrane that was applied to prove that thymus was entirely removed. Based on our results, it had been comparable to 80 open thymectomies (accounting for 88%). The size of the thymic mass is another concern. In our study, tumor size was not significantly different between VATS group and open surgery group. However, we found that, compared to sternotomy group and thoracotomy group respectively, tumor size was smaller in the VATS thymectomy group. Youssef *et al*. (23) had recommended that less than 3 cm thymoma was appropriate for VATS thymectomy approach. Nonetheless, two thymectomies for thymoma with maximal length-diameter about 13 cm were completed through VATS approach while the similar maximal size of tumor existing in open thymectomy group in our hospital. Therefore, we considered the size of thymoma was not an absolute restriction for VATS thymectomy.

The limitations of the present study should not be neglected. First of all, the number of open group was much larger than that of VATS group, so the selection bias was inevitable. Clinical variables were analyzed in the perioperative period in only one institute, which may lead to inaccurate results. So far, multicentric comparative studies with large sample size for thymoma patients between VATS and open thymectomy groups in a long-term have not been reported. In addition, the follow-up recurrence rate after thymectomy, which was an important assessment for VATS approach, was not found in our study. Cheng *et al*. (15) showed that 44 thymoma patients who received VATS thymectomy and followed up for 39.6 months, had no recurrence. Augustin *et al*. (16) also reported nine thymoma patients via VATS thymectomy did not have recurrence, followed up for 25 months. Hopefully, the shortage of our study may inspire us to do further research.

Conclusions

VATS thymectomy is a safe and practicable treatment for early-stage thymoma patients (Massaoka staging I-II) without evident invasion, which has shorter length of hospital stay, reduced blood loss and less restrictions to activities, hence patients will recover sooner. Eagerly, long-term randomized controlled trials in the multicenter are expected to carry out.

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References

1. Chen JL, Weisbrod GL, Herman SJ. Computed tomography and pathologic correlations of thymic lesions. J Thorac Imaging 1988;3:61-5.
2. Qu YJ, Liu GB, Shi HS, et al. Preoperative CT findings of thymoma are correlated with postoperative Masaoka clinical stage. Acad Radiol 2013;20:66-72.
3. Tomaszek S, Wigle DA, Keshavjee S, et al. Thymomas: review of current clinical practice. Ann Thorac Surg 2009;87:1973-80.
4. Detterbeck FC, Zeeshan A. Thymoma: current diagnosis and treatment. Chin Med J 2013;126:2186-91.
5. Tomulescu V, Popescu I. Unilateral extended thoracoscopic thymectomy for nontumoral myasthenia gravis--a new standard. Semin Thorac Cardiovasc Surg 2012;24:115-22.
6. Ng CS, Wan IY, Yim AP. Video-assisted thoracic surgery thymectomy: the better approach. Ann Thorac Surg 2010;89:S2135-41.
7. Landreneau RJ, Dowling RD, Castillo WM, et al. Thoracoscopic resection of an anterior mediastinal tumor. Ann Thorac Surg 1992;54:142-4.
8. Tomaszek S, Wigle DA, Keshavjee S, et al. Thymomas: review of current clinical practice. Ann Thorac Surg 2009;87:1973-80.
9. Jurado J, Javidfar J, Newmark A, et al. Minimally invasive thymectomy and open thymectomy: outcome analysis of 263 patients. Ann Thorac Surg 2012;94:974-81.
10. Blalock A, McGehee AH, Ford FR. The treatment of myasthenia gravis by removal of the thymus. JAMA 1941;18:1529-33.
11. Detterbeck FC. Evaluation and treatment of stage I and II thymoma. J Thorac Oncol 2010;5:S318-22.
12. Liu TJ, Lin MW, Hsieh MS, et al. Video-assisted thoracoscopic surgical thymectomy to treat early thymoma: a comparison with the conventional transsternal approach. Ann Surg Oncol 2014;21:322-8.
13. Pennathur A, Qureshi I, Schuchert MJ, et al. Comparison of surgical techniques for early-stage thymoma: feasibility of minimally invasive thymectomy and comparison with open resection. J Thorac Cardiovasc Surg 2011;141:694-701.

14. Meyer DM, Herbert MA, Sobhani NC, et al. Comparative clinical outcomes of thymectomy for myasthenia gravis performed by extended transsternal and minimally invasive approaches. Ann Thorac Surg 2009;87:385-90.
15. Cheng YJ, Hsu JS, Kao EL. Characteristics of thymoma successfully resected by videothoracoscopic surgery. Surg Today 2007;37:192-6.
16. Augustin F, Schmid T, Sieb M, et al. Video-assisted thoracoscopic surgery versus robotic-assisted thoracoscopic surgery thymectomy. Ann Thorac Surg 2008;85:S768-71.
17. Roviaro G, Rebuffat C, Varoli F, et al. Videothoracoscopic excision of mediastinal masses: indications and technique. Ann Thorac Surg 1994;58:1679-83; discussion 1683-4.
18. Chetty GK, Khan OA, Onyeaka CV, et al. Experience with video-assisted surgery for suspected mediastinal tumours. Eur J Surg Oncol 2004;30:776-80.
19. Bodner J, Wykypiel H, Greiner A, et al. Early experience with robot-assisted surgery for mediastinal masses. Ann Thorac Surg 2004;78:259-65.
20. Cirino LM, Milanez de Campos JR, Fernandez A, et al. Diagnosis and treatment of mediastinal tumors by thoracoscopy. Chest 2000;117:1787-92.
21. Gronseth GS, Barohn RJ. Practice parameter: thymectomy for autoimmune myasthenia gravis (an evidence-based review): report of the Quality Standards Subcommittee of the American Academy of Neurology. Neurology 2000;55:7-15.
22. Zahid I, Sharif S, Routledge T, et al. Video-assisted thoracoscopic surgery or transsternal thymectomy in the treatment of myasthenia gravis? Interact Cardiovasc Thorac Surg 2011;12:40-6.
23. Youssef SJ, Louie BE, Farivar AS, et al. Comparison of open and minimally invasive thymectomies at a single institution. Am J Surg 2010;199:589-93.

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Original Article

Changes of HMGB1 and sRAGE during the recovery of COPD exacerbation

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Background: Acute exacerbation of chronic obstructive pulmonary disease is associated with increased airway and systemic inflammation. However, the correlation between acute exacerbation/convalescence of chronic obstructive pulmonary disease (COPD) and simultaneous changes of high mobility group protein B1 (HMGB1) and soluble RAGE (sRAGE) levels has not been clearly clarified. The aim of this study was to assess these issues.

Methods: A total of 44 COPD patients were recruited. Following a structured interview, plasma levels of HMGB1, sRAGE, fibrinogen and serum level of high-sensitivity C-reactive protein (hsCRP) were measured in patients with acute exacerbation of COPD (AECOPD) within 24 h of hospitalization and pre-discharge (convalescence). All patients were examined with spirometry in convalescence of COPD.

Results: There was a significant decline in plasma HMGB1 ($P<0.01$), sRAGE ($P<0.05$), fibrinogen ($P<0.01$) and serum hsCRP ($P<0.01$) levels from acute exacerbation to convalescence phase of COPD. Changes of sRAGE was significantly correlated with changes of HMGB1 ($r=0.4$, $P=0.007$). COPD disease status correlated with the ratio of HMGB1/sRAGE, but not gender, age, course of disease, smoking history and FEV1% pred. Levels of HMGB1 and sRAGE were the highest in the current smoker group, and significantly decreased in ex-smoker group in both acute exacerbation and convalescence phase of COPD, however, their levels in never smoker group were higher than ex-smoker group in either phase of COPD.

Conclusions: HMGB1 and sRAGE levels were dynamically changed between exacerbation and convalescence phase of COPD, HMGB1 and sRAGE were likely not only a potential marker in COPD exacerbation but also a therapeutic target for COPD treatment.

Keywords: Chronic obstructive pulmonary disease (COPD); high mobility group protein B1 (HMGB1); soluble RAGE (sRAGE); biomarker; exacerbation; convalescence

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Introduction

Chronic obstructive pulmonary disease (COPD) is a leading cause of morbidity and mortality worldwide, which is characterized by irreversible airflow limitation, and usually progressive and associated with abnormal inflammatory responses of the lung to noxious particles or gases (1). Despite the involvement of airway inflammation in the development of COPD, current anti-inflammatory therapies poorly prevent the deterioration of COPD, which indicate that more efforts are need to explore novel molecular mechanisms and targets involved in the disease pathogenesis and progression (2).

High mobility group protein B1 (HMGB1) is an abundant chromatin protein that acts as a cytokine when released into the extracellular milieu by necrotic and inflammatory cells. It is regarded as a marker of tissue injury and a mediator of inflammation. It has been reported that extracellular HMGB1 contributes to the pathogenesis of many inflammatory diseases, such as sepsis, acute lung injury, adult respiratory distress syndrome, cystic fibrosis and systemic lupus erythematosus (3).

HMGB1 has been shown to transduce cellular signals by interacting with at least three receptors: the receptor for advanced glycation end-products (RAGE), toll-like receptors (TLR) 2/4. RAGE is the first identified receptor of HMGB1, binding of HMGB1 to cell surface RAGE results in generation of reactive oxygen species (ROS) and activation of the transcription factor NF-κB, which further promotes cytokines production, all these bioactivators stimulates immune and inflammatory responses (4).

RAGE belongs to the immunoglobulin superfamily of cell surface molecules. RAGE is expressed as both a transmembrane molecule and a soluble molecule. The soluble form seems to be produced by either alternative mRNA splicing or proteolytic cleavage of transmermbrane RAGE (mRAGE). Soluble RAGE (sRAGE) is capable of binding the same RAGE ligands, including HMGB1, it has been proposed that sRAGE acts as a decoy receptor, preventing the interaction of mRAGE with its ligands (5). In the majority of healthy mature tissues, RAGE is expressed at a low basal level, its expression is up-regulated at sites of various pathologies, such as diabetes, atherosclerosis and Alzheimer's disease (5). Pulmonary tissues express relatively high basal levels of RAGE, especially in alveolar epithelial cells (6), suggesting that HMGB1/RAGE pathway may have a number of functions in the lung. Since sRAGE presumably inhibits the activity of HMGB1, it might have potential value in the clinic to treat some lung inflammatory diseases with high level of HMGB1.

Recent studies have reported an enhanced level of HMGB1 in BAL fluid (7), sputum and circulation in patients with COPD (8). Study has shown that the level of plasma sRAGE decreases in patients with COPD in comparison with healthy controls, and its level is even lower in acute exacerbation of COPD (AECOPD) (9), however, the data are less robust. There are fewer studies simultaneously investigating changes of HMGB1, sRAGE and the ratio of HMGB1/sRAGE in acute exacerbation and convalescence phase of COPD, and determining the correlation of the levels of HMGB1, sRAGE and ratio of HMGB1/sRAGE with clinical characteristics of COPD. This study aims to clarify these issues.

Methods

Subjects

The present prospective cohort study was approved by the Research Committee of Human Investigation of Medical College of Xi'an Jiaotong University. All participants were given informed consent. Patients' main hospitalization diagnosis, medical history, and smoking history were collected by physicians. A total of 102 patients with AECOPD were admitted to the Department of Respiratory Medicine of the Second Affiliated Hospital of Medical College, Xi'an Jiaotong University (Xi'an, Shanxi, China) between October 2012 and March 2013. Fifty-eight COPD patients with coronary artery disease, diabetes, autoimmune disease and any other conditions which could affect HMGB1and sRAGE levels were excluded. Forty-four COPD patients were recruited into this study.

Clinical variables

Detailed clinical and demographic data were obtained at the time of hospital admission. Smoking history, hospitalization time, course of disease and exacerbation frequency in previous 3 years were also collected. Laboratory measurements including total leukocyte counts, neutrophils %, serum high-sensitivity C-reactive protein (hsCRP), plasma fibrinogen; arterial blood gas analyses and chest CT were performed.

Criteria for acute exacerbation and convalescence of COPD

AECOPD is defined as the worsening of respiratory symptoms, and is diagnosed based on the presence of an increase in any two major symptoms (dyspnoea, sputum purulence and sputum quantity), or an increase in one major and one minor symptom (wheeze, sore throat, cough and nasal congestion/discharge) for at least two consecutive days according to previously accepted criteria (10). Convalescence of COPD is diagnosed on clinical grounds with the following criteria: symptoms of patient with COPD return to the pre-exacerbation level variability, physician carefully assesses the patient and confirms that the individual is medically stable enough to leave the hospital, but patients are not unstable and prone to acute exacerbation again (11).

Therapy during hospitalization

The routine treatment for AECOPD during hospitalization was as follows: all patients diagnosed with AECOPD were supplemented with low flow oxygen, intravenous infusions of methylprednisolone 0.7-1.4 mg/kg per day for the first 3 to 5 days, and switching to nebulized budesonide (2 mg, 8 hourly) for the following 6 to 9 days. Antibiotics administration was adjusted based on the results of blood

Table 1 The basic information of the study subjects

Parameter	Value
N	44
Sex (male/female)	32/12
Age (years)	68.2±8.5
BMI (kg/m^2)	24.4±4.2
Never/current/ex-smokers	14/19/11
Smoking history (pack-years)*	39.5±17.6
Hospitalization time [days]	14 [12-17]
Exacerbation frequency (times/year)	0.53±0.21
Course of disease (years)	10.3±5.4

BMI, body mass index; *, includes only 30 current/ex-smokers; Values are mean ± standard deviation (SD) or median (range).

tests for inflammatory markers, sputum test, and signs of pneumonia. Intravenous infusions of aminophylline and expectorants were prescribed until patients with AECOPD condition were stable. Inhaled long-acting β2 agonists were prescribed for maintenance therapy upon discharge.

Pulmonary function tests

Spirometry was performed on each subject. Reversibility assessment was conducted in COPD patients by making them inhale a short-acting β_2 agonist equivalent to 200 μg salbutamol. For hospitalized patients with acute exacerbations, spirometry was performed 12 to 17 days after the onset of exacerbation when the patients were stable enough to perform the spirometer maneuver.

Blood sample preparation

Blood was collected at the following two time points: within 24 hours of hospitalization; before discharge from hospital after treatment for 12-17 days in hospital. Blood was drawn and stored by the same researcher in the same department. Blood samples were collected aseptically in ethylenediamine tetraceticacid (EDTA)-anticoagulated tubes and stored at 4 ℃. Samples were centrifuged at 1,000 g at 4 ℃ for 15 min, and plasma was separated and stored at –80 ℃ in aliquots of 1 mL until the measurements were taken.

Measurements of HMGB1, sRAGE, hsCRP and fibrinogen

Plasma HMGB1 and sRAGE levels were determined using commercial enzyme-linked immunosorbent assay (ELISA) kits (KYM, Beijing, China). The detection limit of the kits is 0.1 ng/mL for HMGB1 and sRAGE. Each sample was run in duplicate and compared with a standard curve. The mean concentration was determined for each sample. Fibrinogen and hsCRP were measured immediately in clinic laboratory once blood plasma was acquired.

Statistical analyses

The Statistical Package for Social Sciences (SPSS), version 13.0, was used to analyze the data. Normality of distribution was examined with Kolmogorove-Smirnov test. Normally distributed data were expressed as mean ± standard deviation (SD). Non-normally distributed data were expressed as median (range). Normally distributed data were compared using paired *t*-tests between acute exacerbation and convalescence phase of COPD; non-normally distributed data were analyzed using wilcoxon signed Ranks test. Frequency data were compared with χ^2 test. Correlation of changes of sRAGE and HMGB1 was examined by a Pearson's correlation. The differences of HMGB1 and sRAGE among groups (never smoker, current smokers and ex-smokers) of COPD were analyzed using Mann-Whitney U test. Multiple linear regression analysis were used to assess the relationship between HMGB1/sRAGE and COPD disease status, gender, age, course of disease, smoking history, FEV1% pred.

Results

Patient characteristics

Table 1 summarizes the demographic characteristics of all subjects. A total of 44 patients were evaluated (32 males, 12 females; age 68.2±8.5 years). A total of 30 patients were current or former smokers, they are mostly male, with a consumption of 39.5±17.6 pack-years. The hospitalization time of AECOPD was 14 (range, 12-17) days, and frequency of acute exacerbation in the 3 previous years was 0.53±0.21 times/year.

Laboratory parameters

Total leucocytes counts, neutrophils %, arterial blood gas analysis, hsCRP, fibrinogen were determined and recorded at the time of admission and discharge. Pulmonary function was not performed at the time of admission due to worsening of physical status, and was examined before patients discharge. The laboratory parameters of subjects

Table 2 Laboratory parameters of subjects

Parameter	Acute exacerbation (range)	Convalescence (range)	P
White blood counts (10^9/L)	7.54±3.45	5.68±1.85	<0.01
Neutrophils % (%)	73.60±12.71	63.80±9.51	<0.01
PaO_2 (mmHg)	66.40±8.65	76.42±9.78	<0.01
$PaCO_2$ (mmHg)	42.26±8.20	41.4±4.11	0.346
FEV1% pred (%)	ND	47.5±13.5	ND
FEV1% FVC (%)	ND	46.2±12.2	ND
RV% TLC (%)	ND	48.0±9.3	ND
Serum hsCRP (mg/dL)	4.4 (0.57-21.4)	0.58 (0.3-3.54)	<0.01
Plasma fibrinogen (mg/dL)	358.18±109.97	258.32±60.22	<0.01
Plasma HMGB1 (ng/mL)	0.4668±0.0587	0.4215±0.0555	<0.01
Plasma sRAGE (ng/mL)	4.563±0.547	4.377±0.496	0.03

PaO_2, arterial oxygen pressure; $PaCO_2$, arterial carbon dioxide pressure; FEV1, forced expiratory volume at 1 s; % pred, percent of predicted value; FVC, forced vital capacity; RV, residual volume; TLC, total lung capacity; hsCRP, high-sensitivity C-reactive protein; HMGB1, high-mobility group box1; sRAGE, soluble receptor for advanced glycation end products; ND, not done; Values are mean ± standard deviation (SD) or median (range).

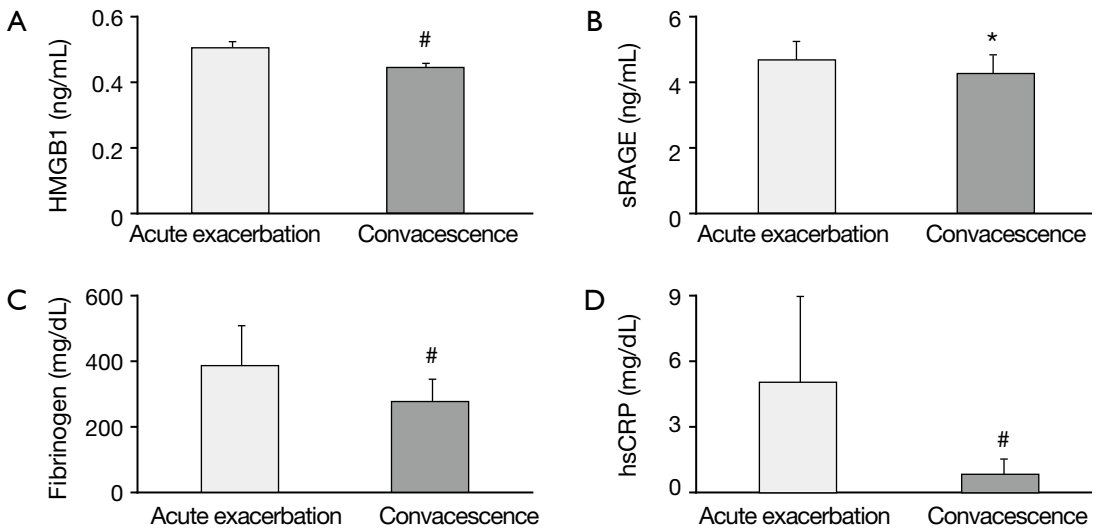

Figure 1 Analysis of concentrations of plasma HMGB1 (A), sRAGE (B), fibrinogen (C), serum hsCRP (D) between acute exacerbation and convalescence of COPD (n=44). Values of HMGB1, sRAGE, fibrinogen are expressed as mean + SD and values of hsCRP are expressed as medians (interquartile range). HMGB1, high mobility group protein B1; sRAGE, soluble receptor for advanced glycation end products; hsCRP, high-sensitivity C-reactive protein; COPD, chronic obstructive pulmonary disease; *, P<0.05; #, P<0.01; SD, standard deviation.

are shown in *Table 2*.

Plasma HMGB1, sRAGE, fibrinogen and serum hsCRP levels in exacerbation and convalescence phase of COPD

The changes of plasma HMGB1, sRAGE, fibrinogen and serum hsCRP level are shown in *Table 2* and *Figure 1A-D*. There was a significant decline in plasma HMGB1 (P<0.01), sRAGE (P<0.05), fibrinogen (P<0.01) and serum hsCRP (P<0.01) level from acute exacerbation to convalescence phase in COPD.

Correlation of the changes of sRAGE and HMGB1 in exacerbation and convalescence phase

The correlation between the change of HMGB1 level

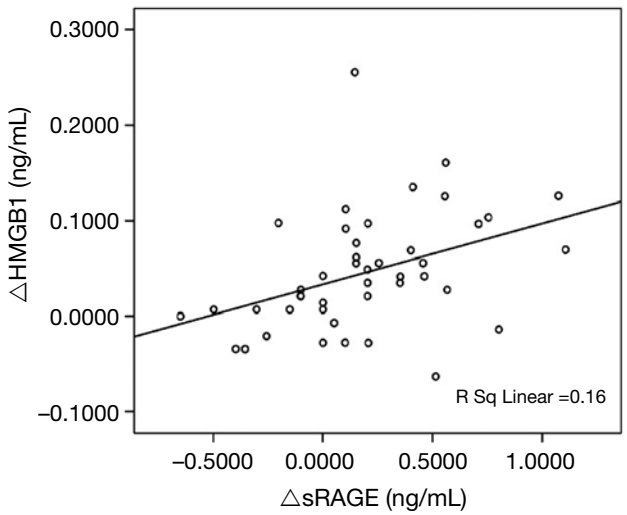

Figure 2 The correlation between the change of HMGB1 and the change sRAGE from acute exacerbation to convalescence in chronic obstructive pulmonary (n=44, r^2=0.16, P=0.007). HMGB1, high mobility group protein B1; sRAGE, soluble receptor for advanced glycation end products.

and the change of sRAGE level in acute exacerbation and convalescence is shown in *Figure 2*. There was a significant positive correlation between the change of sRAGE and change of HMGB1 (r=0.4, P=0.007).

HMGB1/sRAGE ratio correlates with COPD disease status

In the multivariate linear regression analysis with HMGB1/sRAGE as a dependent variable, COPD disease status was found to be an only major influence factor affecting HMGB1/sRAGE ratio (unadjusted r^2=0.102, adjusted r^2=0.036), whereas gender, age, course of disease, smoking history and FEV1% pred did not appear to be significant factors influencing HMGB1/sRAGE ratio (*Table 3*).

HMGB1 and sRAGE levels with smoking

Subjects with COPD were classified into three groups of never smoker, current smoker and ex-smoker according to smoking history. *Table 4* shows that the level of HMGB1

Table 3 Multivariate linear regression of all subjects with HMGB1/sRAGE as the dependent variable

Parameter	B*	β#	t	P
Constant	0.109		7.506	<0.01
Disease statue	–0.007	–0.267	–2.534	0.013
Sex (male/female)	–0.003	–0.113	–0.825	0.412
Age (years)	8.29	0.055	0.444	0.658
Course of disease (years)	6.29	0.056	0.425	0.672
Smoking statue	0.001	0.038	0.270	0.788
FEV1% pred (%)	5.31	–0.057	–0.495	0.622

FEV1, forced expiratory volume at 1 s; % pred, percent of predicted value; Definition of disease statue: acute exacerbation COPD =1, convalescence COPD =2; Sex: male =1, female =2; Smoking statue: never smokers =0, ex-smokers =1, current smokers =2; B*, non-standardised coefficient; β#, standardised coefficient.

Table 4 The effects of smoking on the levels of HMGB1 and sRAGE in COPD patients

Parameter	Never smoker (range)	Ex-smokers (range)	Current smokers (range)	P1	P2	P3
Sex (male/female)	4/10	10/1	18/1	0.002	<0.01	0.685
Age (years)	71.57±7.94	66.63±7.34	66.52±9.08	0.125	0.107	0.973
FEV1% pred (%)	47.28±12.38	43.12±19.23	51.11±10.29	0.518	0.339	0.223
A-HMGB1 (ng/mL)	0.477 (0.376-0.572)	0.404 (0.376-0.522)	0.480 (0.355-0.600)	0.062	0.559	0.028
A-sRAGE (ng/mL)	4.699±0.351	4.193±0.673	4.563±0.512	0.021	0.985	0.043
C-HMGB1 (ng/mL)	0.422±0.047	0.385±0.058	0.443±0.056	0.106	0.177	0.012
C-sRAGE (ng/mL)	4.411 (3.583-5.256)	4.234 (2.652-4.742)	4.487 (3.782-5.360)	0.125	0.402	0.021

FEV1, forced expiratory volume at 1 s; % pred, percent of predicted value; A-HMGB1, high-mobility group box1 levels of acute exacerbation of COPD; C-HMGB1, high-mobility group box1 levels of convalescence of COPD; A-sRAGE, soluble receptor for advanced glycation end products of acute exacerbation of COPD; C-sRAGE, soluble receptor for advanced glycation end products of convalescence of COPD; P1, ex-smokers compared with never smoker; P2, current smokers compared with never smoker; P3, current smokers compared with ex-smokers.

was the highest in current smoker group, and decreased significantly in ex-smoker group in either acute exacerbation or convalescence phase. However, HMGB1 level was higher in never smoker group than ex-smoker group. The change of sRAGE level had a similar pattern.

Discussion

The episode of COPD exacerbation is characterized by an increase of various inflammatory markers in lung, including neutrophils, macrophages and various cytokines (12). The activation of NF-κB plays a critical role in inflammatory responses of COPD exacerbation (13). Studies have shown that extracellular HMGB1 is a critical mediator in late stage of inflammatory responses, HMGB1 signals through RAGE leading to activation of NF-κB and subsequent up-regulation of various leukocyte adhesion molecules, membrane receptor and pro-inflammatory cytokines, such as IL-1β, IL-6, TNF-α and RAGE (14,15). These pro-inflammatory cytokines in turn promote hepatic synthesis of hsCRP and fibrinogen (12). High level of RAGE is expressed in alveolar epithelial cells and alveolar macrophages and is thought to be important in the pathogenesis of COPD (4). Studies have shown that plasma HMGB1 level in COPD patients is significantly greater than that in healthy controls. We showed here that HMGB1 was also associated with the occurrence of AECOPD, its level raised in the AECOPD, and declined in convalescence phase of COPD. It seems that HMGB1 may be not only a potential inflammatory marker in COPD but also a therapeutic target for COPD.

sRAGE corresponds to the extracellular domain of membrane-bound RAGE lacking its cytosolic and transmembrane domains, and exists in extracellular fluids. sRAGE may act as a decoy receptor binding RAGE ligands and preventing them binding to membrane RAGE (5). Numerous studies have reported that a correlation between sRAGE level and inflammatory diseases, for example, sRAGE level was elevated in BAL fluid and plasma in patients with acute lung injury, it was positively correlated with the severity of acute lung injury (16). In our study, we also observed that the level of plasma sRAGE was higher in AECOPD than that of convalescence. These results seem inconsistent with the notion that sRAGE functions to decoy HMGB1. Study by Smith *et al*. suggested that sRAGE was lower in stable COPD than in healthy control, and AECOPD was associated with even lower sRAGE level, sRAGE increased with COPD convalescence (9). This discrepancy may be due to several reasons. In Smith's study, the comorbidities of patients with AECOPD and oxygen therapy in these patients were not counted and analyzed. AECOPD is associated with worsening hypoxia and activation of NF-κB, which increases expression of membrane RAGE (17). Soluble RAGE is generated by proteolytic cleavage of membrane-bound RAGE (18), this process is mediated by disintegrin, metalloprotease (ADAM10) (19) and matrix metalloproteinase-9 (MMP-9) (20). It has also been shown that MMP-9 activity and ADAM10 expression are increased in AECOPD compared to convalescence of COPD (21-23), which stimulate membrane RAGE shedding and promote the release of sRAGE (20). In addition, HMGB1 has also been found to induce RAGE shedding (24).

The results of the present study suggested that gender, age, course of disease, smoking history and FEV1% pred did not significantly affect HMGB1/sRAGE ratio. COPD disease status was found to be an only major factor affecting HMGB1/sRAGE ratio. The ratio of HMGB1/sRAGE was elevated in exacerbation of COPD and declined in convalescence of COPD, suggesting that although both levels of HMGB1 and sRAGE were increased in AECOPD, the elevation of HMGB1 was predominant. Our study further indicated that the levels of HMGB1 and sRAGE in circulation were the highest in currently smoking COPD patients, and the lowest levels were in ex-smoker COPD patients. We inferred that smoking can induce the elevation of HMGB1 levels, which were declined by quitting smoking. Cigarette smoke was an extremely concentrated sources of ROS and reactive nitrogen species (25), which activates inflammasomes leading to releasing HMGB1 (26). As mentioned above, HMGB1 stimulate RAGE shedding and promote the release of sRAGE, the trend of sRAGE level had a similar with HMGB1. However, the reasons for levels of HMGB1 and sRAGE higher in never smoking COPD than ex-smoking COPD were still unclear.

Limitations of this study include small sample size, lack of measurement of lung function prior to treatment of AECOPD, and lack of complete long-term follow-up. Despite such limitations, this study provides valuable preliminary information about changes of HMGB1 and sRAGE in COPD exacerbation episodes.

Conclusions

The present study showed that the levels of plasma HMGB1, sRAGE, fibrinogen and hsCRP were elevated

in AECOPD and tended to decline in convalescence of COPD; the ratio of HMGB1/sRAGE was correlated with status COPD. Meanwhile, the reduction of plasma HMGB1 was correlated with the reduction of plasma sRAGE from acute exacerbation phase to convalescence phase. The concentrations of plasma HMGB1 and sRAGE in patients with COPD were influenced by smoking.

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References

1. Decramer M, Janssens W, Miravitlles M. Chronic obstructive pulmonary disease. Lancet 2012;379:1341-51.
2. Kim V, Rogers TJ, Criner GJ. New concepts in the pathobiology of chronic obstructive pulmonary disease. Proc Am Thorac Soc 2008;5:478-85.
3. Nogueira-Machado JA, de Oliveira Volpe CM. HMGB-1 as a target for inflammation controlling. Recent Pat Endocr Metab Immune Drug Discov 2012;6:201-9.
4. Lotze MT, Tracey KJ. High-mobility group box 1 protein (HMGB1): nuclear weapon in the immune arsenal. Nat Rev Immunol 2005;5:331-42.
5. Maillard-Lefebvre H, Boulanger E, Daroux M, et al. Soluble receptor for advanced glycation end products: a new biomarker in diagnosis and prognosis of chronic inflammatory diseases. Rheumatology (Oxford) 2009;48:1190-6.
6. Demling N, Ehrhardt C, Kasper M, et al. Promotion of cell adherence and spreading: a novel function of RAGE, the highly selective differentiation marker of human alveolar epithelial type I cells. Cell Tissue Res 2006;323:475-88.
7. Ferhani N, Letuve S, Kozhich A, et al. Expression of high-mobility group box 1 and of receptor for advanced glycation end products in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2010;181:917-27.
8. Hou C, Zhao H, Liu L, et al. High mobility group protein B1 (HMGB1) in Asthma: comparison of patients with chronic obstructive pulmonary disease and healthy controls. Mol Med 2011;17:807-15.
9. Smith DJ, Yerkovich ST, Towers MA, et al. Reduced soluble receptor for advanced glycation end-products in COPD. Eur Respir J 2011;37:516-22.
10. Vestbo J, Hurd SS, Rodriguez-Roisin R, et al. An overview of Global Strategy for the Diagnosis, Management and Prevention of Chronic Obstructive Pulmonary Disease (GOLD) (revised 2011). Zhonghua Yi Xue Za Zhi 2012;92:937-8.
11. Koutsokera A, Kiropoulos TS, Nikoulis DJ, et al. Clinical, functional and biochemical changes during recovery from COPD exacerbations. Respir Med 2009;103:919-26.
12. Thomsen M, Ingebrigtsen TS, Marott JL, et al. Inflammatory biomarkers and exacerbations in chronic obstructive pulmonary disease. JAMA 2013;309:2353-61.
13. Di Stefano A, Caramori G, Oates T, et al. Increased expression of nuclear factor-kappaB in bronchial biopsies from smokers and patients with COPD. Eur Respir J 2002;20:556-63.
14. Huttunen HJ, Rauvala H. Amphoterin as an extracellular regulator of cell motility: from discovery to disease. J Intern Med 2004;255:351-66.
15. Li J, Schmidt AM. Characterization and functional analysis of the promoter of RAGE, the receptor for advanced glycation end products. J Biol Chem 1997;272:16498-506.
16. Jabaudon M, Futier E, Roszyk L, et al. Soluble form of the receptor for advanced glycation end products is a marker of acute lung injury but not of severe sepsis in critically ill patients. Crit Care Med 2011;39:480-8.
17. Tafani M, Schito L, Pellegrini L, et al. Hypoxia-increased RAGE and P2X7R expression regulates tumor cell invasion through phosphorylation of Erk1/2 and Akt and nuclear translocation of NF-{kappa}B. Carcinogenesis 2011;32:1167-75.
18. Hudson BI, Carter AM, Harja E, et al. Identification, classification, and expression of RAGE gene splice variants. FASEB J 2008;22:1572-80.
19. Raucci A, Cugusi S, Antonelli A, et al. A soluble form of the receptor for advanced glycation endproducts (RAGE) is produced by proteolytic cleavage of the membrane-bound form by the sheddase a disintegrin and metalloprotease 10 (ADAM10). FASEB J 2008;22:3716-27.
20. Zhang L, Bukulin M, Kojro E, et al. Receptor for advanced glycation end products is subjected to protein ectodomain shedding by metalloproteinases. J Biol Chem 2008;283:35507-16.
21. Gao P, Zhang J, He X, et al. Sputum inflammatory cell-based classification of patients with acute exacerbation of chronic obstructive pulmonary disease. PLoS One 2013;8:e57678.
22. Zeng M, Wen Y, Liu LY, et al. Role of TNF-α, sTNF-R55

and sTNF-R75 in inflammation of acute exacerbations of chronic obstructive pulmonary disease. Respiration 2009;78:399-403.
23. Zhu LB, Zhao ST, Xu TZ, et al. Tumor necrosis factor-α-induced a disintegrin and metalloprotease 10 increases apoptosis resistance in prostate cancer cells. Oncol Lett 2014;7:897-901.
24. Sugaya K, Fukagawa T, Matsumoto K, et al. Three genes in the human MHC class III region near the junction with the class II: gene for receptor of advanced glycosylation end products, PBX2 homeobox gene and a notch homolog, human counterpart of mouse mammary tumor gene int-3. Genomics 1994;23:408-19.
25. Churg A, Cosio M, Wright JL. Mechanisms of cigarette smoke-induced COPD: insights from animal models. Am J Physiol Lung Cell Mol Physiol 2008;294:L612-31.
26. Lamkanfi M, Sarkar A, Vande Walle L, et al. Inflammasome-dependent release of the alarmin HMGB1 in endotoxemia. J Immunol 2010;185:4385-92.

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Original Article

A comparison of ketamine-midazolam and ketamine-propofol combinations used for sedation in the endobronchial ultrasound-guided transbronchial needle aspiration: a prospective, single-blind, randomized study

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Objective: We aimed to compare the effectiveness and safety of ketamine-midazolam and ketamine-propofol combinations for procedural sedation in endobronchial ultrasound guided transbronchial needle aspiration (EBUS-TBNA).

Methods: Sixty patients who were undergoing EBUS-TBNA were included in this study. Patients were randomly divided into two groups. Group 1 was given 0.25 mg/kg intravenous (iv) ketamine, 2 min later than 0.05 mg/kg iv midazolam. Group 2 received 0.125 mg/kg ketamine-propofol mixture (ketofol), 2 min subsequent to injection of 0.25 mg/kg each. Sedation was maintained with additional doses of ketamine 0.25 mg/kg, and ketofol 0.125 mg/kg each in Group 1 and Group 2, respectively. Blood pressure, heart rate (HR), peripheral oxygen saturation, respiratory rate (RR), Ramsay Sedation Score (RSS), and severity of cough were recorded prior to and after administration of sedation agent in the beginning of fiberoptic bronchoscopy (FOB) and every 5 min of the procedure. The consumption of the agents, the satisfactions of the bronchoscopist and the patients, and the recovery time were also recorded.

Results: HR in the 10^{th} min and RSS value in the 35^{th} min of induction in Group 1 were higher than the other group ($P<0.05$). The recovery time in Group 1 was statistically longer than Group 2 ($P<0.05$). There was no statistically significant difference between groups with respect to other parameters ($P>0.05$).

Conclusions: It was concluded that both ketamine-midazolam and ketamine-propofol combinations for sedation during EBUS-TBNA were similarly effective and safe without remarkable side effects.

Keywords: Transbronchial needle aspiration (TBNA); sedation; ketamine; midazolam; propofol

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Introduction

Endobronchial ultrasound guided transbronchial needle aspiration (EBUS-TBNA) is an ultrasonography method which is developed for imaging neighboring tissues next to the airways. It has been used as a first choice for either diagnosis of mediastinal and hilar lymphadenopathies or staging of lung cancer since it is a minimally invasive method with high diagnostic value (1).

However, EBUS-TBNA can cause anxiety in patients, and can lead to hemodynamic instability, in addition to this, it can affect negatively the performance of the bronchoscopist and the comfort of patients. The anxiety of patients is relieved by sedation. A successful sedation protects reflexes, enables patients to follow

instructions and provides a comfortable work area for the bronchoscopist (2).

Sedation level ranges from awake to general anesthesia status depending on the dose of agent. Conscious sedation is mostly preferred for procedures that require fast recovery (3). Midazolam and propofol are commonly used for sedation in all endoscopic procedures. Midazolam, a benzodiazepine, leads to anxiolysis, anterograde amnesia and light hypnosis. It is preferred for its highly amnestic property (4,5). Meanwhile, propofol has both amnestic and antiemetic effects. Propofol is chosen for its rapid onset time and short acting effect as well as for its quick recovery (6). However, both agents don't have analgesic effect. Ketamine, causes fast deep sedation and analgesia, protects respiration and airway reflexes when it is given slowly. It increases heart rate (HR) and blood pressure slightly and causes bronchodilatation. It is a good choice in sedation of the patients with airway sensitivity. On the other hand, ketamine can cause laryngospasm by increasing secretions (7-9). The other side effects such as nausea, vomiting, hallucinations and anxiety may occur by using ketamine alone (7-10).

We think that the sedation used for interventional bronchoscopy is a unique procedure. Moreover, the sedative agents should also have exceptional advantages without having severe side effects. Until now, there is no certain sedation regimen or ideal agent for EBUS-TBNA. We hypothesized that usage of the sedative agents' combination can alleviate this problem.

The addition of ketamine may reduce hypoventilation and dose dependent side effects in patients having procedures under sedation with midazolam or propofol (8-11). Ketamine and propofol in the same injector (ketofol) is used successfully for sedation in emergency department (8,12). To our knowledge, we did not encounter any study showing that ketofol is used to induce sedation for bronchoscopic procedures in the literature. A combination of ketamine and midazolam may result in fewer side effects and shorter recovery time (9). These combinations are supported since each agent balances others hemodynamic and respiratory side effects (9,13,14).

We designed this study to evaluate the clinical efficacy and safety of two different sedative agents (midazolam and propofol) combined with ketamine during conscious sedation for EBUS-TBNA. For this aim, we compared respiratory and hemodynamic effects, agent consumptions, sedation scores, recovery time, side effects, and the satisfaction of the patients as well as the bronchoscopist.

Materials and methods

This prospective study was conducted with the approval of the ethic committee and with the written informed consent of the patients. Sixty patients between 18-70 years old, with American Society of Anesthesiology (ASA) classification of I-II, and without contraindication for conscious sedation were included in this trial. Patients who had allergy against study agents, ischemic heart disease, high level of kidney and liver function tests, electrolyte imbalance, mental disease, central nervous system disease, drug or alcohol dependency, upper respiratory system infection, glaucoma, and porphyria were excluded from the study.

Performance of EBUS

Patients were informed and asked to sign consent forms 24 h before the procedure. Patients were premedicated with 0.5 mg (intramuscular, im) atropine 30 min before the procedure. In the operation room, electrocardiogram (EKG), non-invasive blood pressure, and peripheral oxygen saturation (SpO_2) were monitorized and a peripheral intravenous (iv) cannula was placed. Five minutes (min) before the procedure had started, 2% lidocaine spray was pumped ten times (1 pump =10 mg lidocaine) to the pharenx. A convex probe-EBUS (BF-UC 180F, Olympus, Tokyo, Japan) was used to examine the lymph nodes, and the ultrasound images were processed with a dedicated scanner (EU-ME1, Olympus, Tokyo, Japan). The bronchoscopist used 22-gauge needle to sample the lymph nodes and applied 2% lidocain while the bronchoscope was passing through vocal cords and carina as well as bronches. Total topical lidocaine dose was limited to 200 mg. All patients received 4 L/dk oxygen via nasal canula during the procedure. The oxygen level was increased to 6 L/dk when SpO_2 was less than 90%.

Design of study

Patients were randomly divided into two groups by sealed envelope method. The patients in Group 1 were first given 0.05 mg/kg (iv) midazolam and then 2 min later, given 0.25 mg/kg ketamine (iv). The patients in Group 2 received ketofol (mixture of 1:1 ratio ketamin- propofol). Ketofol was prepared by combining ketamine 1 mL (50 mg/mL), propofol 5 mL (10 mg/mL), and saline 4 mL in a single syringe. 1 mL of ketofol includes ketamine 5 mg and propofol 5 mg. Group 2 first received 0.25 mg/kg (for each agent, ketamine

and propofol) and then 2 min later it was repeated as 0.125 mg/kg. Sedation level was evaluated using Ramsay sedation score (RSS) during the procedure. According to this score: 1, patient anxious, agitated; 2, patient co-operative, orientated; 3, patient responds to verbal stimulation only; 4, patient asleep, rapid response to light stimulation or loud auditory stimulus; 5, patient asleep, slow response to light stimulation or loud auditory stimulus; 6, no response to any stimulation. For both groups, when RSS was 3, it allowed the fiberoptic bronchoscope to pass vocal cords (15). When RSS was 2, additional doses (0.25 mg/kg ketamine in Group 1 and 0.125 mg/ kg ketamine-propofol mixture in Group 2) were given to the patients to maintain sedation.

Mean arterial blood pressure (MABP), HR, SpO_2, respiratory rate (RR), RSS, coughing severity, and doses of the medications were the recorded parameters. These parameters were recorded on the following times: in the beginning, before any sedative was given (T1); 2 min after first sedation (T2) as well as 5 min (T3), 10 min (T4), 15 min (T5), 20 min (T6), 25 min (T7), 30 min (T8), 35 min (T9), 40 min (T10), 45 min (T11), and 50 min (T12).

Severity of cough was evaluated using a three-point scale during the procedure (16). The scaling was as follows: 1, single coughing; 2, more than one episodes of coughing; 3, severe sustained coughing. Complications and side effects were also recorded during the procedure. We used Modified Aldrete Score (MAS) to evaluate recovery (17). Recovery time was determined as the time until MAS 9.

We did not inform the bronchoscopist and the patients about the sedative agents used during the procedure. We asked and assessed the bronchoscopist's satisfaction with the sedation. The answers were: 0, not enough; 1, moderate; 2, good; 3, excellent. Two hours after the procedure, we asked the patients following questions to understand the satisfaction of the patients with the sedation:

(I) Whether he/she remember the EBUS-TBNA procedure.
   (i) No
   (ii) Partially
   (iii) Yes

(II) In general, are you satisfied with the sedation during the procedure?
   (i) Yes, I am
   (ii) No, I am not

(III) Would you let the same sedatives to be used for the next time?
   (i) Yes
   (ii) No

Statistical analysis

All the data were analyzed using "SPSS for Windows 16" program. For non-quantitative data, tables were made and differential analysis between groups was evaluated using chi-square. The independent-sample *t*-test was used for regularly distributed data and the Mann Whitney U test was used for irregularly distributed data to compare groups. To evaluate differences in the groups, we used repeated measurement variance analysis test for regularly distributed data. For irregularly distributed data, we used the Wilcoxon test to evaluate differences in the groups. Correlation was evaluated using correlations test. $P<0.05$ was accepted as statistically significant. The data were combined at the times 15-20 as 20 min; 25-30 as 30 min; 35-40 as 40 min; 45-50 as 50 min. The recovery time at 35 and 60 in values was combined as 30 min.

Results

Sixty patients were included and analyzed in this study (*Figure 1*). *Table 1* shows demographic data, ASA and comorbid conditions of two groups. There was no statistical difference between two groups for these parameters ($P>0.05$).

Median RSS values were found between 2 and 3 in both groups. Number of patients whose RSS was 3 at T9 (35^{th} min of induction) was higher in Group 1 compared to Group 2 ($P<0.05$). There was no difference between two groups for RSS values in the other periods ($P>0.05$).

Mean SpO_2 values are shown in *Table 2*. There was no statistical difference in SpO_2 between two groups ($P>0.05$). When SpO_2 values are compared to the beginning, SpO_2 decreased significantly at T2, T3, T4, T5, T6, T7, T8, T9 and T10 in Group 1 ($P<0.05$). Similarly, significant decrease in SpO_2 level compared to the beginning was detected in Group 2 at T4, T5, T6, T7, T8, T9 and T11 ($P<0.05$). Mean SpO_2 values were not below 90% in both groups during the study.

When RR values were compared, there was no statistical significant difference between two groups ($P>0.05$). In Group 1, RR showed a significant increase compared to the beginning, only at T2 ($P<0.05$). Whereas, there was a significant decrease in RR compared to the beginning level at T8 in Group 2 ($P<0.05$). Respiratory depression (<10 respiration/min) was not observed in both groups.

When we compared MABP values, there was no statistically significant difference between two groups (*Figure 2*)

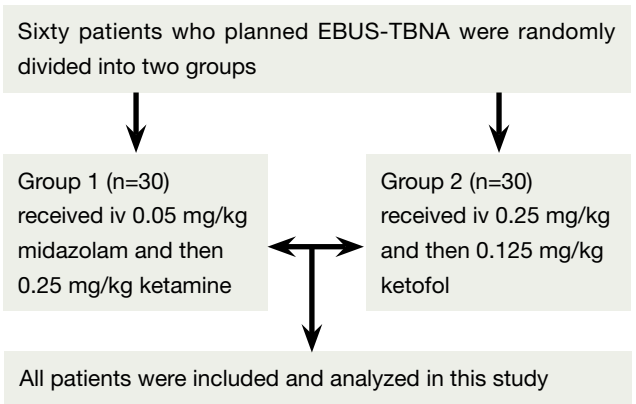

Figure 1 Flow diagram. EBUS-TBNA, endobronchial ultrasound guided transbronchial needle aspiration.

Table 1 Demographic features of the patients in the study groups (Data is expressed as mean ± standard deviation or patient number)

	Group 1	Group 2	P
Age (years)	52.8±13.03	54.4±12.85	0.496
Weight (kg)	72.03±13.60	75.5±13.26	0.882
Male/Female	23/7	22/8	0.766
ASA I/II	14/16	16/14	0.797
Comorbid conditions			
Diabetes mellitus	5	8	0.432
Lung cancer	3	5	

ASA, American Society of Anesthesiology.

(P>0.05). MABP significantly increased compared to the beginning level T2, T4, T5 and T6 after induction in both groups (P<0.05). When HR values were compared between two groups, at T4, there was a significant increase in Group 1 (*Figure 3*, P<0.05). There was no significant difference in HR at the other recording times (P>0.05). In Group 1 and 2, HR increased significantly at the times T2, T3, T4, T5, T6, T7, T8, and T9. In Group 2, HR also increased significantly at T10 (P<0.05).

The coughing scores are shown in *Table 3*. There was no significant difference between two groups for these scores (P>0.05). The mean EBUS-TBNA procedure time was 34±7.81 min in Group 1 and it was 33.67±9.64 min in Group 2. The procedure time was similar between two groups (P>0.05). When two groups were compared according to the side effects, there was no statistical difference between two groups (P>0.05). The characteristics of the patients with complications are shown in *Table 4*. While there was no side effects in Group 1, nausea (n=1), hallucination (n=1), and ventricular extrasystole (n=1) were observed in Group 2. Throughout the study, we did not observe life threatening complication due to EBUS-TBNA procedure or the sedation method.

Mean recovery times were 27.67±4.09 and 25.00±7.31 min in Group 1 and Group 2, respectively. The recovery time was significantly longer in Group 1 when compared to Group 2 (P<0.05).

In the evaluation of the satisfaction of the bronchoscopist with the sedation, Group 1 had significantly more excellent

Table 2 SpO_2 measurements of the patients in the study groups (Data is expressed as mean ± standard deviation)

Time	Group 1		Group 2		P
	Mean ± SD	P	Mean ± SD	P	
T1	97.13±1.94	–	96.70±2.25	–	0.474
T2	94.60±3.82	0.000*	96.23±3.05	0.654	0.112
T3	94.90±2.82	0.000*	95.30±3.05	0.054	0.531
T4	93.93±2.73	0.000*	95.23±3.06	0.023*	0.073
T5	94.73±2.33	0.000*	94.27±3.20	0.002*	0.760
T6	94.57±3.14	0.000*	94.17±2.75	0.001*	0.647
T7	94.36±2.77	0.000*	93.96±2.46	0.001*	0.559
T8	94.29±3.16	0.000*	93.76±2.98	0.003*	0.696
T9	94.24±3.21	0.002*	93.72±2.65	0.009*	0.654
T10	93.22±2.22	0.011*	93.82±2.68	0.068	0.615
T11	94.00±2.16	0.066	93.00±2.28	0.027*	0.386
T12	97.00±2.83	0.317	94.00±2.83	0.180	0.439

*, P<0.05: compared to basal values (T1).

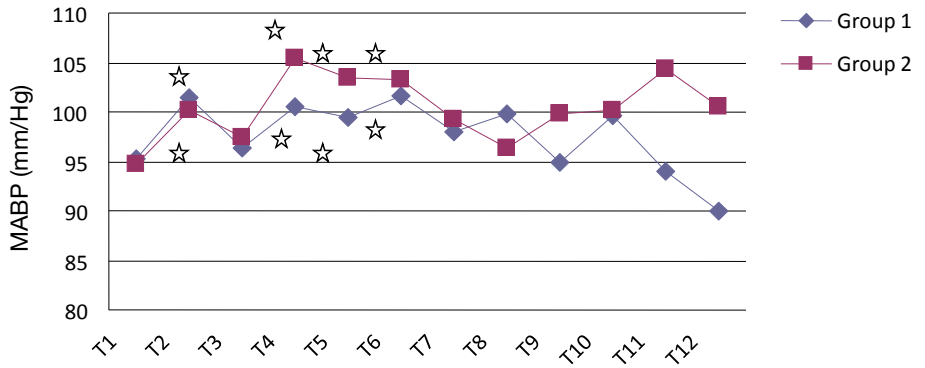

Figure 2 Mean arterial blood pressure values of patients in the study groups. ☆, P<0.05: compared to basal values (T1). MABP, mean arterial blood pressure.

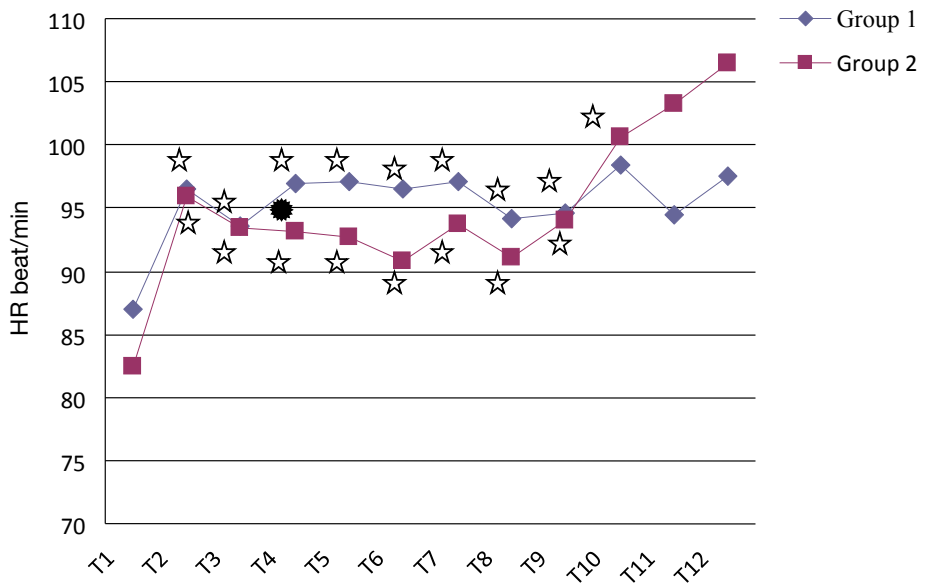

Figure 3 Heart rate (HR) values of patients in the study groups. ☆, P<0.05: compared to basal values (T1). ✹, P<0.05: comparison between the groups.

score compared with Group 2 (*Figure 4*, P<0.05). Groups were similar related to remembering the procedure, patient satisfaction, and given permission for the next time to use the same sedative agents (*Table 5*, P>0.05).

Consumed mean ketamine was similar in two groups (55.7±15.0 mg in Group 1 and 50.6±13.72 mg in Group 2) (P>0.05). Additionally, in Group 1, 3.6±0.7 mg midazolam and in Group 2, 50.6±13.72 mg propofol were used. The repeated doses of each specified time period of the groups was shown in the *Table 6*.

Additional doses were needed for all cases in both groups. The median repeated dose number was 2 (range, 1-8) in Group 1 and 2 (range, 1-4) in Group 2. There was no significant difference in repeated dose number between the groups (P=0.480). The repeated dose number was positively correlated with the body weight both in Group 1 (r=0.308; P=0.098) and Group 2 (r=0.169; P=0.371) without statistical significance.

Discussion

In our study, we compared ketamine-midazolam and ketamine-propofol combinations used for conscious sedation in EBUS-TBNA procedure. We demonstrated that both combinations are similarly effective and safe. In the present study, ketamine's combination with either midazolam or propofol provided good levels of satisfaction for the patients and the bronchoscopist without remarkable

side effects.

Kennedy *et al*. compared general anesthesia and sedation for EBUS-TBNA procedure in the patients with lung cancer (18). They found that sedation is as much comfortable as general anesthesia and it has more advantages regarding recovery time and hospital discharge compared to general anesthesia. Anxiety during the local procedures can stimulate sympathetic system and can cause hypertension, arrhythmia, and increase in myocardial oxygen consumption. Sedative agents are used to relieve anxiety and its' negative effects (19-21). However, some investigators still think that bronchoscopic and endoscopic procedures can be performed without sedation (22,23). They consider that using sedation increases risk of respiratory depression due to combination of different agents, decreases patient's cooperation during the procedure, and causes an increase in both recovery time and the cost of hospitalization (22-25). In a study evaluating FOB with sedation, it was reported that major complication ratio was 0.08-5%. Moreover, half of these complications were due to the sedation itself (26).

Table 3 Coughing scores of patients in the study groups

Time	Coughing scores	Group 1	Group 2	P
T1	–	–	–	–
T2	1	0	1	0.500
	2	30	29	
T3	1	26	26	0.647
	2	4	4	
T4	1	10	13	0.619
	2	20	16	
	3	0	1	
T5	1	14	17	0.303
	2	16	13	
T6	1	18	14	0.278
	2	12	14	
	3	0	1	
T7	1	16	13	0.460
	2	12	12	
T8	1	13	13	0.855
	2	11	7	
	3	0	1	
T9	1	7	6	0.448
	2	10	12	
T10	1	6	6	0.465
	2	3	5	
T11	1	3	2	–
	2	1	4	
T12	1	1	1	–
	2	1	1	

For this reason, anesthetists should evaluate consciousness level carefully. Sedation scores are used to assess consciousness level subjectively, while "bispectral index" (BIS), which measures direct effect of sedation on brain, is used for objective evaluation (27,28). BIS monitoring did not discriminate mild-to-moderate sedation or moderate-to-deep sedation, as measured by the RSS for the patients undergoing procedural sedation (28). There is nothing yet that measures sedation depth quantitatively that can replace the qualitative assessment for procedural sedation (29) Thus, we preferred RSS for the assessment of consciousness level.

The patients should be monitored carefully because of the cardiovascular and respiratory side effects of both local anesthesia and sedation (30). For these reasons, blood pressure, ECG, SpO_2 and RR should be recorded. Routine oxygen support is suggested to prevent hypoxia during fiberoptic bronchoscopy (FOB) under local anesthesia (31). The most important complications are respiratory depression and desaturation owing to the sedative agent (32). We applied continuous nasal oxygen and SpO_2 monitorization both in the operation room and in the recovery room to prevent desaturation due to EBUS-TBNA.

Midazolam and propofol are commonly used for sedation either solely or in combination with other agents (32-35). They can depress cardiovascular and respiratory system depending on the dosage (33-37). The usage of propofol or

Table 4 Side effects and characteristics of the patients with complications

Patient	Group	Side effect	Age (years)	Weight (kg)	Gender	Procedure time	Repeated dose number	ASA
1	Ketofol	Nausea	65	75	Female	40 min	3	I
2	Ketofol	Hallucination	31	90	Male	35 min	2	II
3	Ketofol	Ventricular extrasystole	74	84	Male	40 min	3	I

ASA, American Society of Anesthesiology.

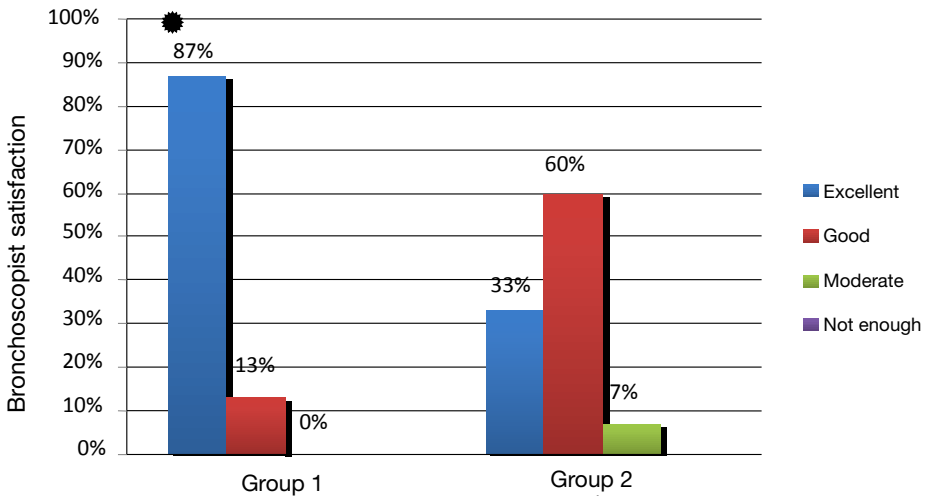

Figure 4 Distribution of patient according to bronchoscopist satisfaction. ✹, P<0.05: comparison between the groups.

Table 5 Comparison of patient satisfaction between the groups

		Group 1		Group 2		P
		n=30	%	n=30	%	
Whether he/she remember the EBUS-TBNA procedure	No	1	3.33	0	0	1.00
	Partially	29	96.7	30	100	
	Yes	0	0	0	0	
General satisfaction	Satisfaction	30	100	30	100	–
	Not satisfaction	0	0	0	0	
Would you let the same sedatives to be used for the next time?	Yes	30	100	30	100	–
	No	0	0	0	0	

EBUS-TBNA, endobronchial ultrasound guided transbronchial needle aspiration.

midazolam for sedation in bronchoscopic procedures can cause severe side effects like hypoxemia, tachycardia, and hypotension (32,35,37).

Even though ketamine protects laryngeal reflexes, its use in adults is limited since it causes increase in HR, hypertension, nausea, vomiting, hallucinations, and anxiety (8,38-40). Ketamine's effects are balanced with combination of midazolam and propofol. Hwang *et al.* showed that patient controlled sedation with ketamine in combination with propofol during FOB provides hemodynamic stability and high patient satisfaction (14). Willman *et al.* reported hypoxia, without the requirement of intubation, in only 3 out of 114 patients receiving ketofol for sedation and analgesia in emergency room (8). Akin *et al.* reported that fewer numbers of patients needed additional dose in ketamine and propofol group than only propofol group in their study performed on pediatric patients (41). While none of the patients suffered from apnea or desaturation in ketamine and propofol group, they observed apnea in six patients and desaturation in four patients in propofol group.

Chudnofsky *et al.* observed apnea in three patients and laryngospasm in one patient in their study performed on 70 adult patients injected with ketamine-midazolam combination for painful procedures (9). In another study, Drummod studied the effects of ketamine and midazolam on airway muscle activities and found that while 10 of 12 patients given midazolam had airway obstruction and respiratory distress, none of the 11 patients injected with ketamine had any respiratory problem (42). In our study, there was no difference between groups regarding to SpO_2 and RR and we did not observe any respiratory depression in our patients.

Willman *et al.* encountered treatment required hypertension and hallucination in three patients in their

Table 6 Comparison of the repeated dose of each specified time period

Time	Sedative agent	Group I/n	Group II/n	P
T1	Midazolam	30/30	0/30	0.000
	Ketofol	0/30	30/30	
T2	Ketamin	30/30	0/30	0.000
	Ketofol	0/30	30/30	
T3	None	29/30	30/30	1.000
	Ketamin	1/30	0/30	
T4	None	11/30	11/30	0.055
	Ketamin	19/30	0/30	
	Ketofol	0/30	19/30	
T5	None	19/30	20/30	0.400
	Ketamin	11/30	0/30	
	Ketofol	0/30	10/30	
T6	None	18/30	17/29	0.185
	Ketamin	12/30	0/29	
	Ketofol	0/30	12/29	
T7	None	19/28	17/25	0.336
	Ketamin	9/28	0/25	
	Ketofol	0/28	8/25	
T8	None	16/23	17/21	0.908
	Ketamin	7/23	0/21	
	Ketofol	0/23	4/21	
T9	None	14/17	12/18	0.113
	Ketamin	3/17	0/18	
	Ketofol	0/17	6/18	
T10	None	5/7	10/11	–
	Ketamin	2/7	0/11	
	Ketafol	0/7	1/11	
T11	None	4/4	5/5	–
T12	None	2/2	2/2	–

study (8). Additionally given midazolam did not cause hypotension, vomiting, and any respiratory distress that required endotracheal intubation. In our study, although both groups were hemodynamically stable, we observed a temporary increase in MABP, and HR when bronchoscope passes vocal cords. It was not clinically significant increase and we think that it can be prevented by increasing topical anesthetic dose.

Laryngospasm, airway obstruction, apnea, a rise in blood pressure and myocardial oxygen demand can be observed as side effects of ketamine (43). During EBUS-TBNA or any other airway procedures, coughing and increase in secretion can happen due to both ketamine and procedure by itself (44). We observed increase in coughing score as during bronchoscope passes the vocal cords in both groups despite sufficient level of sedation. The secretion increase caused by ketamine was not clinically too important and it was tolerated by frequent aspiration.

Mortero *et al*. showed that coadminastration of ketamine attenuates propofol-induced hypoventilation and may provide earlier recovery of cognition (13). In their another study, Akin *et al*. concluded that adding low dose ketamine to propofol did not increase recovery time in 60 patients (ages 1 month-13 year old) who underwent cardiac catheterization (45). Willman *et al*. showed that median recovery time was 15 min (range, 5-45 min) for ketafol (8). Chudnofsky *et al*. founded that mean recovery time for ketamine-midazolam combination as 64±24 min because of higher dose usage (9). The recovery time was approximately 25 min with lower doses in the present study. We attributed that longer recovery time in Group 1 was due to higher RSS at 35th min compared to Group 2 without clinical significance.

Adequate sedation reduces patient anxiety and improves tolerance and satisfaction with the procedure. Putinati *et al*. showed that patient tolerance improved with conscious sedation during FOB without any increased cardiorespiratory risks (19). They found that doctors' satisfaction score is much higher than patients' satisfaction score. They concluded that doctors cannot exactly evaluate patients' responses. Willman *et al*. reported that both patient's and doctor's satisfaction with ketamine-propofol combination were very well (8). Steinfort *et al*. showed that EBUS-TBNA may be safely performed with very high patient satisfaction under conscious sedation (34). Khajavi *et al*. showed that ketamine and propofol combination have more patients satisfaction than fentanil and propofol combinations (46). In our study, sedation was not evaluated as "not enough" for any patient by doctors. Even though, "excellent" response was significantly higher than Group 2, "good and excellent" response was 100% in Group 1 and 93% in Group 2. In general, all of the patients were satisfied. Both groups were similar regarding to patient and doctor satisfaction.

Our study had some limitations. The present study evaluating sedation for EBUS-TBNA was performed in a single center. A multicenter study is required to reveal more objective results and evaluate the clinical efficacy and safety of two different sedative regimens in future. The use of end tidal CO_2 ($ETCO_2$) or transcutaneous CO_2 monitoring would provide additional safety to gauge efficient ventilation

in sedation regimens (29). Although we didn't investigate the cost-effectiveness of the agents, the assessment of the cost-effectiveness could increase the quality of study as well as the efficacy and the safety.

Conclusions

Until now, there is no ideal sedation agent without undesirable effects. The sedative agent should be chosen according to procedure, the age of patient, general health condition, and the experience of bronchoscopist and anesthesiologist. Still there is no sufficient study about the safe sedative agent for EBUS-TBNA. Our study showed that both combinations were adequate and effective to relieve anxiety and to depress hypertension and tachycardia. Besides, there were no side effects like hypoxia, and hypotension indicating that the doses were safe. In conclusion, both ketamine-midazolam and ketamine-propofol combinations in the doses we used provide safe, effective, as well as comfortable conscious sedation for EBUS-TBNA and there was no superiority between two combinations. We think that ketamine's combination with either midazolam or propofol provided good levels of satisfaction for the patients and the bronchoscopist without remarkable side effects.

Acknowledgements

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References

1. Annema JT, Versteegh MI, Veseliç M, et al. Endoscopic ultrasound-guided fine-needle aspiration in the diagnosis and staging of lung cancer and its impact on surgical staging. J Clin Oncol 2005;23:8357-61.
2. Rolo R, Mota PC, Coelho F, et al. Sedation with midazolam in flexible bronchoscopy: a prospective study. Rev Port Pneumol 2012;18:226-32.
3. Whitwam JG, McCloy RF. Principles and Practice of Sedation, 2nd ed. Oxford: Blackwell Scientific,1998:1-54.
4. Friedman AG, Mulhern RK, Fairclough D, et al. Midazolam premedication for pediatric bone marrow aspiration and lumbar puncture. Med Pediatr Oncol 1991;19:499-504.
5. Broennle AM, Cohen DE. Pediatric anesthesia and sedation. Curr Opin Pediatr 1993;5:310-4.
6. Kennedy RM, Luhmann JD, Luhmann SJ. Emergency department management of pain and anxiety related to orthopedic fracture care: a guide to analgesic techniques and procedural sedation in children. Paediatr Drugs 2004;6:11-31.
7. Krauss B, Brustowicz RM. eds. Pediatric Procedural Sedation and Analgesia. Baltimore: Lippincott, Williams &Wilkins, 1999:97-103.
8. Willman EV, Andolfatto G. A prospective evaluation of "ketofol" (ketamine/propofol combination) for procedural sedation and analgesia in the emergency department. Ann Emerg Med 2007;49:23-30.
9. Chudnofsky CR, Weber JE, Stoyanoff PJ, et al. A Combination of Midazolam and Ketamine for Procedural Sedation and Analgesia in Adult Emergency Department Patients. Acad Emerg Med 2000;7:228-35.
10. Nejati A, Moharari RS, Ashraf H, et al. Ketamine/propofol versus midazolam/fentanyl for procedural sedation and analgesia in the emergency department: a randomized, prospective, double-blind trial. Acad Emerg Med 2011;18:800-6.
11. De Oliveira GS Jr, Fitzgerald PC, Hansen N, et al. The effect of ketamine on hypoventilation during deep sedation with midazolam and propofol: A randomised, double-blind, placebo-controlled trial. Eur J Anaesthesiol 2013. [Epub ahead of print].
12. Andolfatto G, Willman E. A prospective case series of single-syringe ketamine-propofol (Ketofol) for emergency department procedural sedation and analgesia in adults. Acad Emerg Med 2011;18:237-45.
13. Mortero RF, Clark LD, Tolan MM, et al. The effects of small-dose ketamine on propofol sedation: respiration, postoperative mood, perception, cognition, and pain. Anesth Analg 2001;92:1465-9.
14. Hwang J, Jeon Y, Park HP, et al. Comparison of alfetanil and ketamine in combination with propofol for patient-controlled sedation during fiberoptic bronchoscopy. Acta Anaesthesiol Scand 2005;49:1334-8.
15. Ramsay MA, Savege TM, Simpson BR, et al. Controlled Sedation with Alphaxalone-Alphadolone. Br Med J 1974;2:656-9.
16. Minogue SC, Ralph J, Lampa MJ. Laryngotracheal topicalization with lidocaine before intubation decreases the incidence of coughing on emergence from general anesthesia. Anesth Analg 2004;99:1253-7.
17. Aldrete JA. The post-anesthesia recovery score revisited. J Clin Anesth 1995;7:89-91.
18. Kennedy MP, Shweihat Y, Sarkiss M, et al. Complete mediastinal and hilar lymph node staging of primary lung cancer by endobronchial ultrasound: moderate sedation or general anesthesia? Chest 2008;134:1350-1.
19. Putinati S, Ballerin L, Corbetta L, et al. Patient

satisfaction with conscious sedation for bronchoscopy. Chest 1999;115:1437-40.
20. Stolz D, Chhajed PN, Leuppi JD, et al. Cough suppression during flexible bronchoscopy using combined sedation with midazolam and hydrocodone: a randomised, double blind, placebo controlled trial. Thorax 2004;59:773-6.
21. Smith I, Avramov MN, White PF. A comparison of propofol and remifentanil during monitored anesthesia care. J Clin Anesth 1997;9:148-54.
22. Colt HG, Morris JF. Fiberoptic bronchoscopy without premedication. A retrospective study. Chest 1990;98:1327-30.
23. Al-Atrakchi HA. Upper gastrointestinal endoscopy without sedation: a prospective study of 2000 examinations. Gastrointest Endosc 1989;35:79-81.
24. Gonzalez R, De-La-Rosa-Ramirez I, Maldonado-Hernandez A, et al. Should patients undergoing a bronchoscopy be sedated? Acta Anaesthesiol Scand 2003;47:411-5.
25. Du Rand IA, Blaikley J, Booton R, et al. British Thoracic Society guideline for diagnostic flexible bronchoscopy in adults: accredited by NICE. Thorax 2013;68:i1-i44.
26. Dreisin RB, Albert RK, Talley PA, et al. Flexible fiberoptic bronchoscopy in the teaching hospital: yield and complications. Chest 1978;74:144-9.
27. Innes G, Murphy M, Nijssen-Jordan C, et al. Procedural sedation and analgesia in the emergency department. Canadian Consensus Guidelines. J Emerg Med 1999;17:145-56.
28. Gill M, Green SM, Krauss B. A study of the Bispectral Index Monitor during procedural sedation and analgesia in the emergency department. Ann Emerg Med 2003;41:234-41.
29. Bahn EL, Holt KR. Procedural sedation and analgesia: a review and new concepts. Emerg Med Clin North Am 2005;23:503-17.
30. Kallio H, Rosenberg PH. Advances in ophthalmic regional anaesthesia. Best Pract Res Clin Anaesthesiol 2005;19:215-27.
31. Milman N, Faurschou P, Grode G, et al. Pulse oximetry during fibreoptic bronchoscopy in local anaesthesia: frequency of hypoxaemia and effect of oxygen supplementation. Respiration 1994;61:342-7.
32. Stolz D, Kurer G, Meyer A, et al. Propofol versus combined sedation in flexible bronchoscopy: a randomised non-inferiority trial. Eur Respir J 2009;34:1024-30.
33. Wright SW, Chudnofsky CR, Dronen SC, et al. Midazolam use in the emergency department. Am J Emerg Med 1990;8:97-100.
34. Steinfort DP, Irving LB. Patient satisfaction during endobronchial ultrasound-guided transbronchial needle aspiration performed under conscious sedation. Respir Care 2010;55:702-6.
35. Clark G, Licker M, Younossian AB, et al. Titrated sedation with propofol or midazolam for flexible bronchoscopy: a randomised trial. Eur Respir J 2009;34:1277-83.
36. Jensen JT, Banning AM, Clementsen P, et al. Nurse administered propofol sedation for pulmonary endoscopies requires a specific protocol. Dan Med J 2012;59:A4467.
37. Oztürk T, Cakan A, Gülerçe G, et al. Sedation for fiberoptic bronchoscopy: fewer adverse cardiovascular effects with propofol than with midazolam. Anasthesiol Intensivmed Notfallmed Schmerzther 2004;39:597-602.
38. White PF, Way WL, Trevor AJ. Ketamine--its pharmacology and therapeutic uses. Anesthesiology 1982;56:119-36.
39. Green SM, Krauss B. The semantics of ketamine. Ann Emerg Med 2000;36:480-2.
40. Song JW, Shim JK, Song Y, et al. Effect of ketamine as an adjunct to intravenous patient-controlled analgesia, in patients at high risk of postoperative nausea and vomiting undergoing lumbar spinal surgery. Br J Anaesth 2013;111:630-5.
41. Akin A, Esmaoglu A, Tosun Z, et al. Comparison of propofol with propofol-ketamine combination in pediatric patients undergoing auditory brainstem response testing. Int J Pediatr Otorhinolaryngol 2005;69:1541-5.
42. Drummond GB. Comparison of sedation with midazolam and ketamine: effects on airway muscle activity. Br J Anaesth 1996;76:663-7.
43. Strayer RJ, Nelson LS. Adverse events associated with ketamine for procedural sedation in adults. Am J Emerg Med 2008;26:985-1028.
44. Heinz P, Geelhoed GC, Wee C, et al. Is atropine needed with ketamine sedation? A prospective, randomised, double blind study. Emerg Med J 2006;23:206-9.
45. Akin A, Esmaoglu A, Guler G, et al. Propofol and Propofol-Ketamine in Pediatric Patients Undergoing Cardiac Catheterization. Pediatr Cardiol 2005;26:553-7.
46. Khajavi M, Emami A, Etezadi F, et al. Conscious Sedation and Analgesia in Colonoscopy: Ketamine/Propofol Combination has Superior Patient Satisfaction Versus Fentanyl/Propofol. Anesth Pain Med 2013;3:208-13.

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Original Article

The role of initial radiologic and clinical manifestations in predicting the prognosis for pneumonia caused by H1N1 influenza virus

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Objective: The aim of this study is to investigate the prognostic values of initial radiologic findings and preexisting medical conditions in pneumonia caused by H1N1 influenza virus that were obtained during the novel swine-origin influenza A (H1N1) virus (S-OIV) pandemic spread.

Methods: Thirty-nine patients hospitalized due to H1N1 infection between September and December 2009 were retrospectively evaluated regarding the radiologic and clinical aspects. The thoracic computed tomography (CT) findings of all patients were assessed and accompanying conditions that may raise the morbidity were stated. The patients were divided into two groups as those who needed the intensive care unit administration and those treated with brief hospitalization; initial radiologic findings and preexisting medical situations of patients were compared among both groups respectively in terms of their prognostic value.

Results: In 39 patients with H1N1 infection (21 males and 18 females; mean age of 53.9±14 in range between 19 and 99 years); the necessity of intensive care was significantly higher in patients with solely chronic obstructive pulmonary disease (COPD) (P=0.008, Odds ratio: 27) or co-existence of COPD and malignity (Odds ratio: 13); however, no statistically significant difference between two groups was observed regarding the radiologic facts or other combinations of accompanying medical conditions in terms of any effects to the prognosis.

Conclusions: In the H1N1 (S-OIV) pandemic, we observed that merely the contribution to the diagnostic process; the radiologic features have no significance as being prognostic indicator. Additionally; the superposition of H1N1 infection in patients with either COPD or COPD by malignity was stated to be a potential risk factor in terms of increased morbidity.

Keywords: H1N1; pneumonia; pandemic; influenza; prognosis

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Introduction

The novel swine-origin influenza (H1N1) virus (S-OIV) generally known as the "swine flu" was first identified in Mexico in April 2009 (1). In 11 January 2009, the World Health Organization (WHO) announced the novel influenza virus (H1N1) global pandemic documented in more than 70 countries. The first series of patients were diagnosed in North America and the disease rapidly spread towards a pandemic (1-3). Similar to the seasonal influenza virus, the virus is spread through hand contact, respiratory tract and aerosol-generating procedures (4). The spectrum of the pandemic influenza A (H1N1) virus infection ranges between non-febrile mild upper respiratory tract disease to severe and even fatal pneumonia. Generally, the condition is often characterized by benign, self-limiting respiratory symptoms; the disease is similar to the other frequently observed viral infections of the respiratory tract and seasonal

influenza that does not cause a more serious disease (5-8).

The aim of the present study is to evaluate the radiological and clinical findings of the patients with H1N1 during the novel swine-origin influenza A (H1N1) virus (S-OIV) pandemic. The radiologic aspects and variable co-existing clinical conditions were quested in terms of being indicators for increased morbidity.

Material and methods

Patients who presented with flu symptoms to our institution in the city of Van in the Eastern Anatolian region of Turkey between September-December 2009 and had to be hospitalized were retrospectively evaluated. Patients who were treated on an outpatient basis and those who did not undergo diagnostic tests for H1N1 although they presented with mild flu symptoms were excluded from the study. Thirty-nine patients with the diagnosis of H1N1 infection confirmed by fluorescent antigen-antibody testing and polymerase chain reaction (PCR) tests were included in the study. Radiography and thoracic computed tomography (CT) examinations were performed; the demographic characteristics, symptoms, laboratory findings and radiological features of all the patients were evaluated. This study was approved by the Institutional Ethics Committee, and informed consents were obtained from all patients.

The CT imaging was carried out using a two cross-sectional CT (SOMATOM Spirit Siemens, Erlangen, Germany) device and at a cross-sectional thickness of 10 mm, at a 130 kV dose and between 50-130 mAs. The images were assessed on the workstation, using the Nova PACS viewer program and a 2.560×2.048 pixel monitor. The CT images' interpretations of all cases were performed by a single radiologist with at least 5 years of thoracic imaging experience.

The hospitalized patients were divided in two groups as those who needed intensive care unit (ICU) administration and those who were required brief hospitalization without advanced mechanical ventilation; ICU management was decided to be necessary for patients defined as the existence of either one of two major, or two of three minor criteria. While the major criteria include need for mechanical ventilation and shock requiring vasopressors; the minor criteria were respiratory rate > or =30 min^{-1}, PaO_2-to-F_iO_2 ratio < or =250, multilobar infiltrates, confusion or delirium, blood urea nitrogen (BUN) > or =20 mg/dL, leukopenia (WBC count <4,000 cells/mm^3), thrombocytopenia (platelet count <100,000 cells/mm^3), hypothermia (core temperature <36 ℃), hypotension requiring aggressive fluid resuscitation criteria for ICU administration were stated to be the requirement of the invasive or non-invasive mechanical ventilation support. In both groups, the radiological features of pulmonary involvement such as extent (>3 lobes or less); location (peripheral or central); and pattern (ground glass opacity-GGO, consolidation, peribronchial thickening, or embolism) were respectively assessed as prognostic index. The pre-existing medical conditions such as obesity, chronic obstructive pulmonary disease (COPD), coronary artery disease and malignancies with or without immunocompromised conditions; moreover the patients' age were considered respectively as if they have significance affect to the prognostic process. The follow-up results of the patients, particularly of the ICU administered cases, were evaluated.

The statistical analysis was performed with the help of the SSPS statistical software (v.16) and independent samples *t*-test and Pearson's Chi-square test were employed. The logistic regression test was used to provide multivariable analysis of the co-existing risk factors and their combinations in terms of significance for the prognostic process. Statistical significance was based on a P value below 0.05.

Results

The mean age was calculated as 53.9±14 years (range, 19-99 years) among the patients, 21 (53.8%) were male and 18 (46.2%) were female. The mean age of the patients who were admitted to ICU was 58.3±14, while the mean age of those followed up on the ward was 52.7±20 years.

When the patients were classified according to their body mass index (BMI), 35 patients (89.7%) were found to be obese (BMI of 30 or greater, 2 were morbidly obese). The independent distribution of risk factors among patients was; coronary artery disease was present in seven patients (17.9%, three patients were with previous coronary by-pass surgery), COPD was present in 8 patients (20.5%), and malignancy was present in three patients (7.7%, two patients had esophageal and gastric cancer respectively; one patient had lymphoma). In terms of the combinations of risk factors that were co-existed with H1N1 pneumonia; the distribution among included patients was summarized in *Table 1*.

Thoracic CT examinations revealed that; unilateral pulmonary involvement was observed in 6 patients (15%), while bilateral pulmonary involvement was detected in 33 patients (85%). Only peripheral pulmonary involvement

Table 1 The distribution of co-existing risk factors with H1N1 pneumonia was summarized

Co-existing conditions with H1N1 pneumonia	Briefly hospitalized group (n=33)	ICU administered group (n=6)
None	2	–
Obesity	18	–
PE*	1	–
Obesity + COPD**	3	2
Obesity + PE	2	–
Obesity + CAD***	3	1
Obesity + Malignity	2	1
COPD + CAD	1	–
Obesity+ PE+ COPD	–	1
Obesity + PE + CAD	1	–
Obesity + PE + COPD + CAD	–	1
Total	33	6

*PE, pulmonary embolism **COPD, chronic obstructive pulmonary disease; ***CAD, coronary artery disease.

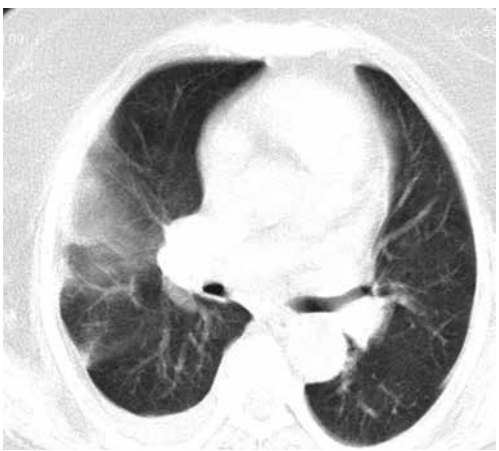

Figure 1 A 60-year-old man with confirmed H1N1 influenza associated pneumonia. Axial computed tomography (CT) image showed ground glass attenuation spreading from perihilar area to subpleural region.

was observed in 14 patients (35.9%), while only central pulmonary involvement was observed in 2 patients (5.1%), and the combination of central and peripheral pulmonary involvement was observed in 23 patients (59%). The involvement according to the lobes was as follows: upper right pulmonary lobe, 20 (51.3%); middle right pulmonary

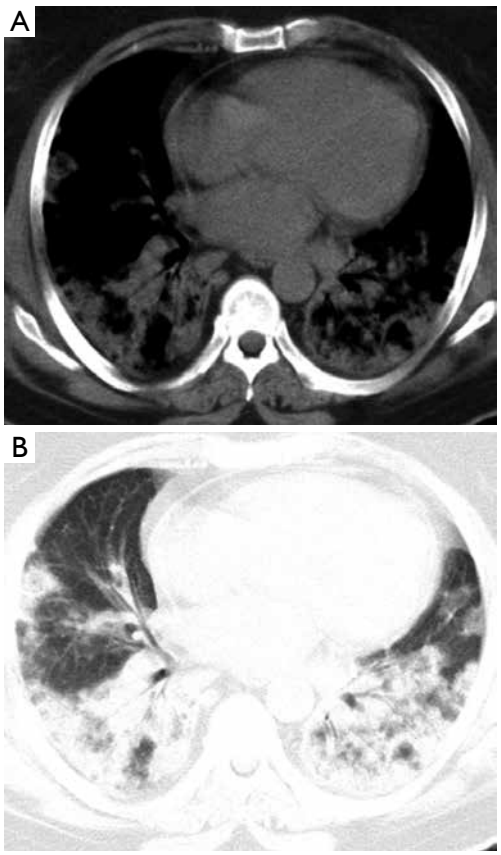

Figure 2 A 57-year-old woman with confirmed H1N1 influenza associated pneumonia. Axial computed tomography (CT) image showed patch consolidations with concomitant peripheral ground glass opacities and coarse reticulation on bilateral lungs at window levels set for (A) mediastinum and (B) parenchyma.

lobe, 29 (74.4%); lower right pulmonary lobe, 28 (71.8%); upper left pulmonary lobe, 19 (48.7%); lingula of the left lung, 24 (61.5%); and lower left pulmonary lobe, 26 (66.7%). The numeric distribution of the pulmonary patterns was as follows: ground glass in 38 patients (97.4%) (*Figure 1*); consolidation in 24 patients (61.5%) (*Figure 2*), peribronchial thickening in 33 patients (84.6%), tree-in-bud sign in 18 patients (46.2%) (*Figure 3*), nodule in 18 patients (46.2%), pleural fluid in 3 patients (7.7%) (*Figure 4*), mediastinal lymphadenopathy in 12 patients (30.8%), atelectasis in 21 patients (53.8%), and pulmonary embolism in 6 patients (15.4%). Embolic occlusions were limited to sub-segmental range (*Figure 4*).

Six patients were admitted to ICU and thirty-three patients who did not require mechanical ventilation were

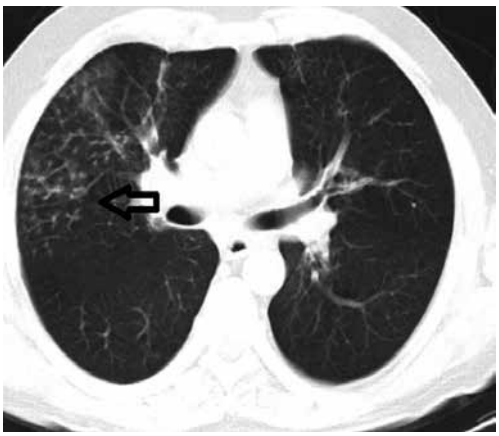

Figure 3 A 60-year-old man with confirmed H1N1 influenza associated pneumonia. Axial computed tomography (CT) image showed tree-in-bud sign (arrow) with concomitant peripheral ground glass attenuation at middle lobe of the right lung.

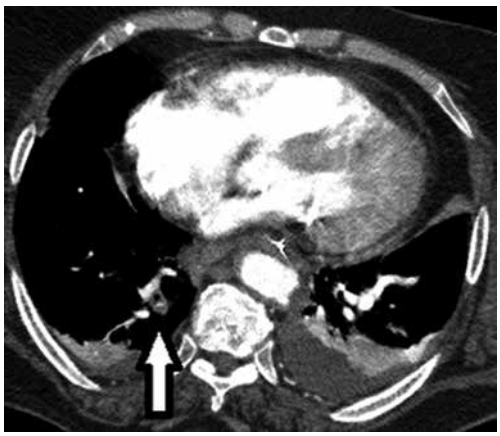

Figure 4 A 63-year-old woman with confirmed H1N1 influenza associated pneumonia. Axial computed tomography (CT) image shows the emboli at lower lobe's segmental branch of right pulmonary artery (arrow). Bilateral pleural effusion and atelectasis of adjacent lung parenchyma can also be determined.

Table 2 The statistical comparison among the patients' ages and patients' radiologic-computed tomography findings in terms of their effects on prognostic process of Influenza A H1N1 pneumonia (P values were calculated by Pearson Chi Square Test)

	Patients with ICU management (n=6)	Patients with brief hospitalization (n=33)	P Value
Mean age	58.33±14.28	52.79±20.44	0.530
Diffuse involvement (involvement >3 lobes)	4	9	0.602
Peripheral pulmonary involvement	2	12	0.412
Central pulmonary involvement	0	2	0.536
Peripheral + central pulmonary involvement	4	19	0.677
GGO*	6	32	0.666
Consolidation	5	19	0.233
Peribronchial thickening	6	27	0.256
Pulmonary Embolism	2	4	0.185

*GGO, ground glass opacity; ICU, intensive care unit.

followed up on the ward. The statistical assessment revealed a significant relationship between the co-existence of solely COPD (P=0.008, Odds ratio: 27) or COPD by malignity (Odds ratio: 13) and increased morbidity rate in the patients with H1N1 pneumonia; besides, no statistically significant correlation was observed between the incidence of necessity of ICU administration and the other investigated parameters including patients' ages, the radiologic features of pulmonary involvement such as extent, location or patterns. The summary of statistical assessment among prearranged patient groups according to CT manifestations was demonstrated in *Table 2*. The co-existing situations such as obesity, coronary artery disease and pulmonary embolism were also considered. Even the combinations of the co-existing factors among patients were included in statistical analysis; no significance by them was revealed that can affect the prognostic process. In the follow-up period; while thirty-three briefly hospitalized patients were discharged within the consequent 24 hours, six patients who were administered in ICU had a mean ICU-stay-

length of 12.67 days ±5.32 (range, 7-21 days) and were discharged without any permanent sequelae. No mortality was occurred.

Discussion

The well described etiologic factor of seasonal influenza that occurs as acute respiratory disease mostly in winter season is an RNA virus from the orthomyxovirus family termed as the "influenza". In humans, the disease is frequently caused by the type A and type B viruses (9). Novel H1N1 influenza viruses are found in North America and Eurasia (1,10). In some cases, the infection in the upper respiratory tract may rapidly progress towards a fatal pulmonary disease. Hospitalization and even mechanical ventilation may be essential in these patients. Thoracic CT plays an important role in characterising the lesions and establishing the extent of the involvement, predicting the prognosis, planning the treatment and following the patient up (11). In the patients when the PCR is negative as the laboratory marker; radiological findings can influence the definite diagnosis and avoid any delays in the treatment protocol (9). According to our experiences, it could not be possible for each patient with suspected symptoms to confirm the H1N1 infection by developed laboratory tests; especially in hospitals located at cities with low socio-economic status. The increased patient number during pandemic periods could be another explanation of this unfortunate situation. Therefore, the predomination about the knowledge of the radiologic findings of the disease has an important role for practical diagnosis to maintain the appropriate medical approach promptly.

In a previous study conducted for this purpose in Mexico, the clinical and epidemiologic characteristics of 18 hospitalized patients due to the diagnosis of S-OIV were described (1). In this study, it has been claimed that the S-OIV infection may lead to severe medical conditions (12 of the 18 patients needed mechanical ventilation) and even death (7 of 18 patients). Also, in contrast to seasonal influenza, the swine flu affected young and middle-aged patients (between 13 and 47 years; 90% of cases were <52 years). The mean age of our cases was 53.9±14, which is higher than such previous study. The mean age of the patients with the requirement of ICU administration was 58.3 years in our study. This evidence may be related to the 76 excluded patients who were treated on outpatient basis without further hospitalization. Among the hospitalized patients who needed ICU administration, only one patient was 32 years old.

In a study reported by Li *et al*. that was conducted on 106 patients (11), the median age was found as 31.7±15.7. The characteristic imaging findings were ground glass opacities (74.5%), consolidations (72.6%), nodular opacities (3.7%), and the reticular opacities (1.6%). These findings had shown similarity with previously reported study by Agarwal *et al*. (12). In our study, there were 38 cases (97.4%) of ground glass, 24 cases (61.5%) of consolidation, 18 cases (46.2%) of nodules, 33 cases of (84.6%) peribronchial thickening, and 18 cases (46.2%) with the tree-in-bud sign. We observed that, the percentage of nodules and tree-in-bud sign were higher. Again, Li *et al*. declared higher percentages of diffuse pulmonary involvement in patients requiring advanced mechanical ventilation (63.3%), when compared with patients lack serious conditions of the disease (19.3%). Our study was also resulted with similar ratios; diffuse pulmonary involvement rates were 66.6% and 43.4% among ICU administered patients and non-ICU patients; respectively. However, the incidences of central, peripheral or both central and peripheral pulmonary involvements among two groups of our study (were 33% and 39%, 0% and 2%, 66% and 57% of 6 critically ill and 33 briefly hospitalized patients; respectively) had shown disparities when compared with the report by Li *et al*. (central involvements were in 63% and 4%, peripheral involvements were in 100% and 8%, and both central and subpleural involvement were in 86% and 29% among 30 critically ill and 76 briefly hospitalized patients; respectively).

From the aspect of the involvement of the lobes by H1N1 influenza pneumonia, the previous study by Li *et al*. had pointed the upper zone in 45%, middle zone in 83% and the lower zone in 96% of patients (11). In our study, involvement in the upper lobes was observed in 51% (upper right lobe: 51%, upper left lobe: 48.7%), involvement in the middle lobes was 81.2% (middle right: 74.4%, left lingula: 71.8%), and involvement in the lower lobes was 89.7% (lower right: 71%, lower left: 66.7%). Consequently, the involvement in the middle and lower lobes were stated with higher incidences in both studies.

Federal advisory committee on Immunization Practices (ACIP) of the United States' Centers for Disease Control and Prevention (CDC) announced the obese and pregnant patients as the emergency risk groups for severe H1N1 infection (13). Another study including ten patients with novel influenza A (H1N1) virus infection and ARDS was pointed out the increased rates for severe complications,

particularly in extremely obese patients (14). Although there was a higher prevalence of obesity in hospitalized patients, no statistically significant difference was observed by our study regarding the poor prognosis due to obesity. The number of hospitalized patients due to H1N1 infection was 39 in our study and interestingly; 35 of them were obese (2 were morbid obese) which can deduce that; in obese patients, H1N1 infection has a higher prevalence for the requirement of hospitalization but no statistical difference between the ratios for brief hospitalization and most advance therapies such as ICU administration and mechanical ventilation was determined.

Pulmonary embolism (PE) has not been reported to be a frequently observed complication of influenza infection (14,15). Among the patients clinically under the suspicion of PE, van Wissen *et al*. have found lower rates of PE evidences (1%) in the patients with influenza A infection (16), Thus, the authors have concluded that the influenza infection was not associated with an increased risk of acute pulmonary embolism. In the study originated from Mexico where the S-OIV (H1N1) was verified in the laboratory, similar result as no higher incidence of PE in H1N1 infected patients was reported (1). In our study, embolisms within segmental branches of pulmonary artery were observed in 6 patients (5.6%). Ohrui *et al.* reported of similar findings that were concluded as pulmonary micro-thromboembolism in two cases (17); thus knowing such particular complication is important for the radiologists to diagnose the pulmonary micro-embolic events when investigating the contrast enhanced thoracic CT images.

In our study, coexisting of H1N1 influenza pneumonia with COPD had an unfavourable prognosis; 4 of 6 patients (66.6%) with the necessity of ICU administration were also suffering from COPD while only 4 of 33 briefly hospitalized patients (12.1%) were with COPD and influenza infection coexistence and the statistical difference between groups was significant (P=0.008). According to our knowledge, this correlation of unfavourable prognostic process of H1N1 influenza pneumonia with accompanying COPD (Odds ratio: 27) or COPD and malignity (Odds ratio: 13) has not previously been reported; and should be confirmed by further studies. We also investigated the prognostic role of coronary artery disease coexistence with H1N1 influenza infection but no relation was determined.

There are a few studies investigated the prognosis of H1N1 infection in patients, especially in children, with co-existing malignant circumstances. Tran *et al.* reported the common interruption of cancer treatment by pH1N1 infection among children with cancer and stem cell transplant recipients (18) and Shah *et al.* declared that the prevention of severe complications of influenza virus can be possible by initiation of antiviral therapy promptly in children with cancer (19). Increased morbidity and mortality in immunocompromised patients with solid tumors was documented by Chemaly *et al*. and immediate antiviral therapy was also suggested to decrease the complication rates (20). Moderate or severe parenchymal involvement mostly without association of bronchiolitis was reported as an indicator of severe viral infection in neutropenic cancer patients (21). In our study; three patients with pre-diagnosed malignancy including the esophageal and gastric cancer in two cases and lymphoma in one case had shown no statistically significant difference when compared with the other patients in terms of prognosis. Only 32-year-old male with co-exiting lymphoma had to be managed in ICU. The explanation of this dilemma when compared with previous studies could be; two patients with esophageal and gastric cancers were in their remission period when referred with influenza infection and no abnormalities in complete blood count including the rough immunity parameters were noticed; besides, slight immunocompromised situation was present in case with lymphoma. However; in our study, the malign situations when combined with COPD had shown statistically significant effect on impoverishing the prognostic process (with an odds ratio of 13). Because of previous studies were mostly included the pediatric population; projected future studies including higher number of H1N1 infected adult patients with coexisting malignancies are necessary to clarify the effects of immunocompromised situations to the prognosis of entity.

The observed radiological findings such as ground glass opacity, consolidation, nodular lesions, tree-in-bud sign, and peribronchial distribution were considered to be non-specific to influenza pneumonia. The radiological differential diagnosis must be based on cryptogenic organizing pneumonia (COP) and chronic eosinophilic pneumonia (CEP) or other viral, fungal and bacterial infections. The radiologic imaging results may be contributory to the laboratory tests especially in cases with reminiscent clinical symptoms and anamnesis of doubtful contamination (22). Additionally, the sudden onset of the H1N1 infection symptoms should be respected as an important factor when distinguishing from other diseases. However, unusual imaging findings such as pleural effusion, lymphadenopathy and lobar consolidation besides the clinical and laboratory results may help in

eliciting the accurate diagnosis (23). The patterns and distribution of the ground glass opacity, consolidations and peribronchovascular or subpleural involvements were found to be highly correlated with H1N1 infection by Aviram *et al*. (24); but we revealed that none of these radiologic features can be considered in estimating the prognosis.

Our study had certain limitations such as the total patient number was limited. We also did not verify S-OIV among the patients who were treated in an outpatient setting. Since we excluded these patients from the study, the demographic and radiological statistics of the patients could be affected by selection bias. No additional comparison of the severity of the disease among ICU administered patients was performed that can be provided by evident predictive scoring systems such as Acute Physiology, Age and Chronic Health Evaluation (I-IV) (APACHI) or Sequential Organ Failure Assessment (SOFA).

Conclusions

Radiologic manifestations and clinical parameters such as age, obesity, co-existence of coronary artery disease or malignancy (with normal immunological parameters in general) does not affect the prognosis of patients with H1N1 influenza pneumonia; besides, the clinicians should be on the alert of unfavourable and rapid progression when considering patients of H1N1 infection with co-existence of solely COPD or COPD by malignity.

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References

1. Perez-Padilla R, de la Rosa-Zamboni D, Ponce de Leon S, et al. Pneumonia and respiratory failure from swine-origin influenza A (H1N1) in Mexico. N Engl J Med 2009;361:680-9.
2. Domínguez-Cherit G, Lapinsky SE, Macias AE, et al. Critically Ill patients with 2009 influenza A(H1N1) in Mexico. JAMA 2009;302:1880-7.
3. Echevarría-Zuno S, Mejía-Aranguré JM, Mar-Obeso AJ, et al. Infection and death from influenza A H1N1 virus in Mexico: a retrospective analysis. Lancet 2009;374:2072-9.
4. Writing Committee of the WHO Consultation on Clinical Aspects of Pandemic (H1N1) 2009 Influenza, Bautista E, Chotpitayasunondh T, et al. Clinical aspects of pandemic 2009 influenza A (H1N1) virus infection. N Engl J Med 2010;362:1708-19.
5. Plessa E, Diakakis P, Gardelis J, et al. Clinical features, risk factors, and complications among pediatric patients with pandemic influenza A (H1N1). Clin Pediatr (Phila) 2010;49:777-81.
6. Komiya N, Gu Y, Kamiya H, et al. Clinical features of cases of influenza A (H1N1)v in Osaka prefecture, Japan, May 2009. Euro Surveill 2009;14. pii: 19272.
7. Park SI, Kim MJ, Hwang HY, et al. Clinical characteristics of children with 2009 pandemic influenza A (H1N1) admitted in a single institution. Korean J Pediatr 2010;53:886-91.
8. Ong AK, Chen MI, Lin L, et al. Improving the clinical diagnosis of influenza--a comparative analysis of new influenza A (H1N1) cases. PLoS One 2009;4:e8453.
9. García-García J, Ramos C. Influenza, an existing public health problem. Salud Publica Mex 2006;48:244-67.
10. Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team, Dawood FS, Jain S, et al. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. N Engl J Med 2009;360:2605-15.
11. Li P, Su DJ, Zhang JF, et al. Pneumonia in novel swine-origin influenza A (H1N1) virus infection: high-resolution CT findings. Eur J Radiol 2011;80:e146-52.
12. Agarwal PP, Cinti S, Kazerooni EA. Chest radiographic and CT findings in novel swine-origin influenza A (H1N1) virus (S-OIV) infection. AJR Am J Roentgenol 2009;193:1488-93.
13. To KK, Wong SS, Li IW, et al. Concurrent comparison of epidemiology, clinical presentation and outcome between adult patients suffering from the pandemic influenza A (H1N1) 2009 virus and the seasonal influenza A virus infection. Postgrad Med J 2010;86:515-21.
14. Centers for Disease Control and Prevention (CDC). Intensive-care patients with severe novel influenza A (H1N1) virus infection - Michigan, June 2009. MMWR Morb Mortal Wkly Rep 2009;58:749-52.
15. Schultz MJ, Haitsma JJ, Zhang H, et al. Pulmonary coagulopathy as a new target in therapeutic studies of acute lung injury or pneumonia--a review. Crit Care Med 2006;34:871-7.
16. van Wissen M, Keller TT, Ronkes B, et al. Influenza infection and risk of acute pulmonary embolism. Thromb J 2007;5:16.
17. Ohrui T, Takahashi H, Ebihara S, et al. Influenza A virus infection and pulmonary microthromboembolism. Tohoku

J Exp Med 2000;192:81-6.
18. Tran D, Science M, Dix D, Portwine C, et al. Pandemic (H1N1) 2009 influenza in Canadian pediatric cancer and hematopoietic stem cell transplant patients. Influenza Other Respir Viruses 2012;6:e105-13.
19. Shah DP, El Taoum KK, Shah JN, et al. Characteristics and outcomes of pandemic 2009/H1N1 versus seasonal influenza in children with cancer. Pediatr Infect Dis J 2012;31:373-8.
20. Chemaly RF, Vigil KJ, Saad M, et al. A multicenter study of pandemic influenza A (H1N1) infection in patients with solid tumors in 3 countries: early therapy improves outcomes. Cancer 2012;118:4627-33.
21. Rodrigues RS, Marchiori E, Bozza FA, et al. Chest computed tomography findings in severe influenza pneumonia occurring in neutropenic cancer patients. Clinics (Sao Paulo) 2012;67:313-8.
22. Punpanich W, Chotpitayasunondh T. A review on the clinical spectrum and natural history of human influenza. Int J Infect Dis 2012;16:e714-23.
23. Guo HH, Sweeney RT, Regula D, et al. Best cases from the AFIP: fatal 2009 influenza A (H1N1) infection, complicated by acute respiratory distress syndrome and pulmonary interstitial emphysema. Radiographics 2010;30:327-33.
24. Aviram G, Bar-Shai A, Sosna J, et al. H1N1 influenza: initial chest radiographic findings in helping predict patient outcome. Radiology 2010;255:252-9.

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Original Article

Levels of 1,25(OH)2D3 for patients with pulmonary tuberculosis and correlations of 1,25(OH)2D3 with the clinical features of TB

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Background: To determine the 1,25-dihydroxyvitamin D3 [1,25(OH)2D3] concentrations to patients with tuberculosis (TB) and whether it influenced the patient's clinical features.

Methods: For the first part, a total of 153 healthy adults and 74 patients with pulmonary TB (PTB) were enrolled. Serum concentrations of 1,25(OH)2D3 were determined by liquid chromatography-tandem mass spectroscopy to examine the 1,25(OH)2D3 concentrations of the two groups from the peripheral blood. If there are differences between the two groups, what follow will increase the experimental group numbers to examine the relationship among the 1,25(OH)2D3 concentrations with the numbers of the lesion area, the tubercule bacilli in sputum and the CD4/CD8 ratio of T lymphocytes in the peripheral blood.

Results: In the first part, the 1,25(OH)2D3 concentrations was lower in patients with TB than in those healthy adults [365.9 (SD 235.7) *vs.* 464.3 (SD 335.6), P<0.05]. In the second part, we increased the sample size to 134 (male 91 cases, female 43 cases). we found that the plasma levels of 1,25(OH)2D3 are not correlated with the numbers of the lesion area and the tubercule bacilli in sputum, but the 1,25(OH)2D3 levels can interact the ratio of CD4/CD8 T lymphocytes, it shows a positive correlation with the ratio of CD4/CD8 T lymphocytes.

Conclusions: The 1,25(OH)2D3 concentrations in TB patients lower than the healthy adults, it might exist as a risk factor during the development of TB or TB might affect the levels of 1,25(OH)2D3. But the different status vitamin D concentration might not affect the numbers of the lesion area, the tubercule bacilli in sputum. It shows a positive correlation with the ratio of CD4/CD8 T lymphocytes. The study will have a significance value to clinical medicine, but further study will need to study the levels of 1,25(OH)2D3 with the TB.

Keywords: Tuberculosis (TB); 1,25-dihydroxyvitamin D3 [1,25(OH)2D3]; clinical features; CD4/CD8 T lymphocytes

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Introduction

Vitamin D has a plethora of functions in the human body, of which the regulation of calcium metabolism is the most well-known. In recent years, the important role for vitamin D in the modulation of the innate immune by its role is as an immunomodulator (1-4). Although the effects of vitamin D on the innate immune response have been established *in vitro* and *ex vivo* (5,6), some data are available evaluating plasma 25(OH)D concentrations (7-12), there is a few concern and studying of its active metabolite 1,25-dihydroxyvitamin D3 [1,25(OH)2D3] *in vivo*. Plasma concentrations of 1,25(OH)2D3, but not 25(OH)D3, is the most active form of vitamin D and is responsible for most of its biological actions, directly up-regulates the production of human cathelicidin antimicrobial peptide (CAMP) from monocytes/macrophages infected with

mycobacterium tuberculosis (TB) (13-15). 1,25(OH)2D3 status had a strong influence by seasonal variation (16). In addition, there are litter studies regarding 1,25(OH)2D3 with clinical outcomes, in this study, we have examined the 1,25(OH)2D3 concentration of the two groups from the peripheral blood and the relationship about the 1,25(OH)2D3 status among the lesion areas (the number of lesion area was divided into six areas through the lower edge of the 2,4 ribs during the chest X-rays), the tubercule bacilli in sputum. Previous *in vitro* and animal studies have suggested that 1,25(OH)2D3 has important immunoregulatory properties, and it has been well documented that T lymphocytes are dominant leukocytes present in TB, and the different immunophenotype of lymphocytes affect the prognosis of TB. Previous *in vitro* and animal studies have suggested that 1,25(OH)2D3 has important immunoregulatory properties and it has been well documented that T lymphocytes are dominant leukocytes present in TB. CD4(+) helper and CD8(+) cytotoxic T cells, two major subsets of T lymphocytes, CD4 T cells become activated and proliferate rapidly secreting cytokines that send signals and maintain active immune response. On the other hand, the CD8 T-cells destroy virally infected cells and tumor cells. The ratio of CD4/CD8 T lymphocyte cell affects the resistance in mycobacterium TB. Previous *in vitro* and animal studies have suggested that 1,25(OH)2D3 has important immunoregulatory properties. So, at the last, we investigated the ratio of CD4/CD8 lymphocyte cell in TB patients, to investigate whether the status of 1,25(OH)2D3 affects the ratio of CD4/CD8 T lymphocyte cell.

Patients and methods

Patients and data acquisition

The patients were recruited from the clinics of Nanjing Chest Hospital Tuberculosis Research Centre (TRC), who were clinically and radiologically diagnosed with PTB, which was confirmed by sputum smear examinations, culture for *M. tuberculosis* (at least two sputum specimens positive or culture for acid-fast bacilli by microscopy sputum smear positive) or clinical features. The test group and control group are come from the Eastern China. All the patients were HIV negative and none of them presented with any other infectious disease or any other organ function impaired. To this end, ethylenediamine tetraacetic acid (EDTA) anticoagulated blood was collected before administration of medicine and centrifuged immediately at 2,000 rpm for 10 min at 4 ℃, after which plasma was stored at –80 ℃ until analysis. Serum concentrations of 1,25(OH)2D3 and the ratio of CD4/CD8 T lymphocyte cell were determined by liquid chromatography-tandem mass spectroscopy, the 24 hours smear were collected to be used for concentration smear and routine culture. The number of lesions and smear-negative TB (≥3 sputum smear investigations is negative) or smear-positive TB (≥3 sputum smear investigations is positive) were obtained from the clinical history of the patients to examine the concentrations of the two groups. The lesion areas, the sputum conversion rate of the PTB were obtained from the clinical history to examine the correlations between the 1,25(OH)2D3 concentrations and these relations. The study was approved by the Institutional Ethics Committee of Nanjing Chest Hospital Tuberculosis Research Centre. Informed consent was obtained from each subject before the start of the study.

Statistical analyses

All analyses were performed using SPSS18.0. Student's *t*-test was applied to calculate the 1,25(OH)2D3 concentrations between the male and female, the sputum negative and positive, the ratio of CD4/CD8 T lymphocyte cell ≥ and ≤1.0, one way analysis of variance (ANOVA) was used to compare the 1,25(OH)2D3 concentrations among the numbers of the lesion areas of two groups. P value less than 0.05 was considered statistically significant.

Results

1,25(OH)2D3 concentrations in the two groups

A total of 74 TB and 153 healthy adults blood were obtained, in the cases group, 46 males and 28 females, in the control group, 91 males and 72 females, among the two groups, there is no significant difference of basal clinical conditions between the two groups (*Table 1*). 1,25(OH)2D3 concentrations were found to be significantly decreased among PTB patients compared to the healthy adults [365.9 (SD 235.7) *vs.* 464.3 (SD 335.6), P<0.05] (*Figure 1*). In the male group, the 1,25(OH)2D3 concentrations were found to be decreased among PTB patients compared to the healthy adults (P<0.05), unfortunately, in the female group, there is no significant difference between the two groups (P>0.05) (*Table 2*).

Table 1 Baseline characteristics of the two groups

	Cases	Controls
Male	46	81
Female	28	72
Body mass index (SD)	19.1 (3.2)	19.5 (3.7)
Total number	74	153

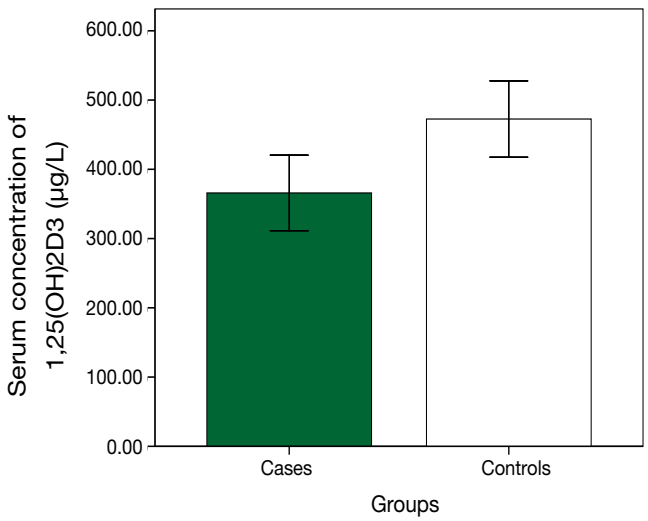

Figure 1 The plasma concentrations of 1,25(OH)2D3 of the two groups. Green stands for cases group, white stands for control group, the plasma concentrations of 1,25(OH)2D3 of the control group was greater compare with the test group. 1,25(OH)2D3, 1,25-dihydroxyvitamin D3.

Table 2 Ages and the plasma concentrations of 1,25(OH)2D3 of the two groups

	Cases		Controls		P
	Mean	SD	Mean	SD	
Age (years)	31.2	10.4	33.07	10.6	0.193
1,25(OH)2D3 (μg/L)					
Total	365.9	235.7	464.3	335.6	0.010
Male	340.7	175.3	429.1	315.8	0.037
Female	407.1	508.7	310.2	356.2	0.188

1,25(OH)2D3, 1,25-dihydroxyvitamin D3.

Correlation between plasma concentrations of 1,25(OH)2D3 and the numbers of the lesion area, the smear-negative or positive and the ratio of the ratio of CD4/CD8 T lymphocyte cell

In the second part, we increase the number of TB cases to 134, males 91, females 43, the most common status 1,25(OH)2D3 is 200-600 ng/mL (*Figure 2*). It showed that the status of 1,25(OH)2D3 was not correlation with the different numbers of the lesion areas, the smear-negative or positive, but it shows a positive correlation with the ratio of CD4/CD8 T lymphocytes with the status of 1,25(OH)2D3 [331.0 (SD 49.7) *vs.* 394.8 (SD 235.7), P<0.05] (*Table 3*).

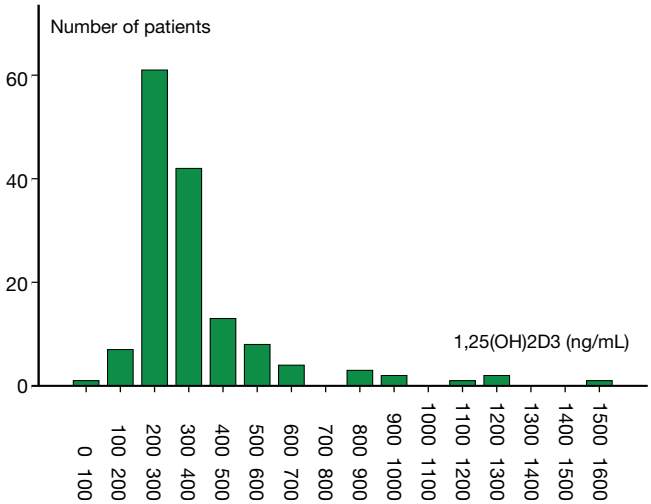

Figure 2 The number of patients of different levels of 1,25(OH)2D3 in the TB group. 1,25(OH)2D3, 1,25-dihydroxyvitamin D3; TB, tuberculosis.

Discussion

In the present study, vitamin D status was evaluated in view of its immune-regulatory role in susceptibility to microbial infection (17). Lots of researches have shown that 1,25(OH)2D3 can increase the monocytes/macrophages to killing mycobacterium TB (18,19). We have shown that the patients with TB had comparatively prevalence of concentrations decreased compared with those healthy adults (365.9 *vs.* 464.3, P=0.010), supported the evidence that the TB patients reflects their poor immune status compared to healthy adults (20,21). Suggest the need for vitamin D supplementation in such patients to prevent or to adjuvant the antibiotic therapy for control of TB. There has been much hope that vitamin D might fulfill at least some of these actions as a potential adjunctive treatment in active or latent TB. As see in the *Figure 2*, the concentrations of 200-600 μg/L were take the most percentage both in the TB groups. In the cases group the male patients shows the

Table 3 The basic clinical and the relationship among plasma concentrations of 1,25(OH)2D3 and the numbers of the lesion areas, the smear-negative or positive and the ratio of CD4/CD8 T lymphocyte cell

Group	n	Mean	SD	P
Total	134	368.4	216.1	0.228
Male	91	354.8	184.5	
Female	43	400.9	267.3	
Smear (-) or (+)				0.105
(+)	56	301.9	96.0	
(–)	70	406.5	261.4	
The ratio of CD4/CD8				0.008
(I) ≤1.0	70	331.0	49.7	
(II) ≥1.0	64	394.8	235.7	
The numbers of lesion area				
(I) 1-2	47	343.9	193.4	(I) and (II) P=0.107
(II) 2-4	32	394.6	238.4	(II) and (III) P=0.192
(III) 4-6	55	387.2	217.5	(I) and (III) P=0.547

1,25(OH)2D3, 1,25-dihydroxyvitamin D3.

1,25(OH)2D3 concentrations decreased than those healthy male adults (340.7 *vs.* 429.1, P<0.05), but in the female group, there is no significant difference between the two groups (P>0.05) (*Table 2*). These findings question the role of estrogen levels maybe affect the 1,25(OH)2D3 status and the active metabolite 1,25(OH)2D3 *in vivo* easy to be influenced by the environment (22). We also studied the different concentrations of 1,25(OH)2D3 on the numbers of the lesion areas and the smear-negative or positive of TB patients. It revealed that there were no significant difference between the concentrations of 1,25(OH)2D3 with the different lung fields. In the last part, we found that the 1,25(OH)2D3 levels can interact the ratio of CD4/CD8 T lymphocytes, it shows a positive correlation with the ratio of CD4/CD8 T lymphocytes, this findings also coincide with the results of Torres's (23). This result also broad our mind that maybe the 1,25(OH)2D3 can improve the immune system of TB patient.

Several limitations of our study deserve attention. First, we studied only the activate concentrations of 1,25(OH)2D3 in the two groups, and have not studied the 25(OH)D3, However, to our knowledge, there are litter data that indicate the concentrations of 1,25(OH)2D3 and its effects on the imaging feature of TB. Secondly, we have only studied the different status of 1,25(OH)2D3 that can affect the ratio of CD4/CD8 T lymphocytes, the higher status of 1,25(OH)2D3, the higher ratio of CD4/CD8 T lymphocytes, this result will broaden our mind that the 1,25(OH)2D3 affects the ratio of CD4/CD8 T lymphocytes subtypes and let the person proved susceptible to TB. Further study will need to study the 1,25(OH)2D3 with the immune system.

Conclusions

In conclusion, the main finding of our study is that the 1,25(OH)2D3 concentrations were lower in patients with TB than in those healthy adults. And the 1,25(OH)2D3 levels can interact the ratio of CD4/CD8 T lymphocytes, it shows a positive correlation with the ratio of CD4/CD8 T lymphocytes. These data provide further evidence that lower of 1,25(OH)2D3 status may predispose to TB or the 1,25(OH)2D3 concentrations might exist as a risk factor during the development of TB, however, it is impossible to investigate the causal relationship between vitamin D3 and susceptibility for TB in this paper. Questions about the relation between TB and 1,25(OH)2D3 concentrations are largely unexplored, it can be concluded that vitamin D supplementation is a much needed, low cost, effective, and safe intervention strategy for breast cancer prevention that should be implemented.

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References

1. Selvaraj P, Harishankar M, Singh B, et al. Effect of vitamin D3 on chemokine expression in pulmonary tuberculosis. Cytokine 2012;60:212-9.
2. Khoo AL, Chai L, Koenen H, et al. Translating the role of vitamin D3 in infectious diseases. Crit Rev Microbiol 2012;38:122-35.
3. Baeke F, Etten EV, Overbergh L, et al. Vitamin D3 and the immune system: maintaining the balance in health and disease. Nutr Res Rev 2007;20:106-18.
4. Yang HF, Zhang ZH, Xiang LB, et al. 25(OH)D(3) affects the maturation and function of mouse bone marrow-derived dendritic cells stimulated by Mycobacterium bovis BCG. PLoS One 2012;7:e48062.

5. Salahuddin N, Ali F, Hasan Z, et al. Vitamin D accelerates clinical recovery from tuberculosis: results of the SUCCINCT Study [Supplementary Cholecalciferol in recovery from tuberculosis]. A randomized, placebo-controlled, clinical trial of vitamin D supplementation in patients with pulmonary tuberculosis'. BMC Infect Dis 2013;13:22.
6. Cao S, Luo PF, Li W, et al. Vitamin D receptor genetic polymorphisms and tuberculosis among Chinese Han ethnic group. Chin Med J (Engl) 2012;125:920-5.
7. Tiwari S, Pratyush DD, Gupta B, et al. Prevalence and severity of vitamin D deficiency in patients with diabetic foot infection. Br J Nutr 2013;109:99-102.
8. Wagner CL, McNeil R, Hamilton SA, et al. A randomized trial of vitamin D supplementation in 2 community health center networks in South Carolina. Am J Obstet Gynecol 2013;208:137.e1-13.
9. Major JM, Graubard BI, Dodd KW, et al. Variability and reproducibility of circulating vitamin D in a nationwide U.S. population. J Clin Endocrinol Metab 2013;98:97-104.
10. Gallo S, Comeau K, Vanstone C, et al. Effect of different dosages of oral vitamin D supplementation on vitamin D status in healthy, breastfed infants: a randomized trial. JAMA 2013;309:1785-92.
11. Abrams SA. Targeting dietary vitamin D intakes and plasma 25-hydroxyvitamin D in healthy infants. JAMA 2013;309:1830-1.
12. Biancuzzo RM, Clarke N, Reitz RE, et al. Serum concentrations of 1,25-dihydroxyvitamin D2 and 1,25-dihydroxyvitamin D3 in response to vitamin D2 and vitamin D3 supplementation. J Clin Endocrinol Metab 2013;98:973-9.
13. Sato E, Imafuku S, Ishii K, et al. Vitamin D-dependent cathelicidin inhibits Mycobacterium marinum infection in human monocytic cells. J Dermatol Sci 2013;70:166-72.
14. Yuk JM, Shin DM, Lee HM, et al. Vitamin D3 induces autophagy in human monocytes/macrophages via cathelicidin. Cell Host Microbe 2009;6:231-43.
15. Fabri M, Stenger S, Shin DM, et al. Vitamin D is required for IFN-gamma-mediated antimicrobial activity of human macrophages. Sci Transl Med 2011;3:104ra102.
16. Kox M, van den Berg MJ, van der Hoeven JG, et al. Vitamin D status is not associated with inflammatory cytokine levels during experimental human endotoxaemia. Clin Exp Immunol 2013;171:231-6.
17. Sudfeld CR, Giovannucci EL, Isanaka S, et al. Vitamin D status and incidence of pulmonary tuberculosis, opportunistic infections, and wasting among HIV-infected Tanzanian adults initiating antiretroviral therapy. J Infect Dis 2013;207:378-85.
18. Jo EK. Innate immunity to mycobacteria: vitamin D and autophagy. Cell Microbiol 2010;12:1026-35.
19. Selvaraj P, Prabhu Anand S, Harishankar M, et al. Plasma 1,25 dihydroxy vitamin D3 level and expression of vitamin d receptor and cathelicidin in pulmonary tuberculosis. J Clin Immunol 2009;29:470-8.
20. Gallagher JC, Peacock M, Yalamanchili V, et al. Effects of vitamin D supplementation in older African American women. J Clin Endocrinol Metab 2013;98:1137-46.
21. Tostmann A, Wielders JP, Kibiki GS, et al. Serum 25-hydroxy-vitamin D3 concentrations increase during tuberculosis treatment in Tanzania. Int J Tuberc Lung Dis 2010;14:1147-52.
22. Jamali Z, Asadikaram G, Mahmoodi M, et al. Vitamin D status in female students and its relation to calcium metabolism markers, lifestyles, and polymorphism in vitamin D receptor. Clin Lab 2013;59:407-13.
23. Zofková I, Kancheva RL. The effect of 1,25(OH)2 vitamin D3 on CD4+/CD8+ subsets of T lymphocytes in postmenopausal women. Life Sci 1997;61:147-52.

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Original Article

Glutathione and nitrite levels in induced sputum at COPD patients and healthy smokers

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Objectives: The role of oxidative stress at the pathogenesis of chronic obstructive pulmonary disease (COPD) is known. The aim of this study is to investigate the oxidative stress with sputum induction that is a simple method in COPD patients and healthy smokers.

Methods: Sputum induction was performed in 21 COPD patients (10 stable, 11 acute exacerbations), nine healthy smokers, and ten healthy non-smokers. Glutathione, NO_2^- levels, and cell counts at sputum, and plasma NO_2^- contents were evaluated in all subjects.

Results: Mean sputum glutathione and NO_2^- levels were significantly higher in acute exacerbations with COPD patients than healthy smokers (P=0.007 and P<0.001 respectively), and non-smokers (P<0.001 and P<0.001 respectively). On the other hand, sputum glutathione and NO_2^- levels did not show significant differences between stable and acute exacerbations with COPD patients. Although, sputum glutathione levels were higher in stable COPD patients than healthy smokers', no statistically significant difference was established. In addition, sputum glutathione levels were significantly higher in healthy smokers than non-smokers (P<0.001).

Conclusions: As a result, we can say that oxidative stress increases not only in COPD patients but also in healthy smokers. In addition, sputum induction that is a simple method can be used to demonstrate to show oxidative stress.

Keywords: Glutathione; nitrite; induced sputum; chronic obstructive pulmonary disease (COPD)

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Introduction

Chronic obstructive pulmonary disease (COPD) is a major cause of chronic morbidity and mortality throughout the world. The pathophysiology of airway obstruction in COPD is multifactorial, and involves neutrophilic airway inflammation, protease-antiprotease imbalance, oxidative stress, T cell predominant interstitial inflammation, and recurrent infection (1-4).

The major risk factor for COPD is cigarette smoking, which is one of the most potent oxidants. Other factors that may exacerbate COPD, such as air pollutants, infections, and occupational dusts also have the potential to produce oxidative stress (5).

Oxidants present in cigarette smoke can stimulate alveolar macrophages to produce reactive oxygen species and to release a number of mediators, some of which attract neutrophils and other inflammatory cells into the lungs. Neutrophils and macrophages are known to migrate in increased numbers into the lungs of cigarette smokers, compared to nonsmokers and can generate reactive oxygen species via the NADPH oxidase system (6).

Activated inflammatory cells are also an important source of free radicals in the lungs (7). To scavenge these radical species, airway surfaces are covered with a thin liquid film

Table 1 Demographic characteristics and lung function data of all groups

	Group 1	Group 2	Group 3	Group 4
n	10	11	9	10
Age (years)	54.20±4.78	55.55±4.18	51.22±4.09	50.60±4.35
Sex (M/F)	9/1	10/1	9/–	9/1
Smoking (pack-year)	51.90±20.26[a]	52.72±17.93[a]	20.33±8.77	–
FEV_1 (% pred)	45.70±12.46[a,b]	32.18±9.99[a,b]	87.66±9.44	93.00±11.01
ΔFEV_1 (%)	–0.09±0.09	0.03±0.06	0.05±0.23	0.01±0.12

ΔFEV_1 (%), post-pre sputum induction FEV_1 values; [a], P<0.001 compared with group 3; [b], P<0.001 compared with group 4.

(epithelial lining fluid) containing a number of antioxidants. One of these antioxidants is glutathione, a tripeptide containing an SH group (8,9). Determining airway glutathione content is of use when investigating oxidative stress in the lung (10).

Nitric oxide (NO) has been used as a marker of airway inflammation and indirectly as a measure of oxidative stress. After production, NO can be exhaled, metabolized to nitrite and nitrate (NO_2, NO_3), or interact with superoxide to form peroxynitrite. The reaction of NO with $O_2^{\cdot-}$ limits the usefulness of this marker in COPD, except perhaps to differentiate from asthma (5,11).

Airway inflammation in COPD can be demonstrated by examination of induced sputum. Sputum induction is a safe and successful method in subjects with COPD patients (12). It is particularly useful as a noninvasive technique when expiratory airflow limitation precludes airway sampling via bronchoscopy (10).

The aim of this study was to assess oxidative stress in stable COPD patients and healthy smokers on induced sputum glutathione, NO_2, and serum NO_2 contents.

Materials and methods

Subjects

A total of 40 subjects were studied (*Table 1*).

COPD patients

Twenty-one COPD patients who satisfied the GOLD criteria (13) were randomly selected from the outpatient clinic of our hospital. They had a history of smoking (>20 pack years) and irreversible airflow limitation [reversibility <10% predicted forced expiratory volume in one second (FEV_1) after 200 μg inhaled salbutamol]. They received no medication during the spirometric study and sputum induction. Ten patients (group 1) were clinically stable and none had a history of respiratory infection for at least 4 weeks before the study. Eleven patients (group 2) were studied within 3 hours of admission to the hospital with acute exacerbation of their condition. Exacerbation of COPD was defined by GOLD (13) (increased breathlessness, cough, and sputum production). None of the patients was taking antioxidant medication, which could affect bronchial glutathione levels.

Healthy smokers and non-smokers subjects

A total of 19, age-matched, healthy volunteers [nine smokers (group 3) and ten lifelong non-smokers (group 4)] were randomly selected from the hospital staff. None of the subjects had a history of respiratory or allergic disease, all of their baseline spirometric parameters were normal as predicted for age, sex, and height, none had a history of upper respiratory tract infection in the previous 6 weeks, and none was taking regular medication.

This study was approved by the local ethics committee, and all subjects gave written informed consent.

Pulmonary function tests

Pulmonary function parameters (FEV_1, FVC) were measured with SuperSpiro spirometer (Micromedical Limited, UK).

Sputum induction

Sputum induction was performed according to a method previously described (10). Before and after sputum induction, spirometric analysis was performed. In addition, the safety of this procedure was monitored by measuring peak expiratory flow rates (PEFR). If PEFR decreased by 25% the procedure would have been stopped.

Sputum induction was performed by having the subject inhale hypertonic saline (3% NaCl) for maximum

20 minutes from an ultrasonic nebuliser (Porta-Neb compressor, Medic-Aid Sidestream nebuliser chamber, mass median diameter 3.18 μm (Medic-Aid Limited, UK)). Before each sputum expectoration, all subjects rinsed their mouths with distilled water. The first portion of sputum was discarded, and the inhalation procedure was continued for maximum 20 minutes longer. At least five sputum samples were obtained from each subject. Process was terminated before 20 minutes if collected enough samples. Expectorated sputum was collected in sterile plastic tubes and placed on ice to slow down metabolic processes, which might result in loss of glutathione.

Sample processing

Sputum samples were processed within 30 minutes of collection. Volumes were measured, and visible salivary contamination was removed from sputum. Samples were diluted with three volumes of chilled phosphate buffered saline (PBS: all reagents were purchased from Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and dispersed by gently agitating the tube and aspirating the sample with a wide bore pipette. Supernatants were obtained by centrifugation (300 g, 15 minutes, 4 ℃) and transferred to another vial by filtering through multiple layers of cotton gauze. Additional centrifugation (800 g, 5 minutes, 4 ℃) ensured the removal of the remaining cell debris and mucus. Aliquots of the supernatants were placed on ice and assayed immediately for glutathione.

The pellet was diluted with three volumes (per gram of pellet) of freshly prepared 6.5 mM dithiothretiol (DTT) in PBS, vortexed, and incubated at 37 ℃ for 15 minutes with occasional mixing. The cell suspension was dispersed, filtered through two layers of cotton gauze, and pelleted by centrifugation (300 g, 10 minutes, 25 ℃). An aliquot of the cells was cytospin at 1,500 g for 3 minutes at 25 ℃ (Cytopsin 3, Shandon, Frankfurt/Main, Germany). Cytospins were stained with May-Gruenwald-Giemsa dye and blindly analyzed by a pathologist blind to the clinical characteristics. At the first reading, the percentage of squamous cells was determined, and at the second reading differential cell counts of non-squamous cells (ciliated cells, macrophages, neutrophils, lymphocytes, eosinophils) were evaluated. The squamous cells, bronchial epithelial cells, macrophages, neutrophils, eosinophils, lymphocytes, ciliated cells were counted in ten area on slide by objective (Olympus Bx50), and then the mean counts of each area were taken. The cases without bronchial epithelial cell and/or alveolar macrophages were excluded in evaluation. Only sputum samples with non-squamous cell viability of more than 60% and 60% or fewer squamous cells (<50%) were analyzed. Any samples of sputum contaminated with blood were excluded from analyses.

Glutathione measurement

The total glutathione level of induced sputum samples was measured using an enzymatic recycling assay (14,15). The standard and sample solutions were added to an equal volume of DTNB and 50 μL of this mixture (final concentrations of DTNB 0.25 mM) were pipetted into a 1 mL cuvette, followed by glutathione reductase and NADPH (final concentrations 1 U/mL and 0.22 μM respectively). The reaction mixture was equilibrated, and the kinetic reaction was followed for two minutes at 412 nm (Techcomp Ltd., UV-VIS 8500 spectrophotometer, Hong Kong).

NO_2^- measurement

Nitrite was measured in sputum and plasma samples, as previously described, using the Griess reaction (16).

Statistical analysis

Data were analyzed using the statistical package for the social sciences (SPSS) software statistical program. Results were given as group means ± standard deviations (SD). Statistical analysis was performed using nonparametric Kruskal-Wallis 1-way ANOVA test for multiple-group comparisons; Mann-Whitney U test was performed to test any observed differences for significance. The significance of correlations was evaluated by determining Spearman rank correlation coefficients. A Pvalue of <0.05 was considered statistically significant.

Results

Sputum induction

All subjects tolerated the sputum induction well. Only a very small decrease in FEV_1 was observed in stable COPD patients upon induction (P>0.05, *Table 1*), and there were no further complications.

Cell counts in sputum

The differential cell percentages in all groups appear in

Table 2 The differential of cell percentages in all groups

	Group 1	Group 2	Group 3	Group 4
Macrophages (%)	29.21±4.40[a,b]	22.91±8.41[b]	46.59±9.54[c]	56.91±10.71
Neutrophils (%)	60.17±5.62[a,b]	70.76±10.16[b]	42.44±7.48	35.53±10.70
Lymphocytes (%)	5.11±2.33	4.01±3.14	5.49±4.77	3.62±3.38
Eosinophils (%)	2.94±2.51	1.47±1.29	3.73±2.88	2.68±3.08
Ciliated cells (%)	2.55±2.02	0.83±0.78	1.72±1.39	1.23±1.69

*, all values presented mean ± SD; [a], P<0.05 compared with group 2; [b], P<0.001 compared with group 3, and 4; [c], P<0.05 compared with group 4.

Table 3 Sputum GSH, NO_2, and serum NO_2 levels in all groups

	Group 1	Group 2	Group 3	Group 4
Sputum GSH (μmol)	2.17±0.59[a]	2.96±0.91[a,d]	1.86±0.52[a]	0.37±0.11
Sputum NO_2 (μmol)	463.82±74.58[b]	534.90±90.87[b]	345.40±26.64	343.43±36.62
Serum NO_2 (μmol/L)	317.21±40.34[c]	361.13±45.57[e]	334.49±28.50[e]	279.58±43.75

*, all values presented mean ± SD; [a], P<0.001 compared with group 4; [b], P<0.001 compared with group 3, and 4; [c], P<0.05 compared with group 2; [d], P<0.01 compared with group 3; [e], P<0.01 compared with group 4.

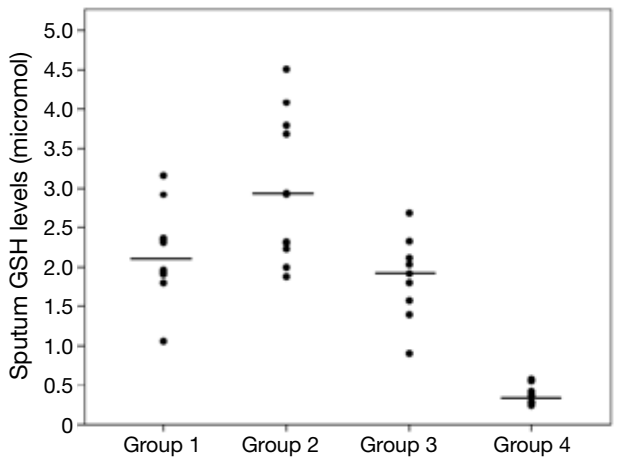

Figure 1 Sputum GSH levels in all groups.

Table 2. The percentage of neutrophils was significantly higher in COPD patients than healthy smokers and non-smokers (P<0.001). The highest percentage of neutrophils was in the acute exacerbations. There were no significant differences in the percentage of lymphocytes, eosinophils, and ciliated cells in the four groups.

Glutathione and NO_2 contents

Sputum glutathione, NO_2, and serum NO_2 levels are shown in *Table 3* and *Figures 1-3*. The highest sputum glutathione, NO_2, and serum NO_2 levels were in the acute exacerbations of COPD patients. Sputum glutathione levels were significantly lower in non-smoker subjects than in stable and acute exacerbations of COPD patients and the healthy smoker group (P<0.001).

The NO_2 contents of sputum samples in both COPD groups (stable and acute exacerbations) were statistically significantly higher than in the smoker and non-smoker groups (P<0.001).

Although the sputum glutathione and NO_2 levels in acute exacerbations of COPD patients were higher than stable COPD patients, statistical analyses were not significant.

Serum NO_2 levels in the acute exacerbations group were significantly higher than the stable COPD and non-smoker groups (P<0.05 and P<0.001 respectively). When compared to the stable COPD and healthy smoker groups, no significant differences were determined.

Given only the patients with COPD, it has been found that there is no correlation between the levels of sputum glutathione, NO_2, and serum NO_2 and sputum neutrophil percentages (r=0.020, r=0.341, and r=0.346, respectively; P>0.05 for all parameters). Similarly, it has been found that there is no relationship between these parameters to FEV_1 values (r=–0.218, r=–0.376, and r=–0.243, respectively; P> 0.05 for all parameters).

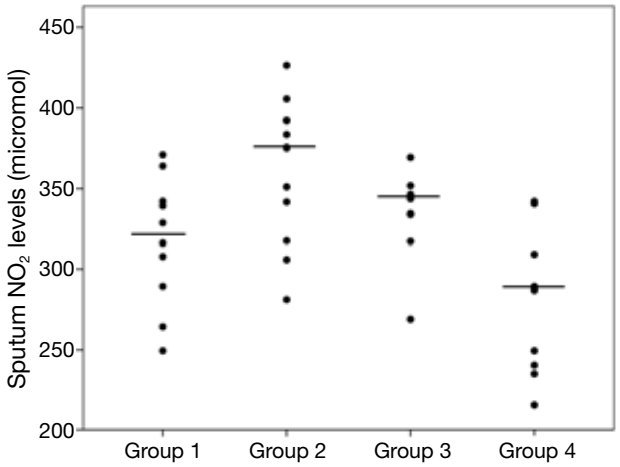

Figure 2 Sputum NO_2 levels in all groups.

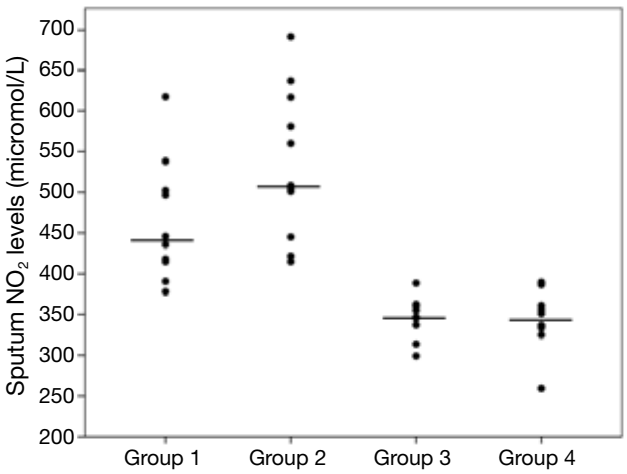

Figure 3 Serum NO_2 levels in all groups.

Discussion

This study was conducted with COPD patients during a stable and acute exacerbation period. Smoker and non-smoker healthy volunteers revealed that oxidative stress was increased in COPD patients. We observed that oxidative stress was also increased in the healthy smoker subjects, although not as significant as in the COPD patients.

COPD is an inflammatory disease, which is characterized by progressive airflow restriction and chronic inflammation affecting the alveoli. Neutrophils are the most significant cells responsible for inflammation. Oxidative stress is the key factor in pathogenesis of the disease. Activated inflammatory cells in the airways, air pollution and smoking are oxidant sources. Previous studies indicated that neutrophil ratio increased in induced sputum in COPD patients and was directly proportional with oxidative stress. Therefore, in the future, neutrophils may be a potential therapeutic target in COPD patients (17,18). Supporting this finding, Serviddio *et al.* (19) found that eosinophils were greater and oxidative stress was lower in COPD patients with reversibility. In our study, we also found that neutrophil ratio increased both in stable COPD patients and in COPD patients during the exacerbation period.

NO is one of the most important oxidative substances in airways. NO levels were detected to be high in the exhalation air of asthma patients. However, a difference could not be found between healthy subjects and particularly stable COPD patients (17,20). Whereas, NO products were showed an increase in induced sputum of COPD patients. Another study revealed that peroxynitrit inhibitor activity decreased in sputum (21). In another study, NO products increased as exhaled NO level decreased, while disease severity increased in stable patients (22). Therefore, measurement of NO products in sputum seems to be a better method than NO measurement in exhalation air to indicate oxidative stress in COPD patients. In our study, we saw that both sputum and serum NO_2 levels were high in COPD patients. Interestingly, we detected that serum NO_2 levels were as high in COPD patients as they were in healthy smoking subjects. This result suggests that smoking leads to systemic oxidative stress, although clinically evident airway restriction has not yet developed. In the study of Rahman *et al.* (23), superoxide anion production was found to increase in neutrophils obtained from the peripheral blood of COPD patients during the acute exacerbation period and decreased to normal levels in the stable period. Similarly, in our study, serum NO_2 levels were very high in the exacerbation period and reduced significantly in the stable period. The difference was statistically significant.

Ziora *et al.* (22) found that there was no association between sputum NO_2 products and FEV_1 values. This study was conducted only in patients in the stable period. Another study showed a negative correlation between exhaled NO levels and FEV_1 levels in stable COPD patients (24). Brindicci *et al.* (25) detected a negative correlation between alveolar NO levels and FEV_1 values and reported that alveolar NO levels could reflect inflammation and remodeling in the periphery

of the lungs. In our study, although sputum and serum NO_2 levels statistically are higher than healthy individuals, statistically no significant relationship between FEV_1 and these parameters was not detected in COPD patients. This result has shown that sputum and serum NO_2 levels alone cannot be used to suggest the degree of airway obstruction. However, it should be noted that our study population is small and our patients had advanced diseases.

Glutathione is one of the most important substances as an antioxidant against oxidative load in the lungs. In fact, glutathione is the main molecule of the intracellular antioxidant system. Extracellular glutathione level is very low. However, it is different in the lungs. A very thin respiratory tract lining fluid (RTLF) was detected in studies. RTLF mainly includes an ample amount of protein and lipid (like surfactant). This fluid is also rich in antioxidants, mainly glutathione. Measurement of glutathione levels in the airways is quite beneficial for investigating oxidative stress in the lungs (26,27).

Antioxidant production increases as a defense mechanism as oxidative stress increases. An increase in BAL glutathione levels were detected in chronic smokers (28). An increase was found in sputum glutathione levels in studies conducted with COPD patients (29). In the study of Drost *et al*. (30), BAL glutathione levels were shown to increase in stable COPD patients and healthy smokers, and it was shown to decrease during the severe and very severe exacerbation periods. Sputum glutathione levels were high in COPD patients in our study, too. However, different from other studies, we detected the highest glutathione levels in cases in the exacerbation period. This finding may be an indication of the increased antioxidant response from increased oxidative stress.

The fact that glutathione loses its stability in a very short amount time is one of the challenges of glutathione level measurement in induced sputum. In a study, sputum glutathione levels were found to be effected from time and were reduced statistically significantly at the 4th hour following sample collection. Therefore, it is recommended to examine sputum glutathione levels as quickly as possible and to freeze the samples until examined (31). Therefore, we initiated examination procedures within 30 minutes of sample collection.

Sputum induction is a method frequently used to show inflammation in the airways. It is preferred in many studies concerning pulmonary diseases, as it is non-invasive. It is reported to be a reliable method (10,12). Our study also revealed that FEV_1 values did not show a statistically significant difference after sputum induction.

In summary, we detected that oxidative stress increased both in the stable and exacerbation periods of COPD patients, and oxidative stress developed in healthy smokers, although clinically evident airway restriction hadn't yet developed. In addition, we may state that sputum induction method is a successful, easy, and reliable method to examine oxidative stress in this patient group.

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References

1. Fischer BM, Pavlisko E, Voynow JA. Pathogenic triad in COPD: oxidative stress, protease-antiprotease imbalance, and inflammation. Int J Chron Obstruct Pulmon Dis 2011;6:413-21.
2. Repine JE, Bast A, Lankhorst I. Oxidative stress in chronic obstructive pulmonary disease. Oxidative Stress Study Group. Am J Respir Crit Care Med 1997;156:341-57.
3. Saetta M, Baraldo S, Corbino L, et al. CD8+ve cells in the lungs of smokers with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 1999;160:711-7.
4. Bresser P, Out TA, van Alphen L, et al. Airway inflammation in nonobstructive and obstructive chronic bronchitis with chronic haemophilus influenzae airway infection. Comparison with noninfected patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2000;162:947-52.
5. MacNee W. Oxidative stress and lung inflammation in airways disease. Eur J Pharmacol 2001;429:195-207.
6. Rahman I, MacNee W. Role of oxidants/antioxidants in smoking-induced lung diseases. Free Radic Biol Med 1996;21:669-81.
7. Mak JC. Pathogenesis of COPD. Part II. Oxidative-antioxidative imbalance. Int J Tuberc Lung Dis 2008;12:368-74.
8. Rahman I, MacNee W. Lung glutathione and oxidative stress: implications in cigarette smoke-induced airway disease. Am J Physiol 1999;277:L1067-88.
9. Cantin AM, Bégin R. Glutathione and inflammatory disorders of the lung. Lung 1991;169:123-38.
10. Dauletbaev N, Rickmann J, Viel K, et al. Glutathione in induced sputum of healthy individuals and patients with asthma. Thorax 2001;56:13-8.
11. Sugiura H, Ichinose M. Nitrative stress in inflammatory

lung diseases. Nitric Oxide 2011;25:138-44.
12. Brightling CE, Monterio W, Green RH, et al. Induced sputum and other outcome measures in chronic obstructive pulmonary disease: safety and repeatability. Respir Med 2001;95:999-1002.
13. Global Initiative for Chronic Obstructive Lung Disease. Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Pulmonary Disease. Updated 2010:1-92.
14. Akerboom TP, Sies H. Assay of glutathione, glutathione disulfide, and glutathione mixed disulfides in biological samples. Methods Enzymol 1981;77:373-82.
15. Buhl R, Vogelmeier C, Critenden M, et al. Augmentation of glutathione in the fluid lining the epithelium of the lower respiratory tract by directly administering glutathione aerosol. Proc Natl Acad Sci U S A 1990;87:4063-7.
16. Verdon CP, Burton BA, Prior RL. Sample pretreatment with nitrate reductase and glucose-6-phosphate dehydrogenase quantitatively reduces nitrate while avoiding interference by NADP+ when the Griess reaction is used to assay for nitrite. Anal Biochem 1995;224:502-8.
17. Zhou M, Liu Y, Duan Y. Breath biomarkers in diagnosis of pulmonary diseases. Clin Chim Acta 2012;413:1770-80.
18. Rytilä P, Plataki M, Bucchieri F, et al. Airway neutrophilia in COPD is not associated with increased neutrophil survival. Eur Respir J 2006;28:1163-9.
19. Serviddio G, Carpagnano GE, Rollo T, et al. Evidence of lower oxidative stress in the air spaces of patients with reversible COPD. Int J Immunopathol Pharmacol 2006;19:617-28.
20. Al-Ali MK, Howarth PH. Exhaled nitric oxide levels in exacerbations of asthma, chronic obstructive pulmonary disease and pneumonia. Saudi Med J 2001;22:249-53.
21. Kanazawa H, Shiraishi S, Hirata K, et al. Imbalance between levels of nitrogen oxides and peroxynitrite inhibitory activity in chronic obstructive pulmonary disease. Thorax 2003;58:106-9.
22. Ziora D, Dworniczak S, Kaczmarczyk G, et al. Correlation of exhaled nitric oxide with nitrogen oxides and selected cytokines in induced sputum of chronic obstructive pulmonary disease patients. J Physiol Pharmacol 2007;58 Suppl 5:791-9.
23. Rahman I, Skwarska E, MacNee W. Attenuation of oxidant/antioxidant imbalance during treatment of exacerbations of chronic obstructive pulmonary disease. Thorax 1997;52:565-8.
24. Ansarin K, Chatkin JM, Ferreira IM, et al. Exhaled nitric oxide in chronic obstructive pulmonary disease: relationship to pulmonary function. Eur Respir J 2001;17:934-8.
25. Brindicci C, Ito K, Resta O, et al. Exhaled nitric oxide from lung periphery is increased in COPD. Eur Respir J 2005;26:52-9.
26. Kelly FJ. Gluthathione: in defence of the lung. Food Chem Toxicol 1999;37:963-6.
27. Cantin AM, North SL, Hubbard RC, et al. Normal alveolar epithelial lining fluid contains high levels of glutathione. J Appl Physiol (1985) 1987;63:152-7.
28. Morrison D, Rahman I, Lannan S, et al. Epithelial permeability, inflammation, and oxidant stress in the air spaces of smokers. Am J Respir Crit Care Med 1999;159:473-9.
29. Beeh KM, Beier J, Koppenhoefer N, et al. Increased glutathione disulfide and nitrosothiols in sputum supernatant of patients with stable COPD. Chest 2004;126:1116-22.
30. Drost EM, Skwarski KM, Sauleda J, et al. Oxidative stress and airway inflammation in severe exacerbations of COPD. Thorax 2005;60:293-300.
31. Beier J, Beeh KM, Kornmann O, et al. Stability of glutathione in induced sputum: impact of freezing. Respiration 2003;70:523-7.

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Original Article

Higher level of heme oxygenase-1 in patients with stroke than TIA

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Background: There is a reverse relationship between serum bilirubin level and incidence of stroke, heme oxygenase-1 (HO-1) can catalyze heme into bilirubin, it is unknown the association of HO-1 level with risk of stroke.

Methods: Sixty patients with stroke and fifty patients with transient ischemic attack (TIA) were recruited. Serum level of HO-1, total and direct bilirubin, alanine transaminase, live function, lipid profile and infection status of patients were measured.

Results: Significant differences were found between two groups in terms of serum levels of HO-1 (163.6±58.7 *vs.* 141.2±49.7, P=0.032), total bilirubin (10.1±4.6 *vs.* 15.8±2.7, P<0.001), direct bilirubin (3.2±2.1 *vs.* 5.9±1.2, P<0.001), fasting glucose (6.7±3.1 *vs.* 4.9±1.3, P<0.001), cholesterol (4.4±1.1 *vs.* 3.9±0.8, P=0.005) and diastolic blood pressure (DBP) (84.9±9.4 *vs.* 81.3±9.2, P=0.046). In multivariate analysis, serum direct bilirubin (OR, 2.83; P<0.001), total bilirubin (OR, 1.82, P=0.001), DBP (OR, 0.88, P=0.041), and fasting glucose (OR, 0.34, P<0.001) were independent predictors of stroke.

Conclusions: Serum HO-1 level is higher in patients with stroke than TIA, but the bilirubin level is lower in patients with stroke than TIA and is an independent predictor of stroke. Further studies are warranted to clarify the underlying link among HO-1, bilirubin and stroke.

Keywords: Heme oxygenase-1 (HO-1); stroke; bilirubin

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Introduction

Heme oxygenases (HO) are microsomal enzymes that include heme oxygenase-1 (HO-1), HO-2 and HO-3. The activity of HO-1, also designated HSP32, is significantly induced by numerous stimuli, including heme, heavy metals, hormones, oxidative stress (1,2) and traumatic brain injury (3).

Tenhunen and coworkers (4) initially reported on the presence of HO in liver microsomes. HO catalyzes the first and rate-limiting step in the oxidative degradation of heme (Fe-protoporphyrin-IX) to carbon monoxide (CO), ferrous iron (Fe^{2+}), and biliverdin-IX (*Figure 1*) (5-7). HO is not a heme protein in itself but uses heme as both its active center and substrate. CO activates cGMP to promote vasodilation, CO exerts a potent anti-inflammatory effect in various disease settings, and biliverdin is subsequently converted by biliberdin reductase to bilirubin (1,5,6), which can serve as an anti-oxidant, both of which may contribute to the reported protective role of HO-1 in cerebral ischemia and subarachnoid hemorrhage (5,8,9). In addition to its role in heme catabolism, HO-1 plays important roles in various pathophysiological states associated with cellular stress. Both HO-1 and bilirubin have the antiatherogenic properties (1,2,10).

Stroke is one of the leading cause of death and a major cause of disability in stroke patients. Transient ischemic attack (TIA) is a brief episode of neurologic dysfunction resulting from focal temporary cerebral ischemia not associated with cerebral infarction (11). It is reported that

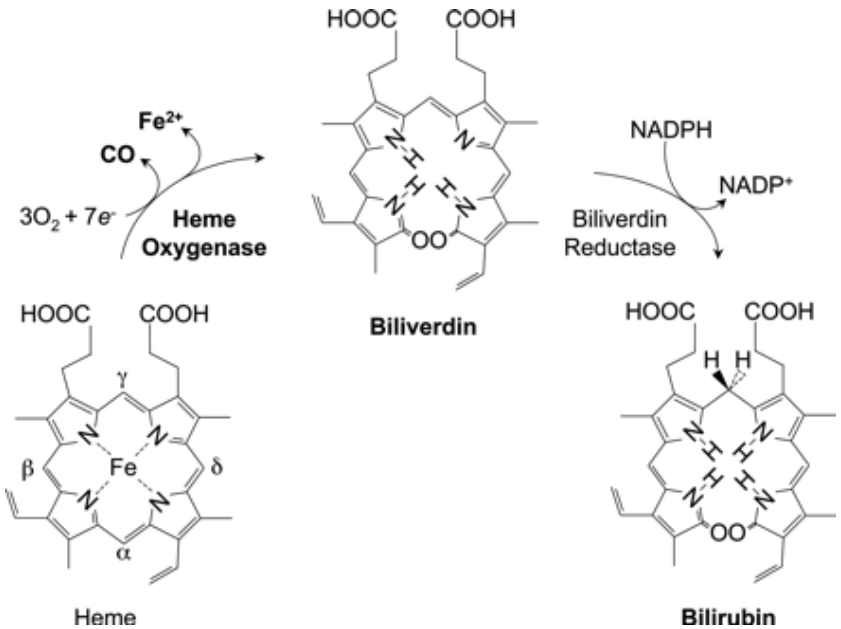

Figure 1 Heme is metabolized by heme oxygenase and biliverdin reductase with the production of CO, iron, biliverdin, and bilirubin. CO, carbon monoxide.

level of bilirubin is reversely associated with the ischemic cerebral infarction (2,12-15). However, little is known about the relationship of serum level of HO-1 with the risk of stroke or TIA. In this study, we compared the serum level of HO-1 in stroke patients with TIA patients in order to find the association of HO-1 with risk of stroke.

Methods and patients

Patients

The investigation was approved by the Clinical Research Ethics Committee of the hospital, and all patients gave written informed consent. Patients with first-ever or recurrent acute ischemic stroke or TIA consecutively admitted to hospital between May 2011 to June 2013 were studied.

Inclusion and exclusion conditions

The study inclusion criteria were well-documented clinical presentation and computed tomography or magnetic resonance imaging of brain, first or recurrent acute ischemic stroke or TIA occurring within seven days prior to admission; the capability and willingness to provide informed consent. The exclusion criteria for the study were hemorrhagic stroke, active liver disease, 2 times higher than normal value of alanine transaminase, bilirubin >34.2 umol/L, or albumin <3.5 mg/dL, hepatobiliary diseases, Gilbert syndrome (12), cancer, severe aphasia or physically unfit for physical examination, stroke or TIA over 7 days before admission, failure to give the informed consent.

Blood pressure was measured in a seated position with a mercury sphygmomanometer. Type 2 diabetes mellitus (DM2) was defined as fasting blood glucose over 7.8 mmol/L or self-reported DM2 or currently on medication for DM (16). Hypertension was defined as a systolic BP of at least 140 mmHg or a diastolic BP of at least 90 mmHg, or a self-reported diagnosis of hypertension and currently on medication to control BP (17). Infection was defined as white blood cell count >10×10^9/L and or neutrophile (%) >70%.

Laboratory test

Venous blood was taken within 24 hours after admission, and plasma was obtained by centrifugation at 1,000 g at 4 ℃ for 20 minutes. All aliquots were stored at –70 ℃ for study. Complete blood counts, fasting glucose, lipid profiles and liver function were measured by automated biochemical profiling. Serum HO-1 was measured with a commercial enzyme linked immunosorbent assay (ELISA) kit (Cusabio, catalog no CSB-E08266h, Barksdale, USA). Briefly, Add

Table 1 Basic characteristics of patients with stroke or TIA

Variable	Stroke	TIA	P value
Age (years)	68.3±11.9	70.9±12.7	0.275
Male, n (%)	41 (68.3)	36 (72.0)	0.676
Hypertension, n (%)	38 (63.3)	35 (70.0)	0.461
DM, n (%)	25 (41.7)	24 (48.0)	0.506
Dyslipidemia, n (%)	17 (28.3)	10 (20.0)	0.312
Current smoker, n (%)	20 (33.3)	16 (32.0)	0.88
Alcohol drinker, n (%)	17 (28.3)	14 (28.0)	0.97
Infection, n (%)	37 (61.7)	31 (62.1)	0.971
HO-1 (μmol/L)	163.6±58.7	141.2±49.7	0.032
Total bilirubin (μmol/L)	10.1±4.6	15.8±2.7	<0.001
direct bilirubin (μmol/L)	3.2±2.1	5.9±1.2	<0.001
ALT (IU/L)	15.6±5.2	16.3±3.6	0.452
Fasting glucose (mmol/L)	6.7±3.1	4.9±1.3	<0.001
Cholesterol (mmol/L)	4.4±1.1	3.9±0.8	0.005
LDL (mmol/L)	2.7±0.8	2.4±0.8	0.052
HDL (mmol/L)	1.2±0.2	1.1±0.2	0.903
SBP (mmHg)	129.9±16.4	128.1±9.9	0.475
DBP (mmHg)	84.9±9.4	81.3±9.2	0.046
WBC ($\times 10^9$)	6.6±2.3	5.6±1.5	0.010
Neutrophile %	61.4±9.9	60.4±9.1	0.587

Data are presented as n (%) or means ± SD; TIA, transient ischemic attack; DM, diabetes mellitus; HO-1, heme oxygenase-1; ALT, alanine transaminase; LDL, low density lipoprotein; HDL, high density lipoprotein; SBP, systolic blood pressure; DBP, diastolic blood pressure; WBC, white blood cell count.

100 μL prepared standards and samples in triplicate to wells of Anti-HO-1 immunoassay plate, incubate for 2 hours at 37 ℃. Remove the liquid of each well, don't wash. Add 100 μL of biotin-antibody HO-1 to each well. Incubate for 1 hour at 37 ℃. Aspirate each well and wash, repeating the process two times for a total of three washes. Wash by filling each well with 200 μL of wash buffer and let it stand for 2 minutes. After the last wash, remove any remaining wash buffer. Add 100 μL of HRP-avidin to each well. Incubate for 1 hour at 37 ℃. Add 90 μL of TMB substrate to each well. Incubate for 15 minutes at 37 ℃ in the dark. Add 50 μL of stop solution to each well, gently tap the plate to ensure thorough mixing. Determine the optical density of each well within 5 minutes with a microplate reader at 450 nm. Plot the HO-1 standard curve and calculate HO-1 sample concentrations.

Statistical analyses

Mean and SD was used to express the normal distributed data. Median (inter-quartile range, IQR) for non-normal distributed data. Categorical data were analyzed by the chi-squared test. Changed in levels of HO-1 and bilirubin levels were evaluated using 2-way repeated measures ANOVA. A multivariate logistic analysis was performed. A probability of less than 0.05 was considered as statistically significant. A software SPSS (13.0) was used for analysis.

Results

Patients' characteristics

A total of 110 consecutive patients were enrolled in this study, 60 of 110 patients had stroke and 50 patients had TIA. *Table 1* shows the baseline characteristics of all patients, all the data including HO-1, bilirubins and blood pressures are normally distributed. *Table 2* demonstrates the results of univariate analysis of risk factors for stroke, serum level of HO-1, total and direct bilirubin, fasting glucose,

Table 2 Univariate analysis of risk factors for stroke

Variable	95% CI	P value
Heme oxygenase-1	(1.96,42.95)	0.032
Total bilirubin	(–7.09,–4.29)	0.001
Direct bilirubin	(–3.33,–2.07)	0.001
Fasting glucose	(0.905,2.66)	<0.001
Cholesterol	(0.154,0.856)	0.005
DBP	(0.072,7.11)	0.046

CI, confidence interval; DBP, diastolic blood pressure.

Table 3 Multivariate regression analysis for stroke

Variable	Odds ratio	95% CI	P value
Direct bilirubin	2.83	1.595-5.017	<0.001
Total bilirubin	1.82	1.289-2.555	0.001
White blood cell count	0.35	0.152-0.811	0.014
Glucose	0.34	0.190-0.612	<0.001
DBP	0.88	0.001-0.995	0.041

CI, confidence interval; DBP, diastolic blood pressure.

cholesterol and diastolic blood pressure (DBP). A significant difference in both groups was found in terms of serum level of HO-1, total bilirubin, direct bilirubin, fasting glucose, and DBP (P<0.05, *Table 2*). In comparison with TIA, serum level of HO-1, fasting glucose, cholesterol, DBP were higher (P<0.001), whereas levels of total bilirubin and direct bilirubin were lower in patients with stroke (P<0.001). No significant difference in both groups was found in terms of age, sex, history of hypertension, diabetes mellitus, dyslipidemia and proportion of alcohol drinker and cigarette smoker, LDL, HDL, and systolic blood pressure (P>0.05).

In order to study the association of serum HO-1, bilirubin levels with stroke, multivariate logistic regression analysis was performed. In regression model, following adjustment for age, gender, history of hypertension, DM, dyslipidemia, HDL, LDL, serum levels of direct bilirubin, total bilirubin, fasting glucose, white blood cell count, and DBP were significant independent predictor of stroke (P<0.05; *Table 3*).

Discussion

The major finding of our study showed that serum level of HO-1 was higher in patients with stroke than TIA, and serum level of both total and direct bilirubin was lower in patients with stroke than TIA. The underlying pathophysiological role has not been understood and requires further investigation. Regarding the level of human serum HO-1 with cardiovascular disorder, Idriss *et al*. (18) reported that HO-1 is raised in stable coronary artery disease and acute coronary artery syndromes. To the best of our knowledge, no data are available for the distribution of human HO-1 in literature, and this is the first paper to report the association of HO-1 level with the risk of stroke.

Tenhunen and coworkers (4) were the first to report on the presence of HO in liver microsomes capable of degrading heme to bilirubin, and this activity was subsequently dissociated from cytochrome P-450 (5-7,9). Three isoenzymes of HO-1 have been found, HO-2 and HO-3 are constitutively expressed isoforms, HO-1 is induced in most cells, oxidative conditions, heme, cytokines and NO donors can induce HO-1 gene expression (5). HO-1 catalyzes the rate-limiting step in the heme catabolism, forming CO, iron, and biliverdin that is subsequently reduced to bilirubin (1,2). In our study, we found that the HO-1 level was higher in stroke group than TIA group, but the total and direct bilirubin level were lower in stroke group than TIA group. High level of HO-1 can be explained that stroke patients have been exposed to a high degree of oxidative stress such as atherosclerosis, infection, hypertension, which leads to an upregulation of HO-1.

Morsi *et al*. (19) reported that HO-1 expression and its activity in human endothelia cells are present only in advanced atherosclerosis and the degree of its expression increases with severity of atherosclerosis. Expressed HO-1 in atherosclerotic lesions can ameliorate oxidative stress and inhibit inflammatory processes in the vessel wall (1). The leukocyte mRNA expression of HO-1 is reported to be associated with the severity of coronary heart disease (20). HO-1 level may reflect the general status of patients' oxidative stress. Serum bilirubin might have some protective function against risk of stroke in men (21).

Numerous studies showed that there is a reverse relationship of bilirubin level with incidence of strokes (2,6-9), which is true in our study, the bilirubin level in stroke patients was lower than TIA patients. However, high serum level of HO-1 in stroke patients in our group were expected to catalyze heme and produce high level of bilirubin theoretically, but surprisingly, the results of serum level of bilirubin was lower, the controversial relationships of HO-1 and bilirubin with the risk of stroke have not been completely understood, and require further study. At least, the activity of HO-1 may not be linearly related

to the formation of bilirubin; another reason may be the upregulated HO-1 enzyme may not be fully activated.

Stroke can be caused by ruptured unstable carotid plaques (11), or thrombosis formation in cerebral arteries. It is reported that approximately 70% of the patients with stroke are cerebral infarction, 15% were hemorrhagic stroke and 15% were TIA (22). Patients with TIA may fully progress into stroke (22). Oxidative stress such as infection induces more circulating neutrophils and macrophages in blood, produces more inducible cytokines and activates HO-1 activity, moreover, immune responses to infection contribute significantly to the formation of atherogenesis. Blood circulation in small atherosclerotic arteries becomes slower especially on condition of low systemic blood pressure and or dehydration, and thereby causes the ischemic stroke.

We have to mention that a limitation was existed in our study. A small number of patients included in current study, many confounder factors of patients, and lack of variables of stroke prognosis may bias the results, and account for the discordance of HO-1 and bilirubin level with risk of stroke. A NIH Stroke Scale should be included in future study.

Conclusions

In summary, our study demonstrates that serum HO-1 level is higher in stroke patients than TIA patients, infection rate is associated with stroke patients. However, bilirubin level is lower in stroke patients. HO-1 may be a general marker to reflect the oxidative stress of patients. Underlying mechanism of HO-1 versus bilirubin with risk of stroke deserves further investigation. A larger sample size of patients from multiple medical centers with long-term follow up will detect significant findings in the risk of stroke.

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References

1. Platt JL, Nath KA. Heme oxygenase: protective gene or Trojan horse. Nat Med 1998;4:1364-5.
2. Novotný L, Vítek L. Inverse relationship between serum bilirubin and atherosclerosis in men: a meta-analysis of published studies. Exp Biol Med (Maywood) 2003;228:568-71.
3. Okubo S, Xi G, Keep RF, et al. Cerebral hemorrhage, brain edema, and heme oxygenase-1 expression after experimental traumatic brain injury. Acta Neurochir Suppl 2013;118:83-7.
4. Tenhunen R, Marver HS, Schmid R. The enzymatic conversion of heme to bilirubin by microsomal heme oxygenase. Proc Natl Acad Sci U S A 1968;61:748-55.
5. Ren S, Liu H, Licad E, et al. Expression of rat liver tryptophan 2,3-dioxygenase in Escherichia coli: structural and functional characterization of the purified enzyme. Arch Biochem Biophys 1996;333:96-102.
6. Ren S, Correia MA. Heme: a regulator of rat hepatic tryptophan 2,3-dioxygenase? Arch Biochem Biophys 2000;377:195-203.
7. Stocker R, Perrella MA. Heme oxygenase-1: a novel drug target for atherosclerotic diseases? Circulation 2006;114:2178-89.
8. Sharp FR, Zhan X, Liu DZ. Heat shock proteins in the brain: role of Hsp70, Hsp 27, and HO-1 (Hsp32) and their therapeutic potential. Transl Stroke Res 2013;4:685-92.
9. Abraham NG, Kappas A. Pharmacological and clinical aspects of heme oxygenase. Pharmacol Rev 2008;60:79-127.
10. Juan SH, Cheng TH, Lin HC, et al. Mechanism of concentration-dependent induction of heme oxygenase-1 by resveratrol in human aortic smooth muscle cells. Biochem Pharmacol 2005;69:41-8.
11. Ren S, Fan X, Peng L, et al. Expression of NF-κB, CD68 and CD105 in carotid atherosclerotic plaque. J Thorac Dis 2013;5:771-6.
12. Perlstein TS, Pande RL, Creager MA, et al. Serum total bilirubin level, prevalent stroke, and stroke outcomes: NHANES 1999-2004. Am J Med 2008;121:781-788.e1.
13. Li RY, Cao ZG, Zhang JR, et al. Decreased serum bilirubin is associated with silent cerebral infarction. Arterioscler Thromb Vasc Biol 2014;34:946-51.
14. Luo Y, Li J, Zhang J, et al. Elevated bilirubin after acute ischemic stroke linked to the stroke severity. Int J Dev Neurosci 2013;31:634-8.
15. Xu T, Zhang J, Xu T, et al. Association of serum bilirubin with stroke severity and clinical outcomes. Can J Neurol Sci 2013;40:80-4.
16. Copeland KC, Silverstein J, Moore KR, et al. Management of newly diagnosed type 2 Diabetes Mellitus (T2DM) in children and adolescents. Pediatrics 2013;131:364-82.
17. James PA, Oparil S, Carter BL, et al. 2014 evidence-based guideline for the management of high blood pressure in adults: report from the panel members appointed to the Eighth Joint National Committee (JNC 8). JAMA

2014;311:507-20.
18. Idriss NK, Lip GY, Balakrishnan B, et al. Plasma haemoxygenase-1 in coronary artery disease. A comparison with angiogenin, matrix metalloproteinase-9, tissue inhibitor of metalloproteinase-1 and vascular endothelial growth factor. Thromb Haemost 2010;104:1029-37.
19. Morsi WG, Shaker OG, Ismail EF, et al. HO-1 and VGEF gene expression in human arteries with advanced atherosclerosis. Clin Biochem 2006;39:1057-62.
20. Vítek L. Does hyperbilirubinemia protect from coronary heart disease? Am J Cardiol 2001;88:1218.
21. Kimm H, Yun JE, Jo J, et al. Low serum bilirubin level as an independent predictor of stroke incidence: a prospective study in Korean men and women. Stroke 2009;40:3422-7.
22. Mohr JP, Albers GW, Amarenco P, et al. American Heart Association Prevention Conference. IV. Prevention and Rehabilitation of Stroke. Etiology of stroke. Stroke 1997;28:1501-6.

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Original Article

EGFR immunoexpression, RAS immunoexpression and their effects on survival in lung adenocarcinoma cases

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Background: The impacts of epidermal growth factor receptor (EGFR) immunoexpression and RAS immunoexpression on the survival and prognosis of lung adenocarcinoma patients are debated in the literature.

Methods: Twenty-six patients, who underwent pulmonary resections between 2002 and 2007 in our clinic, and whose pathologic examinations yielded adenocarcinoma, were included in the study. EGFR and RAS expression levels were examined by immunohistochemical methods. The results were compared with the survival, stage of the disease, nodal involvement, lymphovascular invasion, and pleural invasion. Nonparametric bivariate analyses were used for statistical analyses.

Results: A significant link between EGFR immunoexpression and survival has been identified while RAS immunoexpression and survival have been proven to be irrelevant. Neither EGFR, nor RAS has displayed a significant link with the stage of the disease, nodal involvement, lymphovascular invasion, or pleural invasion.

Conclusions: Positive EGFR immunoexpression affects survival negatively, while RAS immunoexpression has no effect on survival in lung adenocarcinoma patients.

Keywords: Lung adenocarcinoma; epidermal growth factor receptor (EGFR); RAS; survival

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Introduction

Epidermal growth factor receptor (EGFR), also known as *ErbB1* is an intracellular transmembrane glycoprotein, which has intrinsic tyrosine kinase activity (1,2). When the ligand is bound to the cell, autophosphorylation occurs in the intracytoplasmic segment, which activates intracellular tyrosine kinase (1). As a consequence RAS-RAF-MAPK (mitogen activated protein kinase) signal transduction pathway is activated (3). EGFR overexpression causes tumor cell growth, tumor invasion, angiogenesis and eventually metastasis (2). A relation between EGFR overexpression and cellular adhesion, inhibition of apoptosis and resistance to chemotherapy have been identified (4). *EGFR* gene mutations have been confirmed to play role in development of pulmonary adenocarcinoma (5). EGFR expression can be detected in non-small cell lung cancer (NSCLC) cases with immunohistochemical staining (6). EGFR overexpression has been shown to have negative impacts on prognosis in NSCLC patients by a recent meta-analysis (2,7,8).

RAS is the human analog of a gene, which is coded by a retrovirus and causes sarcoma in rats (9). RAS is activated by binding guanosine triphosphate and facilitates intracellular signal transduction (10). *K-RAS* gene codon 12 mutation has been identified in approximately 40% of lung adenocarcinoma cases. RAS mutation plays an important role in cell growth and inhibition of apoptosis (11). RAS can be detected with immunohistochemical techniques in NSCLC cases (12). A meta-analysis has suggested that RAS has no effect on prognosis in NSCLC cases (13).

This study aims at identifying the presence of EGFR

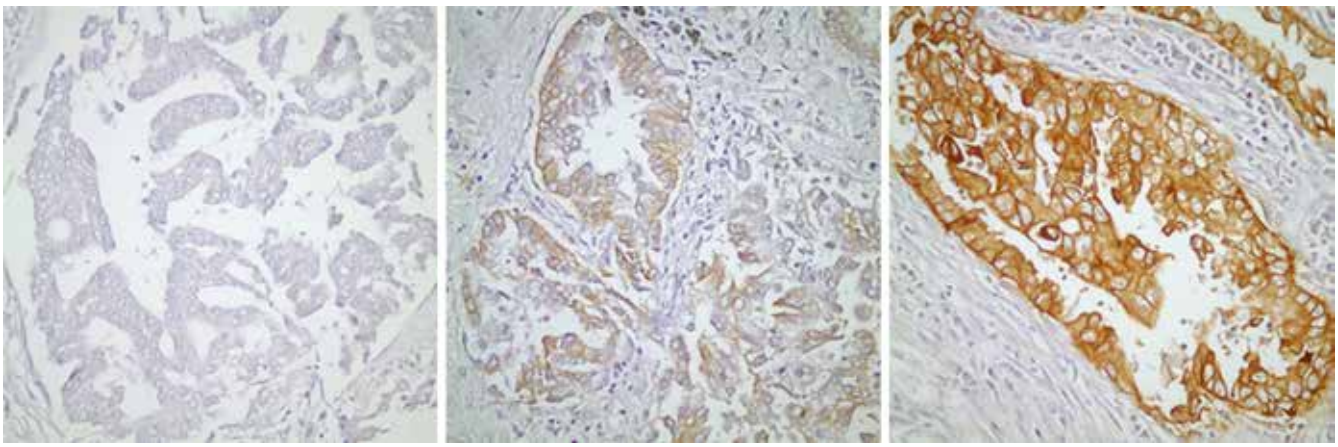

Figure 1 EGFR immunoexpression at tissue level (negative, weak, strong expressions respectively at ×400 magnification). EGFR, epidermal growth factor receptor.

immunoexpression and RAS immunoexpression and their effects on survival in pulmonary adenocarcinoma cases.

Materials and methods

Patients

Twenty-six patients, who underwent complete anatomical resection and mediastinal lymph node dissection due to bronchial adenocarcinoma at Hacettepe University Hospital, Department of Thoracic Surgery between 2002 and 2007, were included in the study. The study was conducted upon approval of local ethics committee of our university. The preoperative diagnostic and metastatic workup was carried out for each patient and the resectable cases were operated on. At least one year follow up survival data was recorded for all patients. Stage of the disesase, lymph node involvement, lymphovascular invasion, and pleural invasion were noted for each patient according to the pathological examination reports after the surgery. EGFR and RAS immunoexpressions were examined using the paraffin blocks of the pathological specimens.

Immunohistochemistry

Twenty of the patients that are included in the study had tissue microarray (TMA) of their pathological specimens. TMA of the 20 patients and the routine paraffin blocks of the remaining six patients were used for the preparation of 5 μm slides. Antigen retrieval procedure and avidin-biotin-peroxidase method were used for all prepared sections. 21E1-1 mouse, monoclonal antibody (ImmunoVision Technologies, USA) and RB-1627P rabbit, polyclonal antibody (Neomarkers, Fremont, CA, USA) were used respectively for EGFR and RAS. Immunohistochemical results were evaluated by using Zeiss Axioskop 2. For EGFR, the intensity of the staining was grouped as: absent (score 0), weak (score 1), medium (score 2) and strong (score 3). The percentage of the staining was grouped as: 0-10% (score 1), 11-50% (score 2) and 51-100% (score 3). The degree of immunoexpression was categorized by the multiplication of intensity score by the percentage score. A multiplication score of 0 was considered to be negative; 1, 2 and 3 were considered to be weak; and 4 and 6 were considered to be strong expressions for EGFR (*Figure 1*). The sections were grouped as weak and strong expressions according to the nuclear staining patterns of RAS (*Figure 2*).

Statistical analysis

Since the number of the patients included in the study was less than 30, nonparametric analyses were used. The relationships of EGFR immunoexpression and RAS immunoexpression with survival, stage of the disease, lymphovascular invasion, and pleural invasion were analyzed using Kendall's tau_b ve Spearman's rho tests in the SPSS 11.5 program.

Results

Twenty-six patients included in the study consisted of 18 (69.2%) male and 8 (30.8%) female participants. The

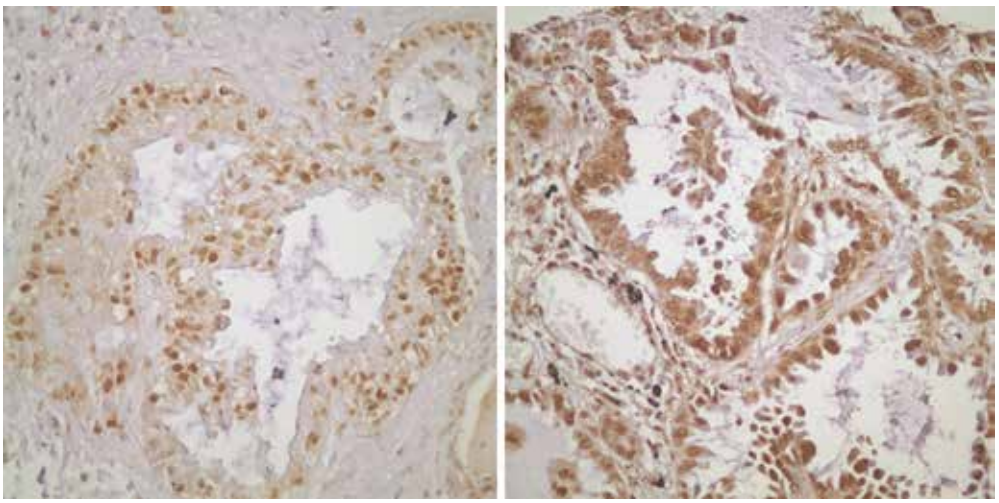

Figure 2 RAS immunoexpression at tissue level (weak and strong expressions respectively at ×400 magnification).

Table 1 EGFR immunoexpression, RAS immunoexpression and the clinicopathological parameters of the patients

		Number of patients				
		EGFR immunoexpression			RAS immunoexpression	
		Absent	Weak	Strong	Weak	Strong
Stage	IA	2	3	1	1	5
	IB	5	4	1	2	8
	IIA	0	0	1	0	1
	IIB	0	1	0	0	1
	IIIA	4	1	2	3	4
	IIIB	1	0	0	0	1
Nodal involvement	N0	7	8	2	3	14
	N1	0	0	1	0	1
	N2	5	1	2	3	5
Lymphovascular involvement	Positive	6	1	1	3	5
	Negative	6	8	4	3	15
Pleural invasion	Positive	7	5	2	4	10
	Negative	5	4	3	2	10

EGFR, epidermal growth factor receptor.

mean age of patients was 56.3±10.4 [35-74]. At the time of the study, 18 (69.2%) patients were alive and 8 (30.8%) had passed away. According to the staging conducted after the pathological examination of the resected specimens; 6 (23.1%) patients were Stage IA, 10 (30.8%) patients were Stage IB, 1 (3.8%) patient was Stage IIA, 1 (3.8%) patient was Stage IIB, 7 (26.9%) patients were Stage IIIA and 1 (3.8%) patient was Stage IIIB. Seventeen (65.4%) patients had N0, 1 (3.8%) patient had N1 and 8 (30.8%) patients had N2 disease after the tissue evaluation of the surgically dissected lymph nodes. Seventeen (65.4%) patients had lymphovascular invasion and 9 (34.6%) patients did not have lymphovascular invasion. Twelve (46.2%) patients had pleural invasion, while 14 (53.8%) patients did not.

EGFR immunoexpression was negative for 12 (46.2%) patients, weak for 9 (34.6%) patients and strong for 5 (19.2%) patients. RAS immunoexpression was weak for 6 (23.1%) patients and strong for 20 (76.9%) patients. EGFR immunoexpression, RAS immunoexpression and the clinicopathological parameters of the patients are shown in *Table 1*.

Table 2 Relationship between EGFR immunoexpression and survival

			EGFR	Survival
Kendall's tau_b	EGFR	Correlation coefficient	1.000	–0.400*
		Sig. (2-tailed)	–	0.035
		N	26	26
	Survival	Correlation coefficient	–0.400*	1.000
		Sig. (2-tailed)	0.035	–
		N	26	26
Spearman's rho	EGFR	Correlation coefficient	1.000	–0.421*
		Sig. (2-tailed)	–	0.032
		N	26	26
	Survival	Correlation coefficient	–0.421*	1.000
		Sig. (2-tailed)	0.032	–
		N	26	26

EGFR, epidermal growth factor receptor; *, correlation is significant at the 0.05 level (2-tailed).

Table 3 Relationship between RAS immunoexpression and survival

			RAS	Survival
Kendall's tau_b	RAS	Correlation coefficient	1.000	–0.167
		Sig. (2-tailed)	–	0.403
		N	26	26
	Survival	Correlation coefficient	–0.167	1.000
		Sig. (2-tailed)	0.403	–
		N	26	26
Spearman's rho	RAS	Correlation coefficient	1.000	–0.167
		Sig. (2-tailed)	–	0.414
		N	26	26
	Survival	Correlation coefficient	–0.167	1.000
		Sig. (2-tailed)	0.414	–
		N	26	26

Correlation is not significant.

Nonparametric bivariate analyses were used for the evaluation of the statistically significant relationship between the immunoexpression status and survival, stage of the disease, nodal involvement, lymphovascular invasion; and pleural invasion. Strong EGFR immunoexpression had a negative relationship with survival, which is statistically significant at α =0.05 level according to Kendall's tau_b and Spearman's rho tests (Kendall's tau_b, r =–0.400; Spearman's rho, r =–0.421; *Table 2*). There was no statistically significant relationship between EGFR immunoexpression and stage of the disease, nodal involvement, lymphovascular invasion, or pleural invasion (Kendall's tau_b, r =–0.075, –0.032, –0.271, –0.037; Spearman's rho, r =–0.088, –0.042, –0.286, –0.039, respectively). RAS immunoexpession had no significant relationship with survival, stage of the disease, nodal involvement, lymphovascular invasion, or pleural invasion. (Kendall's tau_b, r =–0.167, –0.134, –0.194, –0.177, –0.225; Spearman's rho, r =–0.167, –0.147, –0.197, –0.177, –0.225, respectively) (*Table 3*).

Discussion

Lung cancer is one of the leading causes of death in developed countries. The poor prognosis in lung carcinoma

may be due to the patient related factors or the influences associated with the self-nature of the tumor (8). Age, patient status, stage of the disease are among the survival predictors in resectable NSCLC (14). Serum lactate dehydrogenase level, white cell count and neutrophil count are also found to be effective on survival (15). Recently, new techniques to investigate prognosis have been put forward with the advances in molecular biology and cytogenetics (8).

The development and progression of cancer is caused by various factors on the cellular level, such as autonomous growth signals, refraction to growth inhibiting signals, insensitivity to apoptotic signals, unlimited growth potential, angiogenesis, invasion and metastasis (16). Several proto-oncogenes and tumor suppressor genes play a role in these genetic irregularities (17). Some of these genetic irregularities affect the tumor cell behavior much more, and thus can be used as prognostic markers. Molecular prognostic markers reveal themselves as changes in gene copy number, messenger ribonucleic acid (mRNA), and expression levels of proteins (12).

Immunohistochemistry is a quite practical method for detecting the changes in protein expression. It not only shows protein expression in a semi-quantitative manner, but also provides information on the cellular localization of the protein expression. Immunohistochemistry has been used in many different studies as it is involved in literature in this field (12).

EGFR, which has intrinsic tyrosine kinase activity, is a transmembrane protein with an intracellular domain (1). EGFR activates a couple of intracellular signal transduction pathways (7). These pathways cause the cells to transform and grow, inhibit apoptotic signals, and lead to angiogenesis and tumor invasion (18). Kozuki *et al*. have shown mutations of the *EGFR* gene in the development of lung adenocarcinoma (5).

The effect of EGFR expression on survival in NSCLC has been studied by immunohistochemical methods and different results were obtained. In 1997, Rusch *et al.* found EGFR expression to have an effect on survival of NSCLC by using Northern Blot and immunohistochemical methods (19). Volm *et al.* stated that EGFR immunoexpression has a negative effect on survival of squamous cell lung cancer cases (20). In 2000, by conducting an immunohistochemical study, Ohsaki *et al.* have similarly found a negative effect of EGFR expression on survival of NSCLC patients (21). Meert *et al*. conducted a meta-analysis in 2002 by reviewing 16 different studies, which were published between 1989 and 2000. Fourteen of these studies were based on immunohistochemical methods. EGFR expression was found to be positive in 51% NSCLC cases and 46.2% lung adenocarcinoma cases. In a quantitative meta-analysis of eight studies, EGFR expression was shown to be a poor prognostic sign (8). In the current study, the researchers examined the pathological specimens of the patients who were operated for lung adenocarcinoma and grouped them as negative, weak, and strong according to the immunohistochemically detected EGFR expression. Since the total number of the patients was less than 30, the researchers used nonparametric bivariate analyses for the statistical evaluation. Strong EGFR immunoexpression was found to affect survival in a negative fashion. This result is consistent with that obtained by Meert *et al.* in their meta-analysis. In another meta-analysis carried out by Nicholson *et al*. EGFR expression was found to negatively affect the survival in 10-20% of the studies they reviewed (22). In 2005, Niemiec *et al*. showed a statistically significant relationship between EGFR and prognosis (23). The results of the current study are parallel to some of those in the literature. The different results achieved in various studies are dependent on the immunohistochemical methods used. There is no standard evaluation method for EGFR immunoexpression. The scoring is semi-quantitative. The scoring methods depend on the evaluation of staining intensity, percentage of the stained cells, the location of the staining, and the combination of these three parameters (12). The combination process of the staining intensity and percentage of stained cells also varies in different studies. These factors lead to different immunoexpression levels.

Lung adenocarcinoma cases have been shown to bear 40% *K-RAS* gene codon 12 mutation (11). RAS proteins bind GTP. They play a key role in intracellular molecular events. The intrinsic GTPase activity of RAS is lost due to the mutations in tumor cells, and the intracellular signal transduction pathways are continuously stimulated. This gives rise to uncontrolled cell proliferation (13). RAS mutation is important in cell growth and the inhibition of apoptosis (11). The relationship of RAS with prognosis of the disease has been evaluated in various studies using immunohistochemical methods. In NSCLC patients, Harada *et al*. showed RAS to be important for survival, to be an independent prognostic factor, and to be a good marker for understanding the malignant potential of the tumor (24). Miyamato *et al*. used immunohistochemical methods and found RAS expression to be a survival predictor independent of tumor stage (25). In 2005 Mascaux *et al*. published a meta-analysis in which they evaluated the

relationship between RAS expression and survival in lung cancer patients (13). Both immunohistochemistry and other molecular diagnostic methods were used in the studies included in this meta-analysis. In a couple of these studies, there was a statistically significant negative relationship between RAS expression and survival. Yet in a considerable amount of them, there was no relationship. There were also some papers denoting that RAS expression an effect on the metastatic potential of the tumor. The current study did not find a significant relationship between RAS expression and survival. These results are consistent with some of the literature. The studies conducted on a larger number of patients with the use of molecular diagnostic techniques may lead to better results for the evaluation of relationship between RAS and survival.

Conclusions

Positive EGFR immunoexpression affects survival negatively, while RAS immunoexpression has no effect on survival in lung adenocarcinoma patients.

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References

1. Hirsch FR, Scagliotti GV, Langer CJ, et al. Epidermal growth factor family of receptors in preneoplasia and lung cancer: perspectives for targeted therapies. Lung Cancer 2003;41 Suppl 1:S29-42.
2. Nguyen DM, Schrump DS. Growth factor receptors as targets for lung cancer therapy. Semin Thorac Cardiovasc Surg 2004;16:3-12.
3. Alberg AJ, Samet JM. et al. Epidemiology of lung cancer. Chest 2003;123:21S-49S.
4. Jänne PA, Engelman JA, Johnson BE. Epidermal growth factor receptor mutations in non-small-cell lung cancer: implications for treatment and tumor biology. J Clin Oncol 2005;23:3227-34.
5. Kozuki T, Hisamoto A, Tabata M, et al. Mutation of the epidermal growth factor receptor gene in the development of adenocarcinoma of the lung. Lung Cancer 2007;58:30-5.
6. Liu Y, Xu ML, Zhong HH, et al. EGFR mutations are more frequent in well-differentiated than in poor-differentiated lung adenocarcinomas. Pathol Oncol Res 2008;14:373-9.
7. Smith PW, Jones DR. Biology and epidemiology of lung cancer. In: Patterson GA, Cooper JD, Deslauriers J, et al. eds. Pearson's Thoracic and Esophageal Surgery. 3rd Edition, Philadelphia, PA: Churchill Livingstone Elsevier, 2008:708-28.
8. Meert AP, Martin B, Delmotte P, et al. The role of EGF-R expression on patient survival in lung cancer: a systematic review with meta-analysis. Eur Respir J 2002;20:975-81.
9. Johnson BE, Heymach JV. Farnesyl transferase inhibitors for patients with lung cancer. Clin Cancer Res 2004;10:4254s-4257s.
10. Osada H, Takahashi T. Genetic alterations of multiple tumor suppressors and oncogenes in the carcinogenesis and progression of lung cancer. Oncogene 2002;21:7421-34.
11. Jasinski P, Zwolak P, Terai K, et al. Novel Ras pathway inhibitor induces apoptosis and growth inhibition of K-ras-mutated cancer cells in vitro and in vivo. Transl Res 2008;152:203-12.
12. Zhu CQ, Shih W, Ling CH, et al. Immunohistochemical markers of prognosis in non-small cell lung cancer: a review and proposal for a multiphase approach to marker evaluation. J Clin Pathol 2006;59:790-800.
13. Mascaux C, Iannino N, Martin B, et al. The role of RAS oncogene in survival of patients with lung cancer: a systematic review of the literature with meta-analysis. Br J Cancer 2005;92:131-9.
14. Strauss GM. Prognostic markers in resectable non-small cell lung cancer. Hematol Oncol Clin North Am 1997;11:409-34.
15. Kanters SD, Lammers JW, Voest EE. Molecular and biological factors in the prognosis of non-small cell lung cancer. Eur Respir J 1995;8:1389-97.
16. Sieber OM, Heinimann K, Tomlinson IP. Genomic instability--the engine of tumorigenesis? Nat Rev Cancer 2003;3:701-8.
17. Sekido Y, Fong KM, Minna JD. Cancer of the Lung. In: DeVita VT Jr, Hellman S, Rosenberg SA. eds. Cancer, Principles and Practice of Oncology. 7th Edition. Philadelphia, PA: Lippincott Williams & Wilkins, 2005:745-810.
18. Holbro T, Civenni G, Hynes NE. The ErbB receptors and their role in cancer progression. Exp Cell Res 2003;284:99-110.
19. Rusch V, Klimstra D, Venkatraman E, et al. Overexpression of the epidermal growth factor receptor and its ligand transforming growth factor alpha is frequent in resectable non-small cell lung cancer but does not predict tumor progression. Clin Cancer Res 1997;3:515-22.

20. Volm M, Rittgen W, Drings P. Prognostic value of ERBB-1, VEGF, cyclin A, FOS, JUN and MYC in patients with squamous cell lung carcinomas. Br J Cancer 1998;77:663-9.
21. Ohsaki Y, Tanno S, Fujita Y, et al. Epidermal growth factor receptor expression correlates with poor prognosis in non-small cell lung cancer patients with p53 overexpression. Oncol Rep 2000;7:603-7.
22. Nicholson RI, Gee JM, Harper ME. EGFR and cancer prognosis. Eur J Cancer 2001;37 Suppl 4:S9-15.
23. Niemiec J, Kolodziejski L, Dyczek S. EGFR LI and Ki-67 LI are independent prognostic parameters influencing survivals of surgically treated squamous cell lung cancer patients. Neoplasma 2005;52:231-7.
24. Harada M, Dosaka-Akita H, Miyamoto H, et al. Prognostic significance of the expression of ras oncogene product in non-small cell lung cancer. Cancer 1992;69:72-7.
25. Miyamoto H, Harada M, Isobe H, et al. Prognostic value of nuclear DNA content and expression of the ras oncogene product in lung cancer. Cancer Res 1991;51:6346-50.

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Original Article

Evaluating the response of neoadjuvant chemotherapy for treatment of breast cancer: are tumor biomarkers and dynamic contrast enhanced MR images useful predictive tools?

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Objective: In order to evaluate the therapeutic response to neoadjuvant chemotherapy (NAC) for breast cancer, this research focused on the changes in expression of tumor biomarkers and the correlations associated with changes of magnetic resonance imaging (MRI) pre- and post-NAC. We also compared the accuracy of MRI and pathology in terms of residual tumor extent after NAC.

Methods: MRI was performed before and after four courses of cyclophosphamide, epirubicin and paclitaxel (CET) NAC on 114 patients treated in Huashan Hospital (Fudan University) from December 2009 to January 2013. All patients were pathologically diagnosed with invasive breast cancer via core needle biopsy. A series of tumor biomarkers, including P-glycoprotein (P-gp) and Ki-67, was tested by immunohistochemistry in both core needle biopsy and surgical specimens. The changes in tumor biomarker expression and the shrinkage of tumor on MRI were observed. The residual tumor extent after NAC was compared in terms of MRI and histopathology, and the accuracy of MRI was evaluated by both residual tumor extent and by NAC therapeutic effect. Together, these methods enabled a prognostic estimate of NAC.

Results: The P-gp expression before NAC was used to evaluate the therapeutic effect of NAC. The up-regulation of P-gp expression after NAC was associated with poor therapeutic effect (P=0.0011). The expression of Ki-67 was significantly down-regulated (P<0.0001) but it had no association with NAC response (P=0.9645). The mean extent of residual tumor after NAC as seen on MRI was 20.83 mm (±4.14 mm, 95% CI) and that of surgically removed specimens, 18.89 mm (±3.71 mm, 95% CI). The sensitivity of MRI was 95.1%, the specificity was 28.6%, the positive predictive value was 79.6%, and the negative predictive value was 66.7%.

Conclusions: P-gp status was an important factor affecting the pathological complete response (pCR) rate. The change in P-gp expression, from negative to positive following NAC treatment, indicated the emergence of drug resistance resulting from chemotherapy. The down-regulation of Ki-67 was associated with the decline of tumor proliferation. However, compared to the pre-NAC P-gp status, the pre-NAC Ki-67 status had little prognostic value. Additionally, the evaluation of the efficacy of NAC by either MRI or histopathology was inconclusive.

Keywords: Breast cancer; neoadjuvant chemotherapy (NAC); Ki-67; P-glycoprotein (P-gp); pathological complete response (pCR)

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Introduction

In recent years, a number of clinical research results have indicated that neoadjuvant chemotherapy (NAC) has failed to increase overall survival (OS) in patients with early stage breast cancer (1). However, OS of patients with local advanced breast cancer has increased after NAC if they achieved complete pathological response (pCR) (2,3). It would be beneficial to clinical practice if an evaluation system for NAC therapeutic effect could be identified.

Compared with clinical examination, ultrasonography and mammography, dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) is considered the most accurate method for evaluating the extent of residual breast tumor after NAC (4,5). Moreover, the tumor biomarkers Ki-67 (a tumor proliferation marker) and P-glycoprotein (P-gp) are also widely recognized as important histopathologic NAC evaluation tools (6,7).

In order to improve prognostic predictions of patients with breast cancer, we evaluated MRI and tumor biomarkers as indicators of therapeutic response to NAC. We also investigated the correlation of the two methods.

Materials and methods

Patients

Between December 2009 and January 2013, 114 breast cancer patients treated at Huashan Hospital (Fudan University) were enrolled in this study. We used the AJCC (American Joint Committee on Cancer) Breast Cancer Staging System (7th edition, 2009) (8), to classify tumors by TNM staging. All patients were diagnosed with breast cancer based on the tumor tissues obtained by core needle biopsy before NAC. Patient demographics, clinical and imagining examinations, and tumor characteristics were collected.

The inclusion criteria of this study were as follows:

(I) Operable female patients;
(II) AJCC stage II (exclude T0N1M0 and T1N1M0) and AJCC stage III;
(III) No adjuvant treatments (chemotherapy, endocrine therapy, radiology therapy, targeting therapy) before NAC.

The exclusion criteria of this study were as follows:

(I) Pregnancy;
(II) Patients who had a previous history of breast cancer;
(III) AJCC stage IV patients.

Chemotherapy

All patients receiving NAC were given four courses of CET regimen chemotherapy at three week intervals [cyclophosphamide (500 mg/m^2), epirubicin (75 mg/m^2) and paclitaxel (175 mg/m^2)]. All were given by intravenous bolus injection (9).

Pathological examination

The breast cancer tissue was collected by core needle biopsy (Mammotome System, 8 Gauge, Ethicon Inc., USA) before NAC treatment. After four courses of NAC, the surgically removed breast tissue was fixed in neutral buffered formalin and then embedded in paraffin. The specimens were cut into thin sections and stained with hematoxylin and eosin. Determination of P-gp and Ki-67 expression was made immunohistochemically. Standard immunohistochemistry (IHC) techniques were used. P-gp grading was as follows: 0, negative for cell membrane or fewer than 10% of cancer cells positive; 1, slightly positive in more than 10% of cancer cells; 2, moderately positive in more than 10% of cancer cells; 3, markedly positive in more than 10% of cancer cells. Ki-67 positive staining was defined as Ki-67 staining of 14% or more of cancer cell nuclei; Ki-67 negative staining was defined as Ki-67 staining of fewer than 14% of cancer cell nuclei (10).

Histopathologic criteria for assessment of NAC therapeutic response

The histopathologic therapeutic effects were determined based on the histopathologic criteria for assessment of therapeutic response in breast cancer described in the 2007 version of the Japanese Breast Cancer Society (JBCS) (11). In this study, we grouped the histopathologic therapeutic effects into three categories: (I) no pathological response (pNR); (II) partial pathological response (pPR, grade 1 and grade 2); and (III) pCR (grade 3).

MRI acquisition and assessment

To be eligible for the study, all patients were required to receive MRI scans before and after NAC treatment. Two experienced radiologists calculated the MR tumor size measurements using Functool SER software with a AW43 imaging processing workstation (GE Inc., USA). The radiologists were blinded to patient information, including

Table 1 Patient cohort

	Pre-NAC patients			Censored	Post-NAC patients
	Pre-NAC	pCR	mCR		
Total patients	114	28		0	114
P-gp	89	20		0	69
Ki-67	88	19		3	66
MRI	55		6	0	49

NAC, neoadjuvant chemotherapy; pCR, pathological complete response; P-gp, P-glycoprotein; mCR, complete response as determined by MRI; MRI, magnetic resonance imaging.

breast cancer subtype, NAC regimen and final pathological outcome.

In accordance with the response evaluation criteria in solid tumors (RECIST) (12), MRI tumor size was determined by the longest tumor diameter. For multiple tumors, the maximal tumor diameters of the five largest lesions were measured. NAC treatment responses were based on DCE-MRI examinations. Responses were classified as follows: MRI complete response (mCR: no residual cancer); MRI partial response (mPR: ≥30% size reduction); MRI no response (mNR: <30% size reduction, including stable disease (SD); and progressive disease (PD).

Time-signal intensity curves (TIC, a measure of peak intensity from the time of contrast material injection) in DCE-MRI were designed to differentiate benign from malignant enhancing lesions based on region of interest (ROI) analysis. TICs were classified according to the shapes as type I, steady enhancement with a straight or curved time-signal intensity line; type II, plateau of signal intensity; or type III, washout of signal intensity.

Statistical analysis

Correlations between Ki-67, P-gp and pCR were derived using chi-square tests. The Kruskal-Wallis test was used to analyze the correlation between the histopathologic therapeutic responses (pCR, pPR and pNR), and Ki-67/P-gp expression. The NAC therapeutic effect was evaluated using Spearman's rank correlation coefficient. The consistency between MRI responses and histopathologic therapeutic responses was tested using the Kappa test. Data was analyzed by SAS (Version 8.01; Statistical Analysis System Inc., North Carolina, USA). A Pvalue of less than 0.05 was considered statistically significant.

Results

Patient cohort

A total of 114 NAC-treated patients were included in the study. Twenty-eight patients (24.6%) reached a pCR after NAC treatment. At the start of the study, Ki-67 and P-gp were not used as routine test biomarkers; thus, 88 and 89 patients were analyzed for Ki-67 and P-gp expression, respectively, before NAC treatment. There were 19 pCR patients who tested positively for Ki-67 and 20 pCR patients who tested positively for P-gp. Three non-pCR patients were excluded due to the failure of the Ki-67 test. Thus, Ki-67 and P-gp tests were run on 66 and 69 non-pCR patients, respectively, following NAC treatment. Fifty five patients underwent complete MRI therapy before NAC treatment. Among them, six patients achieved a mCR. Thus, the MRI therapeutic response was evaluated in 49 patients after NAC treatment (*Table 1*).

Patient characteristics

The clinical-pathologic characteristics of the 114 patients are shown in *Table 2*. The patients' ages ranged from 29 to 74 years (mean 51.9 years). In terms of menopausal status, 68 patients (59.7%) were postmenopausal, and 46 patients (40.4%) were premenopausal. With regard to the pathologic category, invasive ductal carcinoma (76.3%) was the most commonly observed. With a cut-off for Ki-67 positivity of ≥14% positively stained tumor cells, most of the tumors (65.9%) were classified as having a high level of Ki-67. Fifty-two patients (58.4%) were P-gp positive before NAC. Twenty-eight patients (24.5%) achieved a pCR; 66 patients (57.9%) achieved a pPR, and 20 patients (17.5%) achieved a pPR.

Table 2 Patients' characteristics and clinical characteristics

Factor	pCR (%)	pPR (%)	pNR (%)	Sum (%)	P value
	28 (24.56)	66 (57.89)	20 (17.54)	114	
Age					0.87
<40 y	2 (25.00)	6 (75.00)	0 (0.00)	8 (7.02)	
40-49 y	7 (21.21)	20 (60.61)	6 (18.18)	33 (28.95)	
50-59 y	15 (26.79)	30 (53.57)	11 (19.64)	56 (49.12)	
60-69 y	4 (23.53)	10 (58.82)	3 (17.65)	17 (14.91)	
Menopausal status					0.17
Postmenopausal	15 (22.06)	38 (55.88)	15 (22.06)	68 (59.65)	
Premenopausal	13 (28.26)	28 (60.87)	5 (10.87)	46 (40.35)	
Pathological category					0.0035
Invasive ductal carcinoma	17 (19.54)	54 (62.07)	16 (18.39)	87 (76.32)	
Invasive lobular carcinoma	1 (16.67)	3 (50.00)	2 (33.33)	6 (5.26)	
Medullary carcinoma	2 (50.00)	2 (50.00)	0 (0.00)	4 (3.51)	
Mucinous adenocarcinoma	0 (0.00)	0 (0.00)	2 (100.00)	2 (1.75)	
DCIS with invasive tumor	4 (40.00)	6 (60.00)	0 (0.00)	10 (8.77)	
Others	4 (80.00)	1 (20.00)	0 (0.00)	5 (4.39)	
Tumor biomarker (before NAC)					
Ki67 ≥14%	10 (17.24)	37 (63.79)	11 (18.97)	58 (65.91)	0.196
Ki67 <14%	9 (30.00)	16 (53.33)	5 (16.67)	30 (34.09)	
P-gp +	4 (7.69)	34 (65.38)	14 (26.92)	52 (58.43)	0.024
P-gp –	16 (43.24)	19 (51.35)	2 (5.41)	37 (41.57)	
NAC response					
pCR				28 (24.5)	
pPR				66 (57.89)	
pNR				20 (17.54)	

DCIS, ductal carcinoma in situ; pCR, pathological complete response; pPR, partial pathological response; pNR, no pathological response; NAC, neoadjuvant chemotherapy; P-gp, P-glycoprotein.

Correlations between patient characteristics and NAC histopathologic therapeutic effects

Table 2 shows the results of the univariate analyses for the factors associated with NAC histopathologic therapeutic effects. The association between NAC response versus age (P=0.87) and menopausal status (P=0.17) were not statistically significant. Compared with postmenopausal patients, premenopausal patients achieved slightly higher pCR and pPR rates (pCR: 28.3% *vs.* 22.1%; pPR: 60.9% *vs.* 55.9%), but a lower pNR rate (10.9% *vs.* 22.1%).

The predominant tumor type was invasive ductal carcinoma (76.3%). Patients having medullary carcinoma, or ductal carcinoma in situ (DCIS) with invasive tumors, achieved promising response rates (50% pCR, 50% pPR, and 40% pCR, 60% pPR, respectively). Two mucinous adenocarcinoma patients did not respond. These data indicated that pathologic type of tumor had a significant correlation with the NAC response (P=0.0035).

The pre-NAC Ki-67 expression was not a good predictor of NAC treatment response (P=0.196). P-gp negative patients achieved higher pCR rates than P-gp positive patients (43.2% *vs.* 7.7%, P=0.024).

Change in P-gp and Ki-67 status pre- and post-NAC

Figure 1A indicates that P-gp was up-regulated in 32 patients and down-regulated in 10 patients after NAC treatment. This change was statistically significant (P=0.001). Among the 16 pNR patients, P-gp was up-

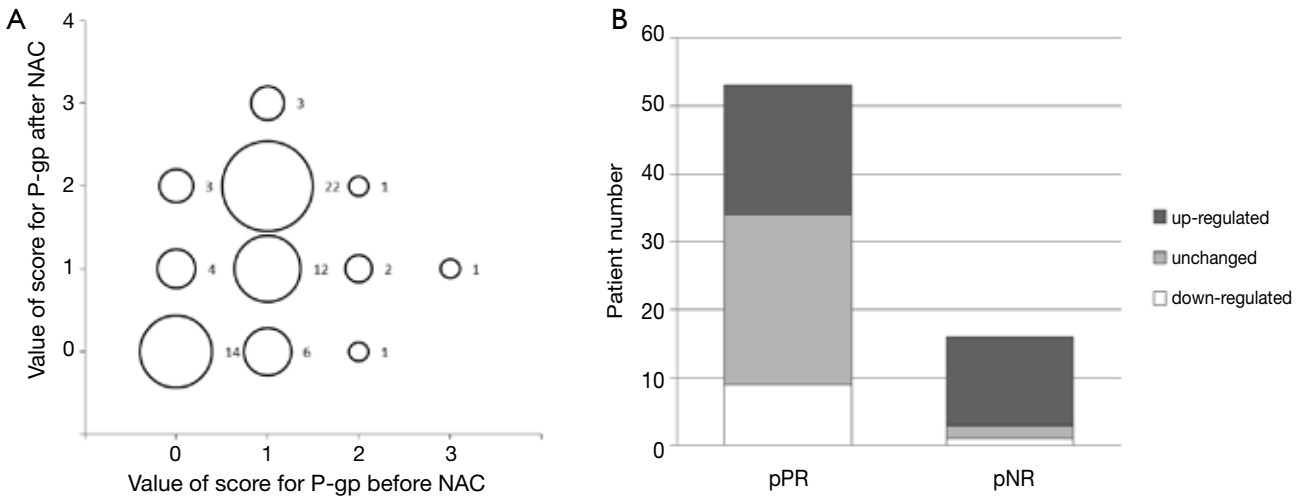

Figure 1 P-gp change pre- and post-NAC and its predictive effect. Bubble plot for IHC score for P-gp before and after NAC in 69 patients with P-gp expression change. The figure added to the bubbles is the number of patients and each bubble's size is determined by the number of patients in the category: (A) the more the patients, the larger the bubble; (B) bar figure displayed the NAC therapeutic effect with P-gp change; white, P-gp down-regulated; light grey, P-gp unchanged; dark grey, P-gp up-regulated. NAC, neoadjuvant chemotherapy; IHC, immunohistochemistry; P-gp, P-glycoprotein; pPR, partial pathological response; pNR, no pathological response.

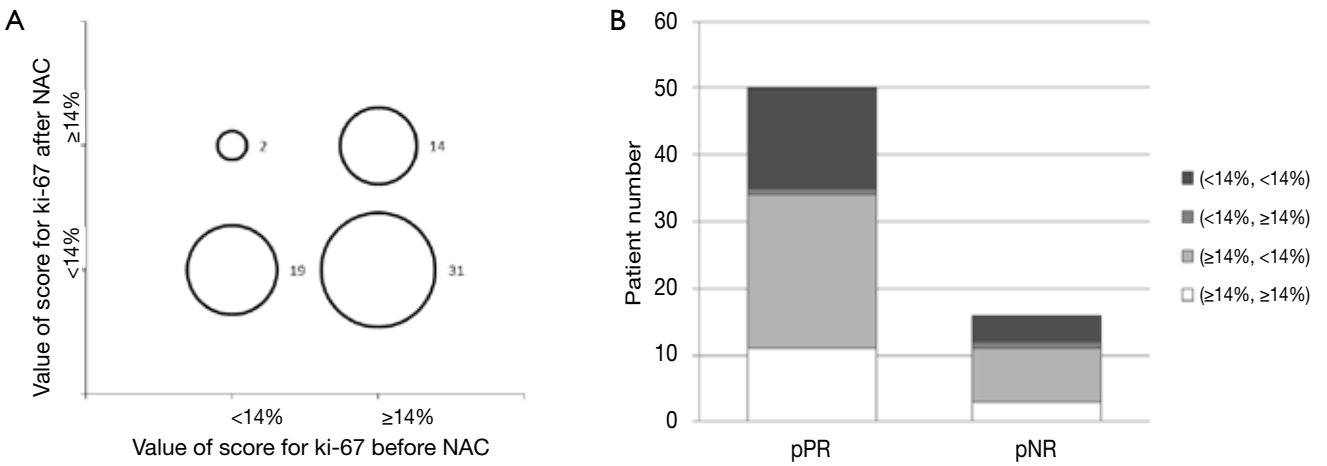

Figure 2 Ki-67 change pre- and post-NAC and its predictive effect. Bubble plot for IHC score for Ki-67 before and after NAC in 66 patients with Ki-67 expression change. The figures added to the bubbles are the number of patients and each bubble's size is determined by the number of patients in the category: (A) the more the patients, the larger the bubble; (B) bar figure displayed the NAC therapeutic effect with Ki-67 change; white, Ki-67 ≥14% both pre-NAC and post-NAC; light grey, Ki-67 ≥14% pre-NAC while <14% post-NAC; medium grey, Ki-67 <14% pre-NAC while ≥14% post-NAC; dark grey, Ki-67<14% both pre-NAC and post-NAC. NAC, neoadjuvant chemotherapy; IHC, immunohistochemistry; pPR, partial pathological response; pNR, no pathological response.

regulated in 13 patients, down-regulated in 1 patient and remained unchanged in 2 patients. Of the 53 pPR patients, P-gp was upregulated in 19, down-regulated in 9 and unchanged in 25. The up-regulation of P-gp was associated with a negative NAC therapeutic effect (P=0.024) (*Figure 1B*).

Figure 2A indicates that Ki-67 was down-regulated in 31 patients, and up-regulated in 2 patients after NAC treatment. The down-regulation of Ki-67 after NAC treatment was statistically significant (P<0.001), but it had no correlation with NAC therapeutic effect (P=0.9645) (*Figure 2B*).

Table 3 Pre-NAC TIC type and NAC therapeutic effect

	MRI therapeutic effect					Histopathologic therapeutic effect				
	mCR	mPR	mNR	Sum	P value	PCR	pPR	pNR	Sum	P value
I	2	7	3	12		4	6	2	12	
II	2	13	6	21		2	16	3	21	
III	2	17	3	22		8	11	3	22	
Total	6	37	12	55	0.637	14	33	8	55	0.503

NAC, neoadjuvant chemotherapy; TIC, Time-signal intensity curves; MRI, magnetic resonance imaging; mCR, complete response as determined by MRI; mPR, MRI partial response; mNR, MRI no response; pCR, pathological complete response; pPR, partial pathological response; pNR, no pathological response.

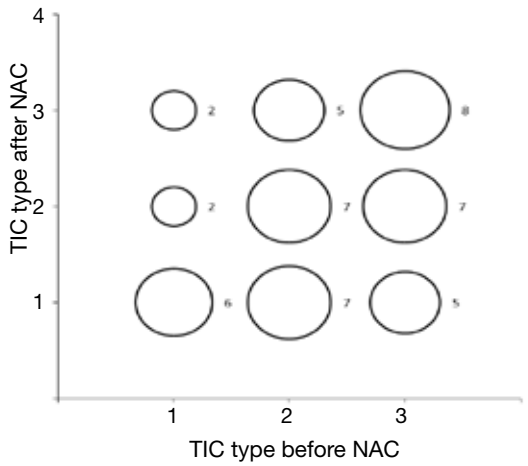

Figure 3 Bubble plot for MRI TIC type conversion before and after NAC in 49 patients with TIC change. The figures added to the bubbles are the number of patients and each bubble's size is determined by the number of patients in the category: the more the patients, the larger the bubble. NAC, neoadjuvant chemotherapy; TIC, Time-signal intensity curves; MRI, magnetic resonance imaging.

Table 4 MRI therapeutic effect compared with histopathologic therapeutic effect

	pCR	pPR	pNR	Sum	P value
mCR	4	1	1	6	
mPR	9	23	5	37	
mNR	1	9	2	12	
Total	14	33	8	55	0.056

pCR, pathological complete response; pPR, partial pathological response; pNR, no pathological response; mCR, complete response as determined by MRI; mPR, MRI partial response; mNR, MRI no response; MRI, magnetic resonance imaging.

Correlations between MRI TIC type and NAC MRI/ histopathologic therapeutic effect

Table 3 indicates that the pre-NAC TIC categories were not predictive for NAC therapeutic effects, by either MRI analysis (P=0.637) or by histopathologic analysis (P=0.503). *Figure 3* shows the change of TIC categories pre-and-post NAC treatment: 21 patients (42.9%) remained unchanged; 19 patients (38.8%) moved into a higher TIC category; and 9 (18.4%) patients moved into a lower TIC category. However, the change of TIC category pre-and-post NAC treatment was not statistically significant (P=0.0872). It had no significant correlation with therapeutic effect, by either MRI analysis (P=0.948) or by histopathologic analysis (P=0.792).

The equivalence of therapeutic effects as analyzed by MRI or histopathology

Table 4 indicates overlap of 4 mCR with 14 pCR patients (28.6%), and 2 mNR with 8 pNR patients (25.0%).Thus, MRI and the pathology response grades did not match well. In patients achieving a partial response, there was a better overlap of 23 mPR with 33 pNR patients (69.7%). However, although the data approached significance, it failed to reach statistical significance (P=0.056).

In *Figure 4*, we classified NAC treatment responses as pCR and non-pCR. Within the pCR group, we found 4 mCR in 14 pCR patients. This specificity was 28.6%. Within the group of 41 non-pCR patients, there were only 2 mCR patients. This sensitivity was 95.1%. Overall, the positive predictive value was 79.6% and the negative predictive value was 66.7%. However, the correlation between mCR and pCR was not statistically significant (P=0.29).

Non-pCR		pCR	
True positive	39	False negative	2
False positive	10	True negative	4
Sensitivity		95.12%	
Specificity		28.57%	
Positive predictive value		79.59%	
Negative predictive value		66.67%	
P value		P=0.29	

Figure 4 The consistency between pCR and mCR. pCR, pathological complete response; mCR, complete response as determined by MRI; MRI, magnetic resonance imaging.

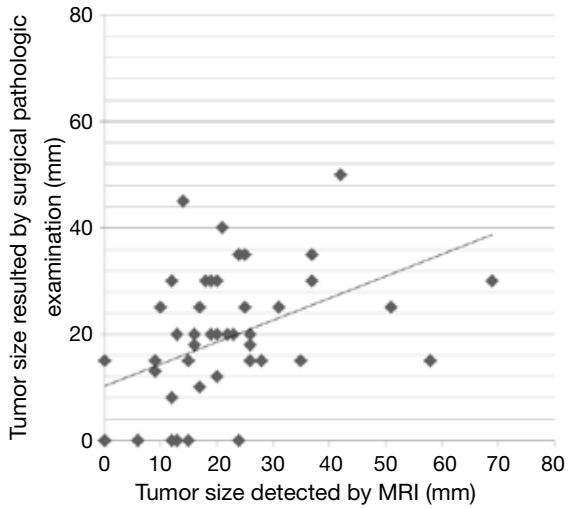

Figure 5 Correlation of pathology as measured by longest diameter of residual tumor (mm) (y-axis) and preoperative MRI as measured by MRI longest diameter (mm) (x-axis). MRI, magnetic resonance imaging.

The average extent of the longest diameter in residual tumor by MRI analysis was 20.83±4.14 mm, and that of the surgically removed specimen, 18.89±3.71 mm. In 18 patients, the residual tumor extent was smaller by MRI analysis than the actual surgically excised specimen (max: –31.0 mm; mean: -10.67 mm). However, in 22 patients, the residual tumor extent by MRI analysis was larger than the actual surgically excised specimen (max: +43.0 mm; mean: +12.77 mm) (*Figure 5*).

Discussion

Earlier studies of the prognostic assessment of breast cancer following NAC have focused on one of two aspects: (I) the biological and pathological analysis of surgical specimens pre/post treatment of NAC (13) or; (II) magnetic resonance imaging (MRI) to evaluate residual tumor extent after NAC (14). Traditional prognostic tumor biomarkers for breast cancer have been well studied. Imaging assessment of NAC treatment response, such as MRI, can provide valuable information about the residual tumor extent and enable better surgical plans to achieve a tumor-free margin. Complete response determined by MRI overlaps with complete response determined by pathological methods, for evaluating NAC response and predicting prognosis. In this current study, we combined the predictive use of pathological biomarkers with imaging findings, to evaluate their consistency and to identify discrepancies. The factors that influence accuracy were elucidated, to better guide clinical practice in the treatment of breast cancer.

Our study investigated DCE-MRI and the expression of tumor biomarkers, in an effort to explore their combined assessment value in NAC-treated breast cancer patients. IHC results indicated that lower pre-NAC P-gp expression was associated with better pCR rates. This suggests that P-gp is a good predictive tool for the efficacy of NAC. In addition, P-gp was significantly up-regulated after NAC treatment, the tendency of which was even more obvious in pNR patients. In other studies, it has been shown that P-gp expression is induced by NAC and its expression confers some degree of drug resistance (15). In this current study, Ki-67 expression was lower after NAC treatment, suggesting a decrease of tumor proliferation; however, its prognostic value for pCR is limited. The accuracy of MRI for evaluating NAC outcome and its consistency with pathological findings are inconclusive. Here, complete response as determined by MRI (mCR) was compared to complete response as determined by IHC (pCR). MRI analysis for pCR prediction was observed with high sensitivity, but with low specificity.

In recent years, proliferative tumor markers, including Ki-67, have been widely investigated as predictive and prognostic factors following NAC treatment. It has been shown that, in the neoadjuvant setting, a high level of Ki-67 expression predicts response to chemotherapy regimens (16), which may explain the high sensitivity of proliferating tumor cells to NAC compounds. Many studies have studied the correlation of Ki-67 to overall response rates (17-19), but with varying and conflicting results. de Azambuja *et al.* (20) analyzed the data from 29 studies, using patients with node-negative and node-positive breast cancer, and

demonstrated that Ki−67 expression is associated with poor OS and disease−free survival. While other studies found that patients with high Ki-67 expression were more likely to achieve pCR (21), or Ki-67 shows no significant trend in relation to OS (22), Klintman *et al*. (23) have concluded that the value of Ki-67, as a prognostic tool, is restricted to ER-positive patients and to those with histological grade 2 tumors. There are some plausible reasons for these discordant results, including varying chemotherapy protocols, heterogeneous patient subtypes, and different Ki-67 standards and scoring systems.

Our study investigated the value of Ki-67 as a predictive factor and found that patients with low Ki-67 status pre-NAC were somewhat more likely to achieve pCR, although these results were not statistically significant. Interestingly, in this current study, most patients demonstrated a decrease of Ki-67 from high pre-treatment levels to low post-treatment levels. Our findings are consistent with previous studies that show Ki-67 low level expression (<3%) in healthy breast tissue (23), and a progressive increase of Ki-67 expression from benign breast disease, to DCIS, to invasive breast cancer (24). Our finding of Ki-67 reduction change suggests that NAC treatment induces a gradual downgrading from invasive carcinoma to DCIS, and may even promote eventual tumor disappearance.

The drug resistance of breast cancer is widely observed during chemotherapy, and various mechanisms have been characterized. Several xenobiotic transporter-related genes have been most frequently implicated as modifiers of NAC in breast cancer (25), and P-gp (encoded by MDR-1) is one of the key factors. P-gp is one member of the transmembrane efflux pump group, which is normally involved in the excretion of toxins from cells. P-gp has a broad range of substrates and exports a number of chemotherapeutics, inducing drug resistance (26). Some studies have investigated the relationship between P-gp expression levels and its prognostic value in breast cancer. Although a number of different conclusions have been drawn, the broad consensus is that P-gp expression can be induced by NAC (27,28). In our study, we found that 46.4% of patients showed P-gp up-regulation following chemotherapy for disease progression, a finding consistent with other studies (28,29). The reasons for this P-gp up-regulation include at least two possibilities. The first is our chemotherapy regimen of anthracycline and taxanes, which are both known substrates for P-gp (26). Here, P-gp negative cells are more sensitive and are killed by NAC treatment, while the primary high P-gp expressing tumor cells survive. The second possibility is acquisition of drug resistance after exposure to NAC treatment. Seven P-gp negative patients became P-gp positive, an indication of drug resistance. Regardless of whether P-gp expression is primary or acquired, high P-gp expression in either the pre- or post-treatment setting predicts poor patient outcome.

Some studies have reported that the decrease of tumor volume and enhancement on DCE-MRI is correlated with histopathologic response (30). Results from the I-SPY 1 trial (31) revealed that MRI findings of tumor reduction are better indicators of pathology than clinical examinations. In contrast, one study by Ko *et al*. (14) reported that ER positive breast cancer of a low nuclear grade might be associated with a poor response following NAC, and MRI might be less accurate in predicting the residual lesion extent of this kind of tumor.

In our study, the residual tumor extent on MRI and the surgically removed specimen size were analyzed. Based on the longest diameter of residue tumor, Pearson's correlation coefficient between the two methods was 0.513 (P=0.0003), indicating a moderate correlation between the two measurements. However, the measurements by MRI included ten false-positive diagnoses. There are several possible reasons for this overestimation. (I) The residual tumor extent on MRI is *in vivo*. The tumor may shrink when fixed in formalin after surgical removal; (II) DCIS is often manifested as enhanced lesions on MRI images, which are similar to invasive tumors and difficult to differentiate from invasive tumors. Small residual lesions after NAC may often be misinterpreted as pCR, and residual DCIS tissue may lead to a false-positive diagnosis. Two of the 10 false positive lesions in our study were revealed to be DCIS, and were included in the pCR category. Although the impact of a DCIS diagnosis might be negligible in terms of prognosis, it may be an important risk factor for local recurrence after breast conserving surgery (32). Therefore, with DCIS tumors, overestimation on MRI is inevitable; (III) In another study, Ko *et al*. reported that a taxane-induced chemotherapy regimen may increase the vascular permeability, and capillary protein leakage could lead to augmented gadolinium uptake by the tumor, producing an over-or underestimation (14).

In conclusion, by studying the change of P-gp and Ki-67 expression from pre- to post-NAC, our investigation demonstrated that NAC treatment may arrest tumor proliferation, but may also potentially increase drug

resistance. The pre-NAC P-gp status provided independent prognostic information for NAC treatment, while the pre-NAC Ki-67 status had little prognostic value. With rapidly evolving MRI technology, imaging assessment has been more widely applied to the evaluation of therapeutic effectiveness; it provides valuable information about the residual tumor. However, since the accuracy of MRI might be affected by many factors—the NAC-induced reactive change in tumor, the molecular character of the tumor, and the influence of chemotherapeutic agents—the discrepancies between imaging results and pathologic findings remain to be settled. In this retrospective study, we did not compare our chemotherapy regimen with more recently developed NAC chemotherapeutics. Therefore, our findings may not be applicable to newer therapeutics and will require further investigation.

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References

1. Alvarado-Cabrero I, Alderete-Vazquez G, Quintal-Ramirez M, et al. Incidence of pathologic complete response in women treated with preoperative chemotherapy for locally advanced breast cancer: correlation of histology, hormone receptor status, Her2/Neu, and gross pathologic findings. Ann Diagn Pathol 2009;13:151-7.
2. Gonzalez-Angulo AM, McGuire SE, Buchholz TA, et al. Factors predictive of distant metastases in patients with breast cancer who have a pathologic complete response after neoadjuvant chemotherapy. J Clin Oncol 2005;23:7098-104.
3. Charfare H, Limongelli S, Purushotham AD. Neoadjuvant chemotherapy in breast cancer. Br J Surg 2005;92:14-23.
4. Londero V, Bazzocchi M, Del Frate C, et al. Locally advanced breast cancer: comparison of mammography, sonography and MR imaging in evaluation of residual disease in women receiving neoadjuvant chemotherapy. Eur Radiol 2004;14:1371-9.
5. Akazawa K, Tamaki Y, Taguchi T, et al. Preoperative evaluation of residual tumor extent by three-dimensional magnetic resonance imaging in breast cancer patients treated with neoadjuvant chemotherapy. Breast J 2006;12:130-7.
6. Tsukamoto F, Shiba E, Taguchi T, et al. Immunohistochemical Detection of P-glycoprotein in Breast Cancer and Its Significance as a Prognostic Factor. Breast Cancer 1997;4:259-63.
7. Dowsett M, Nielsen TO, A'Hern R, et al. Assessment of Ki67 in breast cancer: recommendations from the International Ki67 in Breast Cancer working group. J Natl Cancer Inst 2011;103:1656-64.
8. Edge SB, Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. Ann Surg Oncol 2010;17:1471-4.
9. Yao X, Hosenpud J, Chitambar CR, et al. A phase II study of concurrent docetaxel, epirubicin and cyclophosphamide as a neoadjuvant chemotherapy regimen in patients with locally advanced breast cancer. J Cancer 2012;3:145-51.
10. Cheang MC, Chia SK, Voduc D, et al. Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer. J Natl Cancer Inst 2009;101:736-50.
11. Kurosumi M, Akashi-Tanaka S, Akiyama F, et al. Histopathological criteria for assessment of therapeutic response in breast cancer (2007 version). Breast Cancer 2008;15:5-7.
12. Watanabe H, Okada M, Kaji Y, et al. New response evaluation criteria in solid tumours-revised RECIST guideline (version 1.1). Gan To Kagaku Ryoho 2009;36:2495-501.
13. Railo M, Lundin J, Haglund C, et al. Ki-67, p53, ER receptors, ploidy and S phase as long-term prognostic factors in T1 node-negative breast cancer. Tumour Biol 2007;28:45-51.
14. Ko ES, Han BK, Kim RB, et al. Analysis of factors that influence the accuracy of magnetic resonance imaging for predicting response after neoadjuvant chemotherapy in locally advanced breast cancer. Ann Surg Oncol 2013;20:2562-8.
15. Rudas M, Filipits M, Taucher S, et al. Expression of MRP1, LRP and Pgp in breast carcinoma patients treated with preoperative chemotherapy. Breast Cancer Res Treat 2003;81:149-57.
16. Yerushalmi R, Woods R, Ravdin PM, et al. Ki67 in breast cancer: prognostic and predictive potential. Lancet Oncol 2010;11:174-83.
17. Caudle AS, Gonzalez-Angulo AM, Hunt KK, et al. Predictors of tumor progression during neoadjuvant chemotherapy in breast cancer. J Clin Oncol 2010;28:1821-8.

18. Stuart-Harris R, Caldas C, Pinder SE, et al. Proliferation markers and survival in early breast cancer: a systematic review and meta-analysis of 85 studies in 32,825 patients. Breast 2008;17:323-34.
19. Molino A, Pedersini R, Micciolo R, et al. Relationship between the thymidine labeling and Ki-67 proliferative indices in 126 breast cancer patients. Appl Immunohistochem Mol Morphol 2002;10:304-9.
20. de Azambuja E, Cardoso F, de Castro G Jr, et al. Ki-67 as prognostic marker in early breast cancer: a meta-analysis of published studies involving 12,155 patients. Br J Cancer 2007;96:1504-13.
21. Penault-Llorca F, Abrial C, Raoelfils I, et al. Changes and predictive and prognostic value of the mitotic index, Ki-67, cyclin D1, and cyclo-oxygenase-2 in 710 operable breast cancer patients treated with neoadjuvant chemotherapy. Oncologist 2008;13:1235-45.
22. Pinto AE, Andre S, Pereira T, et al. Prognostic comparative study of S-phase fraction and Ki-67 index in breast carcinoma. J Clin Pathol 2001;54:543-9.
23. Klintman M, Bendahl PO, Grabau D, et al. The prognostic value of Ki67 is dependent on estrogen receptor status and histological grade in premenopausal patients with node-negative breast cancer. Mod Pathol 2010;23:251-9.
24. Allred DC, Mohsin SK, Fuqua SA. Histological and biological evolution of human premalignant breast disease. Endocr Relat Cancer 2001;8:47-61.
25. Amiri-Kordestani L, Basseville A, Kurdziel K, et al. Targeting MDR in breast and lung cancer: discriminating its potential importance from the failure of drug resistance reversal studies. Drug Resist Updat 2012;15:50-61.
26. Leonard GD, Fojo T, Bates SE. The role of ABC transporters in clinical practice. Oncologist 2003;8:411-24.
27. Rivera E. Implications of anthracycline-resistant and taxane-resistant metastatic breast cancer and new therapeutic options. Breast J 2010;16:252-63.
28. Kim B, Fatayer H, Hanby AM, et al. Neoadjuvant chemotherapy induces expression levels of breast cancer resistance protein that predict disease-free survival in breast cancer. PLoS One 2013;8:e62766.
29. Burger H, Foekens JA, Look MP, et al. RNA expression of breast cancer resistance protein, lung resistance-related protein, multidrug resistance-associated proteins 1 and 2, and multidrug resistance gene 1 in breast cancer: correlation with chemotherapeutic response. Clin Cancer Res 2003;9:827-36.
30. Martincich L, Montemurro F, De Rosa G, et al. Monitoring response to primary chemotherapy in breast cancer using dynamic contrast-enhanced magnetic resonance imaging. Breast Cancer Res Treat 2004;83:67-76.
31. Hylton NM, Blume JD, Bernreuter WK, et al. Locally advanced breast cancer: MR imaging for prediction of response to neoadjuvant chemotherapy--results from ACRIN 6657/I-SPY TRIAL. Radiology 2012;263:663-72.
32. Moon HG, Han W, Lee JW, et al. Age and HER2 expression status affect MRI accuracy in predicting residual tumor extent after neo-adjuvant systemic treatment. Ann Oncol 2009;20:636-41.

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Original Article

Effects of yoga training in patients with chronic obstructive pulmonary disease: a systematic review and meta-analysis

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Introduction: Currently, several studies have assessed the effect of yoga training on the management of chronic obstructive pulmonary disease (COPD), but these studies involved a wide variation of sample and convey inconclusive results. Hence, the present study was performed a systematic review and meta-analysis to investigate the efficacy of yoga training in COPD patients.

Methods: PubMed, EMBASE, the Cochrane Library, Google Scholar, and ClinicalTrials.gov databases were searched for relevant studies. The primary outcomes were forced expiratory volume in one second (FEV_1), FEV_1% predicted (% pred). Secondary outcomes included 6-min walking distance (6 MWD), arterial oxygen tension (PaO_2), and arterial carbon dioxide tension ($PaCO_2$). Weighted mean differences (WMDs) and 95% confidence intervals (CIs) were calculated, and heterogeneity was assessed with the I^2 test.

Results: Five randomized controlled trials (RCTs) involving 233 patients fulfilled the inclusion criteria. Yoga training significantly improved FEV_1 (WMD: 123.57 mL, 95% CI: 4.12-243, P=0.04), FEV_1% pred (WMD: 3.90%, 95% CI: 2.27-5.54, P<0.00001), and 6 MWD (WMD: 38.84 m, 95% CI: 15.52-62.16, P=0.001). However, yoga training had no significant effects on PaO_2 (WMD: 1.29 mmHg, 95% CI: –1.21-3.78, P=0.31) and $PaCO_2$ (WMD: –0.76 mmHg, 95% CI: –2.06-0.53, P=0.25).

Conclusions: The current limited evidence suggested that yoga training has a positive effect on improving lung function and exercise capacity and could be used as an adjunct pulmonary rehabilitation program in COPD patients. However, further studies are needed to substantiate our preliminary findings and to investigate the long-term effects of yoga training.

Keywords: Chronic obstructive pulmonary disease (COPD); yoga; pulmonary function; meta-analysis

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Introduction

Chronic obstructive pulmonary disease (COPD) is an important cause of morbidity and mortality and poses a major public health problem. By 2020, COPD is predicted to rank as the third leading cause of death worldwide, whereas its social burden will rank fifth (1-3). COPD is characterized by irreversible airflow obstruction, a gradual decline in lung function, loss of lung tissue, reduced quality of life, and high rates of mortality. The Global Initiative for Chronic Obstructive Lung Disease (GOLD) management includes a reduction in symptoms, complications, and exacerbations, improved exercise tolerance, improved health status, and reduced mortality (2). Recent evidence-based clinical practice guidelines and statements have shown that pulmonary rehabilitation is widely accepted as the most effective non-pharmacotherapy in the management of COPD (4). Research have indicated that various exercises,

such as upper extremity exercise (5), Tai Chi (6), and yoga training (7), can relieve dyspnea, improve lung function, and improve the quality of life of COPD patients. Furthermore, a physical therapist-assisted intensive flexibility training that focuses on stretching and rib cage mobilization can significantly improve 6-min walking distance (6 MWD) (8).

Yoga originated in ancient India, and may denote the union between the individual self and the transcendental self. The body's organs and systems are cleansed through *asanas* (postures) and *pranayama* (controlling the breath). Along with meditation, yoga *asanas* and *pranayama* have become popular in the West, and the practice of yoga has become "westernized." Postures are taught as ends in themselves, that is, to heal an illness, reduce stress, or to look better (9). Yogic exercises have been shown to have positive effects on people with asthma (10,11), cardiac diseases (12), diabetes (13), tuberculosis (14), depressive disorders (15), osteoarthritis (16), and pleural effusion (17).

A number of clinical trials have suggested that yoga training may improve the pulmonary function of patients with COPD (18,19), but the quality of these studies have not been evaluated systematically. Therefore, we undertook a systematic review and meta-analysis of available randomized controlled trials (RCTs) to assess the efficacy of yoga training on pulmonary function and other clinical endpoints in patients with COPD.

Methods

Data sources and search strategy

The following electronic databases were searched: PubMed, Embase databases, Cochrane Central Register of Controlled Trials, Google Scholar, and ClinicalTrials.gov (until Jan 2014). The employed keywords were "yoga" and "COPD," or "yoga" and "COPD". The searches were limited to English publications in humans as well as RCTs. Bibliographies of all potentially relevant studies, articles (including unpublished data and meta-analyses), and international guidelines were manually searched. Furthermore, we also attempted to contact the authors of potentially relevant studies to obtain additional information.

Study selection

The following inclusive selection criteria inPICOS order involved the following: (I) population: patients with COPD; (II) intervention: yoga training with or without other treatments; (III) comparison intervention: any type of control; (IV) outcome measures: the primary outcomes were forced expiratory volume in one second (FEV_1) and FEV_1% predicted (% pred), whereas secondary outcomes included 6 MWD, arterial oxygen tension (PaO_2), and arterial carbon dioxide tension ($PaCO_2$); and (V) study design: RCT reported in a full paper article.

Data extraction

For each candidate article, Xun-Chao Liu and Lei Pan recorded the characteristics of the patients being studied, i.e., first author, year of publication, COPD stage, sample size of the study population (intervention/control), grade, staging, age, study design, Jadad scale, interventions (i.e., style of intervention, training frequency, exercise time, and duration), outcome parameters, and their results. Liang Dong checked all of the data. Disagreements were resolved by discussion.

Quality assessment and risk-of-bias assessment

The methodological quality of each research was evaluated using the Jadad scale (20). A score ≤2 indicates low quality, whereas a score ≥3 indicates high quality (21). The risk of bias was assessed using the *Cochrane Handbook for Systematic Reviews of Interventions* (http://ims.cochrane.org/revman). Two authors (Xun-Chao Liu and Lei Pan) subjectively reviewed all studies and assigned a value of 'high,' 'low,' or 'unclear' to the following: (I) selection bias (was there adequate generation of the randomization sequence? was allocation concealment satisfactory?); (II) blinding (i.e., performance bias and detection bias) (was there blinding of participants, personnel, and outcome assessment?); (III) attrition bias (were incomplete outcome data sufficiently assessed and dealt with?); (IV) reporting bias (was there evidence of selective outcome reporting?); and (V) other biases (was the study apparently free of other problems that could place it at a high risk of bias?).

Statistical analysis

This systematic review and meta-analysis is reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (22). We used Revman version 5.1 software for all data and statistical analyses. For continuous outcomes, the pooled mean difference was calculated by using the weighted

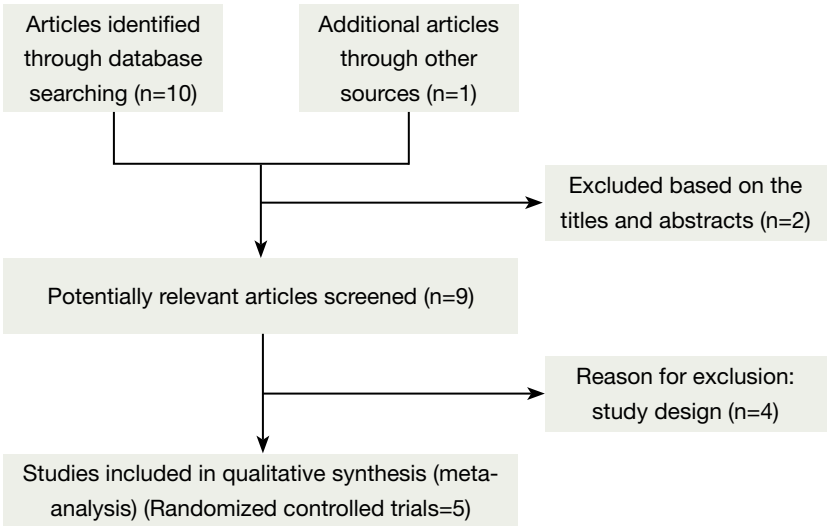

Figure 1 Search strategy and flow chart of screened, excluded, and eventually analyzed articles.

mean difference (WMD). Heterogeneity was assessed with the I^2 statistic and defined as low ($I^2 \leq 25\%$), moderate ($25\% < I^2 \leq 50\%$), or high ($I^2 > 50\%$) (23,24). A random-effects model was undertaken whether heterogeneity was high or not. Potential publication bias was not assessed because of the limited number of studies (<10) included in each analysis. A P value of <0.05 was considered statistically significant. The overall treatment effect was compared with its minimal clinically important difference (MCID).

Results

Bibliographic search

Figure 1 shows the study search process according to PRISMA guidelines (22). Of the 11 studies retrieved from the initial search, six met our inclusion criteria (7,18,19,25-27), but one study was excluded upon secondary analysis (27). The reasons for exclusion are presented in *Figure 1*. Finally, five RCTs, which had a combined cohort size of 233 participants, were selected for this meta-analysis, and their main characteristics are presented in *Table 1*. The sample size per RCT ranged from 30 to 100. These studies were published between 1978 and 2012, and their duration ranged from 12 weeks to 9 months. Four RCTs reported FEV_1 (18,19) or FEV_1% (7,25), and two RCTs reported 6 MWD (7,25). Meanwhile, two RCTs reported PaO_2 and $PaCO_2$ (18,19). The mean Jadad score of the studies included was 2.4 (SD=0.89). The risk of bias analysis is presented in *Table 2*.

Primary outcomes

Two RCTs reported primary outcomes as FEV_1 (18,19), and another two RCTs reported FEV_1% pred (7,25). The aggregate results of these studies suggested that yoga training was associated with a significant improvement in FEV_1 [WMD: 123.57 mL, 95% confidence interval (CI): 4.12-243, P=0.04] (*Figure 2*) and FEV_1% (WMD: 3.90, 95% CI: 2.27-5.54, P<0.00001) (*Figure 3*). The test for heterogeneity was not significant (FEV_1: P for heterogeneity=0.68, I^2=0%; FEV_1%: P for heterogeneity=0.39, I^2=0%). We subsequently performed sensitivity analyses to explore potential sources of heterogeneity. The mean changes of FEV_1 were greater than the MCID (>100 mL) (28).

Secondary outcomes

Meanwhile, two RCTs reported results in terms of 6 MWD (7,25). The aggregate results of these studies suggested that yoga training was associated with a statistically significant improvement in 6 MWD (WMD: 38.84 m, 95% CI: 15.52-62.16, P=0.001). The results for the heterogeneity test was not significant (P for heterogeneity=0.08, I^2=67%) (*Figure 4*). Subsequently, the mean changes of 6 MWD were greater than the MCID (>26 m) (29). Two RCTs reported PaO_2 and $PaCO_2$ (18,19), and these studies suggested that yoga training was not associated with a significant difference on PaO_2 (WMD: 1.29 mmHg, 95% CI: –1.21-3.78, P=0.31) or $PaCO_2$ (WMD: –0.76 mmHg, 95% CI: –2.06-0.53,

Table 1 Characteristics of randomized controlled trials included in the meta-analysis

First author	Patients No. (I/C), Grade, Staging	Age (years), (Mean, I/C)	Form or style		Protocol	Duration	Study design/ Jadad score
			Yoga group	Control group			
Donesky-Cuenco *et al*./2009	29 (14/15), Stable, NR	69.9±9.5	Pranayama, asana	Usual-care control	60 min/per time 2 times/w	12 w	RCT/2
Soni *et al*./2012	60 (30/30), Stable, Mild or Moderate	30-60	Pranayama, asana	Conventional treatment	45 min/d	2 mon	RCT/2
Kulpati *et al*./1982	75 (25/50), NR, NR	50.6/48.65	Pranayama	Conventional treatment and breathing exercises	30 min/per time 2 times/d	12 w	RCT/2
Tandon *et al*./1978	24 (12/12), Stable, NR	<65	Yogic breathing exercises and postures	Physiotherapy breathing exercises	60 min/per time 1-4 w: 3 times/w; 5-8 w: 2 times/w; 9 w-9 mo: 2 times/w	9 mon	RCT/2
Katiyar *et al*./2006	45 (23/22), Stable, severe	53.3/51.1	Pranayama	Usual-care control	30 min/per time 6 times/w	3 mon	RCT/4

I/C, intervention/control; RCT, randomized controlled trial; NR, not reported.

Table 2 Assessing risk of bias

Study/year	Sequence generation	Allocation concealment	Blinding	Incomplete outcome data addressed	Selective outcome reporting	Free of other bias
Donesky-Cuenco *et al*./2009	Unclear	No	No	Yes	Yes	Unclear
Soni *et al*./2012	Unclear	No	No	Yes	Yes	Unclear
Kulpati *et al*./1982	Unclear	No	No	Yes	Yes	Unclear
Tandon *et al*./1978	Unclear	No	No	Yes	Yes	Unclear
Katiyar*et al*./2006	Yes	No	Yes	Yes	Yes	Unclear

P=0.25). The test for heterogeneity was not significant (PaO_2: P for heterogeneity=0.47, I^2=0%; $PaCO_2$: P for heterogeneity=0.23, I^2=31%) (*Figures 5,6*).

Discussion

This study is the first meta-analysis to evaluate the effects of yoga training on COPD patients. Our results suggested that yoga training has a positive improvement effect on lung function and exercise capacity and could be used as an adjunct pulmonary rehabilitation program for COPD patients.

Currently, no drugs could hinder the progress of COPD, but lung training and pulmonary rehabilitation have been shown to reduce disability in many chronic respiratory diseases and have become valuable means of COPD treatment (30-33). Studies have indicated an increase in tidal volume and FVC, reduction in respiratory rate, increase in FEV_1, FEV_1%, maximum voluntary ventilation, and breath holding capacity after short-term yoga practice (34,35). Furthermore, studies suggested that yoga training may improve exercise capacity, prevent lung function decline, improve quality of life, and reduce dyspnea in patients with COPD (36,37). However, these studies did not provide adequate data or sufficient clinical evidence to support the beneficial effects of yoga training on these relevant findings.

Our results suggested that yoga training improved FEV_1 or FEV_1% pred in four studies (7,18,19,25). However, the results required comparison with the MCID, which is defined as the smallest change in the measurement used to evaluate the clinical significance of intervention effects. The

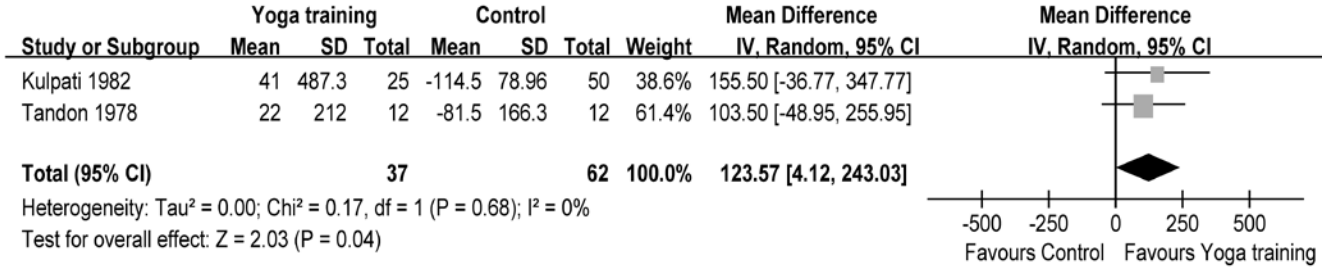

Figure 2 Meta-analysis of randomized controlled trials evaluating effects of yoga training on FEV_1 by the random-effects model. FEV_1, forced expiratory volume in one second.

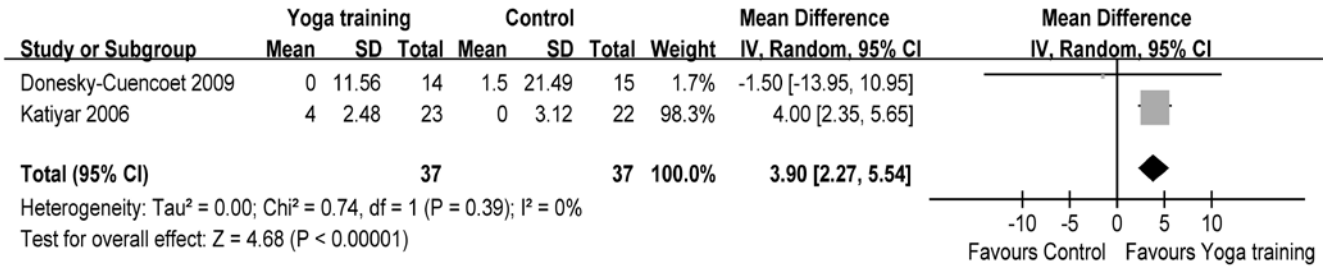

Figure 3 Meta-analysis of randomized controlled trials evaluating effects of yoga training on FEV_1% by the random-effects model. FEV_1, forced expiratory volume in one second.

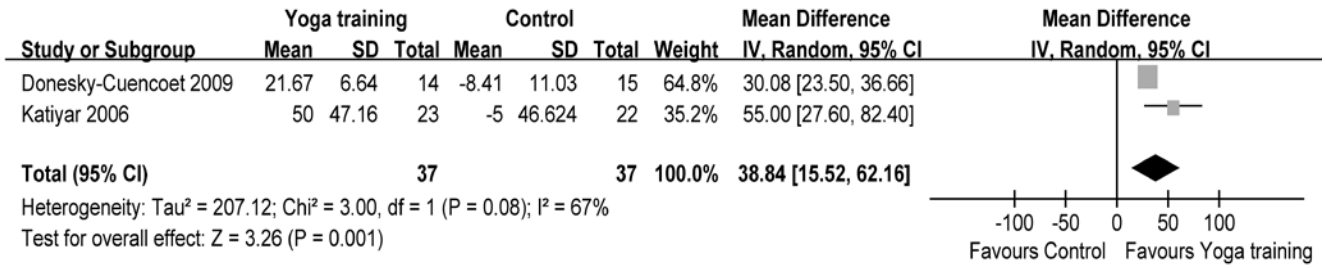

Figure 4 Meta-analysis of randomized controlled trials evaluating effects of yoga training on 6WMD by the random-effects model. WMD, weighted mean difference.

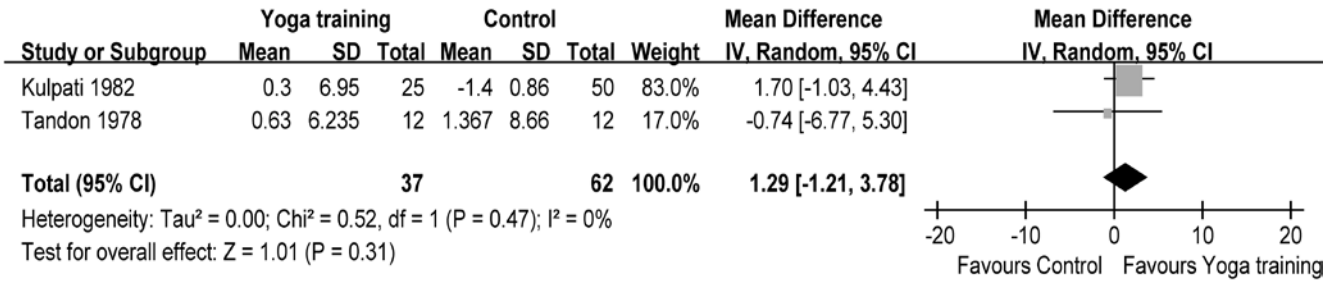

Figure 5 Meta-analysis of randomized controlled trials evaluating effects of yoga training on PaO_2 by the random-effects model. PaO_2, arterial oxygen tension.

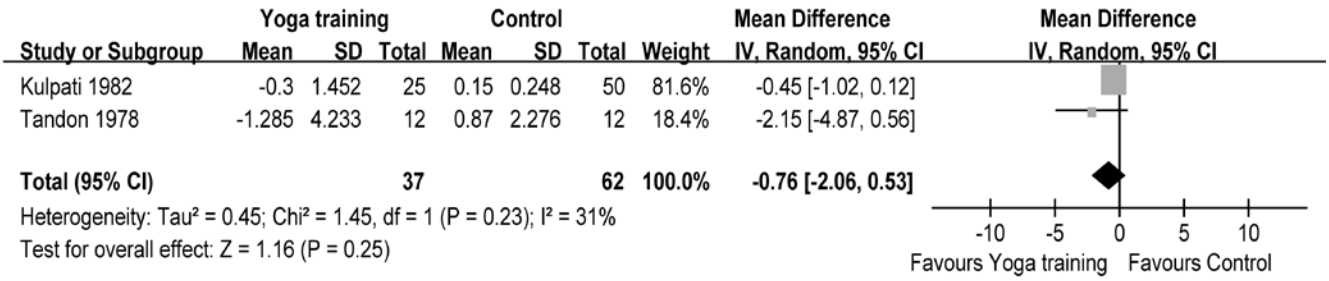

Figure 6 Meta-analysis of randomized controlled trials evaluating effects of yoga training on $PaCO_2$ by the random-effects model. $PaCO_2$, arterial carbon dioxide tension.

MCID is claimed to be a 100-mL change in FEV_1 from baseline (28), and the 123.57 mL increment in FEV_1 in our study is greater than the MCID. This result indicates that yoga training can have a clinical effect on COPD patients. Unfortunately, we failed to compare further the FEV_1% pred with the MCID because of insufficient available data. Our meta-analysis also found that yoga training statistically improved 6 MWD in patients with COPD. The 38.84 m change for 6 MWD was greater than the MCID (≥26 m) (29). However, our meta-analysis showed that yoga training did not affect arterial blood gas analyses, which included PaO_2 and $PaCO_2$. PaO_2 and $PaCO_2$ are affected by various factors, such as temperature factors (38), breathing frequency (39), varying levels of light intensities (40), sampling location, blood volume, and inspection time (41). We believed that PaO_2 and $PaCO_2$ are not suitable evaluation parameters of yoga training in COPD patients because of their instability. However, further study is needed to investigate this noteworthy topic.

The mechanisms of yoga training responsible for its beneficial effects that differ from other forms of exercise have yet to be elucidated. Several factors may be responsible for the beneficial effects seen in the patients undergoing yoga training aside from exercises (42-45). Yoga training aids in toning up general body systems (42), increasing respiratory stamina, relaxing chest muscles, expanding the lungs, raising energy levels, and calming the body (43). Additionally, yoga training improves blood circulation and increases the strength of respiratory muscles (44). Finally, yoga training also helps patients to breathe more deeply by utilizing the shoulder, thoracic, and abdominal muscles efficiently (45).

Yoga training can provide a complementary strategy for patients with COPD. Apart from relaxing tense muscles, yoga can also alleviate mental pressure (15). However further studies are needed to examine the effectiveness of yoga training compared with other breathing exercises. Our study showed follow-up durations that ranged from 12 weeks to 9 months, and the long-term effects of yoga training optimal exercise duration currently remain unknown. Future research should focus on optimizing training intensity, duration, and frequency. Moreover, it should be emphasized that the severity may greatly influence the effect of yoga. However, not all studies have noted the severity of COPD patients. Further research should focus on the relationship between the severity and efficacy in COPD patients, which can help to determine the best yoga exercise prescription and the best suitable patients. Finally, most studies lacked other physiological outcome measures, such as inflammatory biomarkers, continuous monitoring, sensitive measures of change, and peripheral muscle strength. Further studies that focus on these will enrich clinical evidence regarding yoga.

Several limitations are identified in our study. First, included trials significantly varied in terms of interventions protocol, duration, patient populations, severity, and study quality, which limit the conclusive extent for the overall effectiveness of yoga training on FEV_1, FEV_1% pred, 6 MWD, and blood gas analysis in COPD patients. Secondly, our analysis is based on only five RCTs, and only a maximum of two studies were available for the main outcomes. In addition, studies ranged from 1978 to 2012 hence it encompasses a wide time frame which may affect the results as over the years better drugs are available such anticholinergics to ameliorate symptoms of COPD and more standardized and accurate methods are available to measure some clinical endpoints such as lung function and arterial blood gas analysis. Moreover, these studies have a wide variation in patient populations. The smaller sample size of trials may have significantly overestimated the treatment effect. Finally, several missing and unpublished data may lead to bias.

Conclusions

Our meta-analysis suggested that yoga training that lasts from 12 weeks to 9 months may improve lung function and functional exercise capacity in patients with COPD compared with conventional therapy. Moreover, we suggest that yoga could be a useful adjunct pulmonary rehabilitation program for COPD patients. To help clarify the issue, further rigorously designed, larger-scale trials should be conducted to evaluate the long-term effects of yoga training in COPD patients.

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References

1. Viegi G, Pistelli F, Sherrill DL, et al. Definition, epidemiology and natural history of COPD. Eur Respir J 2007;30:993-1013.
2. Vestbo J, Hurd SS, Agustí AG, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. Am J Respir Crit Care Med 2013;187:347-65.
3. Murray CJ, Lopez AD. Mortality by cause for eight regions of the world: Global Burden of Disease Study. Lancet 1997;349:1269-76.
4. Qaseem A, Wilt TJ, Weinberger SE, et al. Diagnosis and management of stable chronic obstructive pulmonary disease: a clinical practice guideline update from the American College of Physicians, American College of Chest Physicians, American Thoracic Society, and European Respiratory Society. Ann Intern Med 2011;155:179-91.
5. Pan L, Guo YZ, Yan JH, et al. Does upper extremity exercise improve dyspnea in patients with COPD? A meta-analysis. Respir Med 2012;106:1517-25.
6. Yan JH, Guo YZ, Yao HM, et al. Effects of Tai Chi in patients with chronic obstructive pulmonary disease: preliminary evidence. PLoS One 2013;8:e61806.
7. Donesky-Cuenco D, Nguyen HQ, Paul S, et al. Yoga therapy decreases dyspnea-related distress and improves functional performance in people with chronic obstructive pulmonary disease: a pilot study. J Altern Complement Med 2009;15:225-34.
8. Yoshimi K, Ueki J, Seyama K, et al. Pulmonary rehabilitation program including respiratory conditioning for chronic obstructive pulmonary disease (COPD): Improved hyperinflation and expiratory flow during tidal breathing. J Thorac Dis 2012;4:259-64.
9. Garfinkel M, Schumacher HR Jr. Yoga. Rheum Dis Clin North Am 2000;26:125-32, x.
10. Manocha R, Marks GB, Kenchington P, et al. Sahaja yoga in the management of moderate to severe asthma: a randomised controlled trial. Thorax 2002;57:110-5.
11. Sabina AB, Williams AL, Wall HK, et al. Yoga intervention for adults with mild-to-moderate asthma: a pilot study. Ann Allergy Asthma Immunol 2005;94:543-8.
12. Jayasinghe SR. Yoga in cardiac health (a review). Eur J Cardiovasc Prev Rehabil 2004;11:369-75.
13. Malhotra V, Singh S, Tandon OP, et al. The beneficial effect of yoga in diabetes. Nepal Med Coll J 2005;7:145-7.
14. Visweswaraiah NK, Telles S. Randomized trial of yoga as a complementary therapy for pulmonary tuberculosis. Respirology 2004;9:96-101.
15. Sharma VK, Das S, Mondal S, et al. Effect of Sahaj Yoga on depressive disorders. Indian J Physiol Pharmacol 2005;49:462-8.
16. Ernst E. Complementary or alternative therapies for osteoarthritis. Nat Clin Pract Rheumatol 2006;2:74-80.
17. Prakasamma M, Bhaduri A. A study of yoga as a nursing intervention in the care of patients with pleural effusion. J Adv Nurs 1984;9:127-33.
18. Tandon MK. Adjunct treatment with yoga in chronic severe airways obstruction. Thorax 1978;33:514-7.
19. Kulpati DD, Kamath RK, Chauhan MR. The influence of physical conditioning by yogasanas and breathing exercises in patients of chronic obstructive lung disease. J Assoc Physicians India 1982;30:865-8.
20. Jadad AR, Moore RA, Carroll D, et al. Assessing the quality of reports of randomized clinical trials: is blinding necessary? Control Clin Trials 1996;17:1-12.
21. Kjaergard LL, Villumsen J, Gluud C. Reported methodologic quality and discrepancies between large and small randomized trials in meta-analyses. Ann Intern Med 2001;135:982-9.
22. Liberati A, Altman DG, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. BMJ 2009;339:b2700.
23. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med 2002;21:1539-58.
24. Higgins JP, Thompson SG, Deeks JJ, et al. Measuring

inconsistency in meta-analyses. BMJ 2003;327:557-60.
25. Katiyar SK, Bihari S. Role of pranayama in rehabilitation of COPD patients–a randomized controlled study. Indian J Allergy Asthma Immunol 2006;20:98-104.
26. Soni R, Munish K, Singh K, et al. Study of the effect of yoga training on diffusion capacity in chronic obstructive pulmonary disease patients: A controlled trial. Int J Yoga 2012;5:123-7.
27. Donesky D, Melendez M, Nguyen HQ, et al. A responder analysis of the effects of yoga for individuals with COPD: who benefits and how? Int J Yoga Therap 2012:23-36.
28. Donohue JF. Minimal clinically important differences in COPD lung function. COPD 2005;2:111-24.
29. Puhan MA, Chandra D, Mosenifar Z, et al. The minimal important difference of exercise tests in severe COPD. Eur Respir J 2011;37:784-90.
30. Lacasse Y, Wong E, Guyatt GH, et al. Meta-analysis of respiratory rehabilitation in chronic obstructive pulmonary disease. Lancet 1996;348:1115-9.
31. Singh V. Effect of respiratory exercises on asthma. The Pink City lung exerciser. J Asthma 1987;24:355-9.
32. Singh V, Wisniewski A, Britton J, et al. Effect of yoga breathing exercises (pranayama) on airway reactivity in subjects with asthma. Lancet 1990;335:1381-3.
33. Ries AL, Bauldoff GS, Carlin BW, et al. Pulmonary Rehabilitation: Joint ACCP/AACVPR Evidence-Based Clinical Practice Guidelines. Chest 2007;131:4S-42S.
34. Makwana K, Khirwadkar N, Gupta HC. Effect of short term yoga practice on ventilatory function tests. Indian J Physiol Pharmacol 1988;32:202-8.
35. Joshi LN, Joshi VD, Gokhale LV. Effect of short term 'Pranayam' practice on breathing rate and ventilatory functions of lung. Indian J Physiol Pharmacol 1992;36:105-8.
36. Behera D. Yoga therapy in chronic bronchitis. J Assoc Physicians India 1998;46:207-8.
37. Fulambarker A, Farooki B, Kheir F, et al. Effect of yoga in chronic obstructive pulmonary disease. Am J Ther 2012;19:96-100.
38. Bradley AF, Severinghaus JW, Stupfel M. Effect of temperature on PCO2 and PO2 of blood in vitro. J Appl Physiol 1956;9:201-4.
39. Kapus J, Ušaj A, Kapus V, et al. The influence of reduced breathing during swimming on some respiratory and metabolic values in blood. KinSI 2002;8:14-8.
40. Olanrewaju HA, Purswell JL, Collier SD, et al. Effect of varying light intensity on blood physiological reactions of broiler chickens grown to heavy weights. Int J Poult Sci 2012;11:81-7.
41. Cunningham DJ, Cormack RS, O'Riordan JL, et al. An arrangement for studying the respiratory effects in man of various factors. Q J Exp Physiol Cogn Med Sci 1957;42:294-303.
42. Udupa KN, Singh RH. The scientific basis of yoga. JAMA 1972;220:1365.
43. Chanavirut R, Khaidjapho K, Jaree P, et al. Yoga exercise increases chest wall expansion and lung volumes in young healthy thais. Thai J Physiol Sci 2006;19:1-7.
44. Posadzki P, Parekh S. Yoga and physiotherapy: a speculative review and conceptual synthesis. Chin J Integr Med 2009;15:66-72.
45. Vedanthan P. Yoga breathing techniques (YBT) in chronic obstructive pulmonary disease (COPD): a preliminary study. Int J Yoga Ther 2003;13:51-4.

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Original Article

Prognostic value of *FGFR1* gene copy number in patients with non-small cell lung cancer: a meta-analysis

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Background: A number of studies have investigated the relationship between fibroblast growth factor receptor1 (*FGFR1*) gene copy number and survival in non-small cell lung cancer (NSCLC) patients. However, conclusions reported by different parties seem to be inconsistent, especially regarding the differences among different histopathologic subtypes. To derive a more precise estimate of the prognostic significance of *FGFR1* gene copy number, we have reviewed published studies and carried out a meta-analysis.

Methods: The meta-analysis was conducted in accordance with PRISMA guidelines. The required data for estimation of individual hazard ratios (HRs) for survival were extracted from the publications and an overall HR was calculated.

Results: We identified 6 eligible studies, all dealing with NSCLC. The global quality score ranged 32.5-80%, with a median of 53.33%. For *FGFR1* amplification in three studies including differed according to histological type, the overall RR was 0.86 which 95% confidence interval (CI) was 0.75 to 0.99 and P value was 0.048. Combined HR for the six evaluable studies was 1.17 (95% CI: 0.95 to 1.43). In the subgroup of squamous cell lung cancer (SQCC), the combined HR was 1.24 (95% CI: 0.89 to 1.73). For the Asian populations' studies, the combined HR was 1.67 (95% CI: 1.1 to 2.52).

Conclusions: *FGFR1* amplification significantly was more frequent in SQCC. *FGFR1* was not associated with poorer survival in patients with NSCLC. Furthermore studies will be needed in terms of survival implications.

Keywords: Lung cancer; meta-analysis; fibroblast growth factor receptor1 (*FGFR1*); prognosis; survival; systemic review

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Introduction

Lung cancer is the most common cause of cancer death in the world (1). Due to the lack of specific symptoms, most of lung cancer patients are in the mid or late stage when they were diagnosed. Although diagnostic approaches, treatment techniques and surgical levels towards lung cancer have been improved greatly in recent years, most lung cancer patients still have bad prognosis with survival rate of five years fluctuating around 15% (2). Therefore, it was of great significance in treatment selection and patient survival rate increase to look for factors relevant to lung cancer prognosis.

Fibroblast growth factor receptor1 (*FGFR1*) recently has become a hot topic in the research of cancer driver gene. In 2010, Weiss *et al.* (3), a German scientist, found out that this gene had a large number of amplification in squamous carcinoma specimen. In many subsequent

studies, researchers analyzed the role *FGFR1* amplification played during lung cancer prognosis; however, conclusions reported by different parties seem to be inconsistent, especially regarding the differences among different histopathologic subtypes. Some believe that squamous cell lung cancer (SQCC) patients with *FGFR1* amplification have poor prognosis; others believe that *FGFR1* expression was not related to prognosis of SQCC or non-small cell lung cancer (NSCLC). We apply Meta-analysis method to the past research results as a comprehensive quantitative analysis in order to evaluate the effects of *FGFR1* amplification on NSCLC prognosis.

Material and methods

Identification and selection of relevant studies

Criteria for eligibility of a study to the meta analysis were: (I) to deal with NSCLC only; (II) to evaluate the correlation between *FGFR1* gene copy number and patient survival and analyse *FGFR1* in the primary tumour (not in metastatic tissue); (III) to be published as a full paper in the English language literature. Studies published in abstract form were excluded; (IV) to find providing sufficient information, such as P value and survival curve, for the estimation of hazard ratio (HR) and 95% confidence interval (CI); (V) measurement methods, including reverse transcription-polymerase chain reaction (RT-PCR), fluorescent in situ hybridization (FISH) and immunohistochemistry (IHC).

Search for studies was performed using the electronic database PubMed, Embase, Web of Science and Google Scholar until March 31, 2014. The search strategy included the keywords or title combined "lung cancer", "lung carcinoma", "*FGFR1*", "Fibroblast Growth Factor Receptor 1", "survival", and "prognosis". All studies matching the eligibility criteria were mentioned previously. Two investigators (Wen Yang and Yan-Wen Yao) independently deal with the relevant studies' data. Any incongruity was settled through discussion until a consensus was reached.

Data extraction

HRs and their 95% CIs were used to combine the data. When these information were described in text or tables, we acquired these values directly from two articles (3,4). When these statistical variables were not given directly in the article, they were calculated from survival curves in four articles (5-8) using the methods which reported by Parmar *et al*. (9). We extracted data on characteristics of studies and patients, measurements, results and so on. In particular, in each report we recorded the first author, country of origin, year of publication, number of patients analyzed, staging of tumor, method of *FGFR1* gene copy number detection, cutoff value, histology, number of patients of *FGFR1* gene amplification, time of follow-up, and OS data. The primary outcome of the meta-analysis was overall survival.

Quality assessment

Two investigators (Wen Yang and Yan-Wen Yao) independently assessed the quality of the selected studies using the European lung cancer working party quality scale for biological prognostic factors for lung cancer (10). This tool comprises four quality parameters: scientific design, laboratory methodology, generalizability and results analysis. Each category had a maximum score from 8 to 10 points, and the overall score was 40 points. The final scores were expressed as percentages, ranging from 0 to 100%. The Higher values were obtained, the better methodological quality was indicated. The scores were compared and a consensus value for each item was reached in meetings of all investigators needed to be present.

Statistical analysis

Survival data from each study were analyzed in terms of the HRs and 95% CI directly or calculated by Kaplan-Meier curves as previously described by Parmar *et al*. (9) and Tierney *et al*. (11).

Statistical heterogeneity was measured using the chi squared Q test and the I^2 statistic. Significant heterogeneity was determined at a P value less than 0.10. I^2 was used to quantify inconsistencies, where a value more than 50% indicates visible heterogeneity. When visible heterogeneity was observed, the random effects model was used. When no visible heterogeneity was observed, the fixed effects model was used for meta-analysis (12). The individual HR estimates were combined into an overall HR, which less than 1 implied a poor survival for the group with *FGFR1* gene amplification by convention. This impact of *FGFR1* gene copy number on survival was considered as statistically significant if the 95% CI for the overall HR did not overlap 1.

We assessed the subgroup additionally, including the histological type (NSCLC or SQCC), country of origin (Asian or non-Asian). The slection of the model of subgroup analysis was based on the convention, as

Table 1 Characteristics of the study populations

First author	Year	Histology	Stage	N pts	Positive N	Positive rate	Method	HR estimation	Cut off	Results
Weiss	2010	SQCC	I-III	155	15	9.68%	FISH	Survival curve	Copy number >4	NS
Rebecca	2012	SQCC	I-IV	226	37	16.37%	FISH	Survival curve	*FGFR1*/CEP8 ratio ≥2.2	NS
Kim	2012	SQCC	I-III	262	34	12.98%	FISH	HR	Copy number >9	Poor prognosis
Sasaki	2012	NSCLC	I-IV	100	32	32.00%	RT-PCR	Survival curve	Copy number >4	NS
Tran	2013	NSCLC	I-IV	264	37	14.02%	Dual-Colour ISH	survival curve	*FGFR1*/CEP8 ratio ≥2.0	NS
Craddock	2013	SQCC	I-IV	121	11	9.09%	FISH	HR	Copy number >5	NS

N pts, number of patients; HR, hazard ratio; NSCLC, non-small-cell lung cancer; SQCC, squamous cell lung cancer; FISH, fluorescence in situ hybridization; PCR, polymerase chain reaction; NS, non-significant.

Table 2 Incidence rate in different histologic type

Author	Non-amplification			Amplification		
	SQCC	Non-SQCC	Sum	SQCC	Non-SQCC	Sum
Weiss	140	77*	217	15	0	15
Rebecca	37	–	37	189	–	189
Kim	228	–	228	34	–	34
Sasaki	38	30	68	27	5	32
Tran	25	139	164	6	24	30
Craddock	99	–	99	22	–	22

SQCC, squamous cell lung cancer. *, Patients with Non-SQCC were exclude for survival analysis.

previously described. Publication bias including funnel plot and Egger's test was performed. By convention, an observed P value less than 0.05 implied a great statistical significance for summary HR and publication biases. Survival rates on the graphical representation of the survival curves were extracted by Engauge Digitizer version 5.0. HRs and their 95% CI were calculated by STATA version 11.0.

Results

Eligible studies and characteristics

Ten publications (3-8,13-16), published between 1997 and 2013, were selected. They all reported the prognostic value for survival of *FGFR1* gene copy number in lung cancer patients, assessing *FGFR1* gene amplification in the primary tumour. One publication (13) of these studies was excluded because the histological type of cohort was small cell lung cancer (SCLC). One publication (14) was excluded because the cohort of patients were NSCLC with brain metastases. Two publications (15,16) was excluded because the technique of the detection was immature. There were six publications eligible for the meta-analysis finally (3-8). The total number of included patients was 1,128, ranging from 100 to 264 patients per study (median number =188). The main characteristics of the six eligible publications are described in *Table 1*. In the six studies included in the analysis, gene copy number of *FGFR1* was evaluated by FISH in five (3-5,7,8) by RT-PCR in one (6). Using cut off values for overexpression chosen by each author, 166 (15.69%) of the 1,128 patients in this meta-analysis had *FGFR1* amplification. Comprehensive analysis for overall *FGFR1* amplification in three studies including differed according to histological type, the overall RR was 0.9 which 95% CI was 0.85 to 0.96 and P value was 0.001 (*Table 2* and *Figure 1*). The outcome showed that significantly more frequent in SQCC.

Qualitative assessment

We assessed the quality of the selected studies using the European lung cancer working party quality scale for biological prognostic factors for lung cancer. Overall, the global quality score ranged 32.5-80%, with a median of 53.33% (*Table 3*).

Meta-analysis

The combined HR for all six eligible studies was 1.17 (95% CI: 0.95 to 1.43) using fixed effects model, indicating that *FGFR1* gene copy number had no significant survival impact in patients with NSCLC (*Figure 2A*). There was no significant heterogeneity between studies (I^2=22.4%, P=0.266) (*Figure 2A*).

When subgrouped according to histological subtypes, the combined HR for the NSCLC studies was 1 (95% CI: 0.67 to 1.49), the pooled HR for SQCC was 1.24 (95% CI: 0.89 to 1.73) (*Figure 2B*).

When subgrouped according to country of origin, the combined HR for the Asian populations' studies was 1.67 (95% CI: 1.1 to 2.52) while for Non-Asian populations' studies was 1.04 (95% CI: 0.82 to 1.73) (*Figure 2C*).

The sensitivity analysis showed that omitting any single study did not influence the pooled HR. We conduct another analysis after the literature using RT-PCR was excluded, then the combined HR was 1.17 and the 95% CI was 0.81-1.68 (*Figure 2D*). The results were consistent with the above-mentioned ones. For publication bias test, a more formal evaluation either using Bgger's test or Egger's test also showed no evidence of significant publication bias (Bgger's test: Z=0.19, P=0.851; Egger's test: coef. =0.068, P=0.908, 95% CI: –1.47-1.6) (*Figure 3*). This suggested absence of publication bias in all studies.

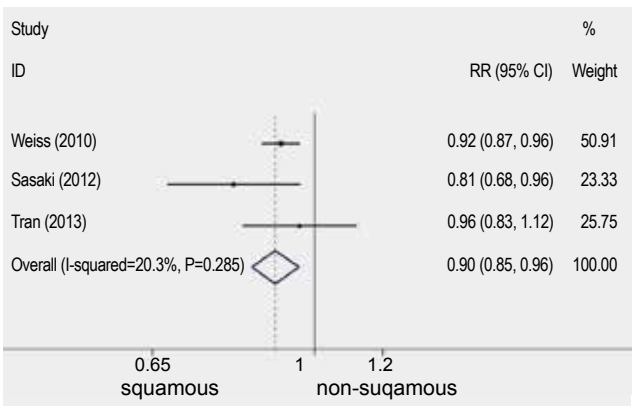

Figure 1 Analysis for overall *FGFR1* amplification in three studies including differed according to histological type. *FGFR1*, fibroblast growth factor receptor1.

Discussion

FGFR is a transmembrane protein of receptor tyrosine kinase which includes *FGFR1*, FGFR2, FGFR3 and FGFR4 (17). FGFRs signal transmission is necessary to normal cell growth and differentiation, participating with physiological processes of angiogenesis, embryogenesis, bone formation, and wound healing, and is quite close to tumor genesis and progression. Among numerous tumors, such as breast cancer, bladder cancer, prostate cancer, NSCLC, FGFR activating mutations or ligand/receptor overexpression causes signal to be continuously activated which is not only closely linked to tumor genesis, progression and poor prognosis but also plays an important role in tumor angiogenesis, tumor invasion and metastasis (18,19). Therefore, FGFR, being closely related to tumor patients' prognosis, is commonly known as the key target of anti-tumor.

There were six publications eligible for the meta-analysis. The heterogeneity test show I^2=22.4% (P=0.266), which indicated no obvious heterogeneity. Then analyzed with fixed effect model, combined HR was 1.17 and 95% CI was 0.95 to 1.43, indicating *FGFR1* amplification was irrelevant to NSCLC prognosis and effect size was similar

Table 3 Results of the methodological assessment by the European Lung Cancer Working Party score

	Weiss	Rebecca	Kim	Sasaki	Tran	Craddock	Average
Scientific design	3	6	7	6	6	6	5.67
Laboratory methodology	5	5	10	8	5	6	6.50
Generalizability	1	4	10	2	6	6	4.83
Results analysis	4	4	5	4	4	5	4.33
	32.50%	47.50%	80.00%	50.00%	52.50%	57.50%	53.33%

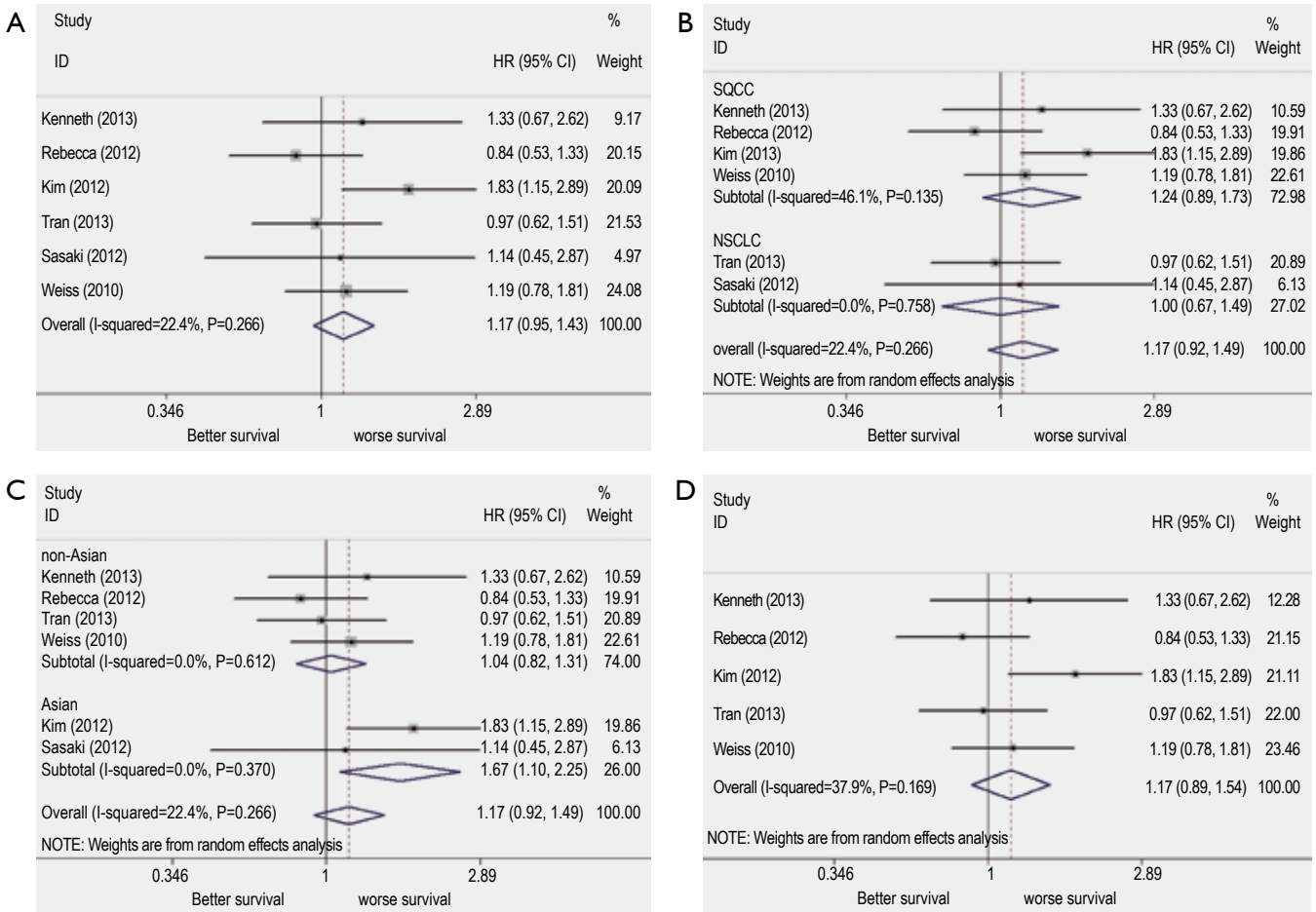

Figure 2 Meta-analysis of hazard ratio (HR) of the effect of *FGFR1* amplification on survival in patients with non-small cell lung cancer (NSCLC). (A) All six eligible studies; (B) Subgroup of histological subtypes; (C) Subgroup of country of origin; and (D) five FISH evaluated studies. *FGFR1*, fibroblast growth factor receptor1; FISH, fluorescence in situ hybridization.

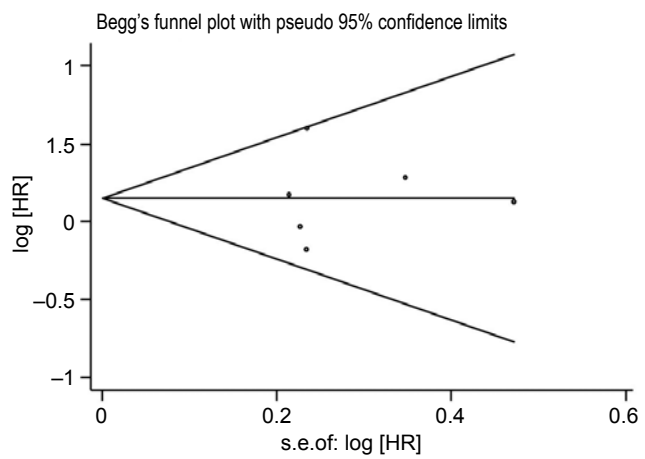

Figure 3 Begg's funnel plot with pseudo 95% confidence limits.

to most of research results. In the former part of this paper, we mentioned that *FGFR1* amplification was abundant in SQCC, while in other types of NSCLC amplification was rare. So was *FGFR1* amplification related to its prognosis on patients of SQCC? Therefore, we conduct subgroup analysis to the data and receive results showing that subgroup combined HR of SQCC was 1.24 and 95% CI was 0.89 to 1.73, indicating influences of *FGFR1* amplification on prognosis of SQCC do not have statistical significance. In racial distinctions, we divide the included people into Asian subgroup and non-Asian subgroup for analysis. Asian subgroup was of combined HR 1.67 and 95% CI was 1.1 to 2.52, while non-Asian subgroup was of combined HR 1.04 and 95% CI was 0.82 to 1.31. Results

from race subgroup analysis show that *FGFR1* amplification showed poor NSCLC prognosis among Asian people; however, researches included were small and there were only two subgroups with research targets of Asian people of total number of 362, so there is a possibility of bias. We need more researches to prove the phenomenon.

Meta-analysis is a quantitative analysis method based on previous research works, greatly influenced by the quality of previous research materials. We refer to the literature quality evaluation standard applied by European lung cancer working party quality scale for biological prognostic factors for lung cancer. Due to the limitations of Meta-analysis itself, there were various biases during the analysis process. The most common bias is publication bias. We adopt Begg's funnel plot and Egger's plot to evaluate publication bias with results showing that bias coefficient was 0.068, 95% CI was 1.47 to 1.6, P=0.908. The 95% CI covers 0 point and P was more than 0.05, indicating publication bias was of no statistical significance. Certainly, it is avoidable that there was omission of literatures in other languages and some negative results being excluded into the analysis, as well as test results undergoing were not to be published yet. The above-mentioned could also become the cause of possible publication bias.

In aspect of technical bias, it is mainly about test methods. In the past, most will adopt RT-PCR method in semi-quantitative gene detection which however imposes higher requirements on sample source and could not verify whether RNA tissue sample ground and extracted is fully a tumor tissue. As the development and sophistication of FISH technology in recent years, PT-PCR application in gene amplification detection is gradually out of historical stage. FISH detection can be conducted by paraffin-embedded tissue and have high correctness in aneuploidy amplification detection of low resolution and high resolution chromosome, with good sensitivity and specificity and objective interpretation of results, making this method become the golden standard of gene amplification detection. Among six literatures included in this paper, out of five adopts FISH technology for detection (incl. Dual-Colour ISH) and one adopts RT-PCR detection. In order to eliminate effects of different detection method on statistical results, we conduct another analysis when the literature using RT-PCR is excluded. The results showed that the combined HR was 1.17 and 95% CI was 0.81 to 1.68, indicating *FGFR1* amplification is not related to NSCLC prognosis. The results were consistent with the above-mentioned ones.

In conclusion, this study conducts statistical analysis to relevant literatures through classic Meta-analysis method. Results show that *FGFR1* has no statistical significance to both NSCLC and SQCC prognosis. Due to small numbers of literatures included and existence of various biases, it is necessary for more research, more detailed data and more standard detection technologies to prove that in order to receive more convincing results.

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References

1. Siegel R, Ma J, Zou Z, et al. Cancer statistics, 2014. CA Cancer J Clin 2014;64:9-29.
2. Wistuba II, Gelovani JG, Jacoby JJ, et al. Methodological and practical challenges for personalized cancer therapies. Nat Rev Clin Oncol 2011;8:135-41.
3. Weiss J, Sos ML, Seidel D, et al. Frequent and focal FGFR1 amplification associates with therapeutically tractable FGFR1 dependency in squamous cell lung cancer. Sci Transl Med 2010;2:62ra93.
4. Kim HR, Kim DJ, Kang DR, et al. Fibroblast growth factor receptor 1 gene amplification is associated with poor survival and cigarette smoking dosage in patients with resected squamous cell lung cancer. J Clin Oncol. 2013;31:731-7.
5. Heist RS, Mino-Kenudson M, Sequist LV, et al. FGFR1 amplification in squamous cell carcinoma of the lung. J Thorac Oncol 2012;7:1775-80.
6. Sasaki H, Shitara M, Yokota K, et al. Increased FGFR1 copy number in lung squamous cell carcinomas. Mol Med Rep 2012;5:725-8.
7. Tran TN, Selinger CI, Kohonen-Corish MR, et al. Fibroblast growth factor receptor 1 (FGFR1) copy number is an independent prognostic factor in non-small cell lung cancer. Lung Cancer 2013;81:462-7.
8. Craddock KJ, Ludkovski O, Sykes J, et al. Prognostic value of fibroblast growth factor receptor 1 gene locus amplification in resected lung squamous cell carcinoma. J Thorac Oncol 2013;8:1371-7.
9. Parmar MK, Torri V, Stewart L. Extracting summary statistics to perform meta-analyses of the published literature for survival endpoints. Stat Med 1998;17:2815-34.
10. Steels E, Paesmans M, Berghmans T, et al. Role of p53 as a prognostic factor for survival in lung cancer: a systematic

review of the literature with a meta-analysis. Eur Respir J 2001;18:705-19.
11. Tierney JF, Stewart LA, Ghersi D, et al. Practical methods for incorporating summary time-to-event data into meta-analysis. Trials 2007;8:16.
12. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986;7:177-88.
13. Schultheis AM, Bos M, Schmitz K, et al. Fibroblast growth factor receptor 1 (FGFR1) amplification is a potential therapeutic target in small-cell lung cancer. Mod Pathol 2014;27:214-21.
14. Preusser M, Berghoff AS, Berger W, et al. High rate of FGFR1 amplifications in brain metastases of squamous and non-squamous lung cancer. Lung Cancer 2014;83:83-9.
15. Volm M, Koomägi R, Mattern J, et al. Prognostic value of basic fibroblast growth factor and its receptor (FGFR-1) in patients with non-small cell lung carcinomas. Eur J Cancer 1997;33:691-3.
16. Mano Y, Takahashi K, Ishikawa N, et al. Fibroblast growth factor receptor 1 oncogene partner as a novel prognostic biomarker and therapeutic target for lung cancer. Cancer Sci 2007;98:1902-13.
17. Wang LY, Edenson SP, Yu YL, et al. A natural kinase-deficient variant of fibroblast growth factor receptor 1. Biochemistry 1996;35:10134-42.
18. Ford MD, Cauchi J, Greferath U, et al. Expression of fibroblast growth factors and their receptors in rat glomeruli. Kidney Int 1997;51:1729-38.
19. Nguyen HB, Estacion M, Gargus JJ. Mutations causing achondroplasia and thanatophoric dysplasia alter bFGF-induced calcium signals in human diploid fibroblasts. Hum Mol Genet 1997;6:681-8.

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Original Article

Correlation between group behavior and quorum sensing in Pseudomonas aeruginosa isolated from patients with hospital-acquired pneumonia

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Background: This study investigated the correlation between the expression of the Las and Rhl quorum-sensing (QS) systems and the communal behavior (motility, biofilm formation, and pyocyanin production) of *Pseudomonas aeruginosa* (*P. aeruginosa*) isolated from patients with hospital-acquired pneumonia.

Methods: We analyzed 138 *P. aeruginosa* isolates from 48 patients (30 men and 18 women; age 68.18±15.08 years). *P. aeruginosa* clinical isolates were assessed for *Las* and *Rhl* gene expression and bacterial motility, biofilm formation, and pyocyanin production.

Results: *P. aeruginosa* swimming, twitching, and swarming motility positively correlated with the expression of *LasI*, *LasR*, and *RhlI* ($P<0.05$) but not with that of *RhlR* ($P>0.05$). At all analyzed time points, a significant positive correlation was found between biofilm formation and the expression of *LasI*, *LasR* ($P<0.01$), and *RhlI* ($P<0.05$ for day 1, $P<0.01$ for days 7 and 14), whereas *RhlR* expression positively correlated with biofilm formation only on day 14 ($P<0.05$). On days 1 and 7, positive correlation was observed between pyocyanin production and the levels of *LasI* and *RhlI* ($P<0.05$). In bacterial clearance cases, the expression of QS-related genes and the group behavior of the pathogen did not correlate ($P>0.05$). However, in cases of persistent *P. aeruginosa* infection, the changes in *LasI* and *LasR* gene expression were positively correlated with those in bacterial motility ($P<0.05$), and the changes in *LasI*, *LasR*, *RhlI*, and *RhlR* expression showed a significant positive association with those in biofilm formation ($P<0.01$).

Conclusions: In patients with hospital-acquired pneumonia, the expression of the *Las* and *Rhl* QS genes was associated with bacterial motility, biofilm formation, and pyocyanin production, suggesting an involvement of the QS genes in the clearance of pathogenic P. aeruginosa in patients.

Keywords: Hospital-acquired pneumonia; *Pseudomonas aeruginosa* (*P. aeruginosa*); group behavior; quorum sensing (QS)

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Introduction

Pseudomonas aeruginosa (*P. aeruginosa*) is one of the most common opportunistic pathogens and a cause of serious hospital-acquired infection (1). This pathogen is persistent and easily forms biofilms, colonizing the body and causing outbreaks of cross-infections and other clinical manifestations. It is now well established that a variety of *P. aeruginosa* phenotypic features, including motility, virulence, and the ability to form biofilms are regulated by quorum-sensing (QS) systems (2,3).

A number of gram-negative bacteria, including *P. aeruginosa*, use acylated homoserine lactone (HSL)-based QS that in *P. aeruginosa* includes adjustable Las and Rhl signal systems. The Las signaling system comprises the *LasR*

and *LasI* genes (4,5) encoding a transcriptional activator LasR and an enzyme LasI, which directs the synthesis of a signal molecule *N*-(3-oxododecanoyl)-$_L$-homoserine lactone (3-oxo-C_{12}-HSL) (6). The Rhl signaling system includes the *RhlI* and *RhlR* genes, which encode butyl-homoserine lactone (N-butyl homoserine lactones, C4-HSL) synthase and the RhlR protein (7). Functionally, 3-oxo-C12-HSL and C4-HSL act as signaling molecules up to a certain concentration and specifically bind to the LasR and RhlR proteins to activate a series of downstream genes. These signaling systems are responsible for the regulation of 11% of the *P. aeruginosa* genome (8-10). However, their role in the control of *P. aeruginosa* group behavior remains unclear.

In this study, we assessed the expression of QS-related genes in *P. aeruginosa* clinical isolates from patients with acute lower respiratory tract infections and analyzed the relationship between the QS signaling systems and *P. aeruginosa* group behavior. We also compared these parameters in clinically controlled and persistent *P. aeruginosa* isolates with the aim of providing a basis for novel therapeutic strategies in the treatment of hospital-acquired *P. aeruginosa* infection.

Materials and methods

Patients and P. aeruginosa clinical isolates

This prospective study (ethics code: 2013) included patients treated from March to November 2010 at the respiratory general ward, emergency intensive care unit (EICU), respiratory intensive care unit (RICU), surgical intensive care unit (SICU), and cardiac surgical intensive care unit (CSICU) of the Shanghai Jiaotong University Affiliated Ruijin Hospital and at the respiratory general ward of the Ruijin Hospital Luwan Branch. Subjects (30 men and 18 women) were recruited among the patients with freshly diagnosed hospital-acquired pneumonia. All participants provided written informed consent. The clinical diagnostic criteria included chest radiography 48 h after the admission prompted by emerging or progressive exudative lesions combined with any two of the following three clinical manifestations: temperature above 38 ℃, high blood leukocytosis, and purulent sputum. Patients with previously diagnosed *P. aeruginosa* infection were excluded from the study.

The analyzed clinical parameters included patients' age, sex, disease complications, antibiotic treatment, and bacterial clearance at days 1, 7, and 14 after the treatment for *P. aeruginosa* infection. Secretions from lower respiratory tract via the endotracheal tube or after morning expectoration were collected in a mouthwash container filled with sterile saline. The isolates were streaked on LB agar and stored in 20% glycerol/LB broth at –80 ℃.

The study endpoints included negative airway secretions, negative *P. aeruginosa* sputum culture within 14 days, or patient death.

Biofilm formation and quantification

P. aeruginosa isolates were grown overnight in LB medium at 37 ℃. The cultures was subsequently diluted with tryptone broth (TB) to OD_{600} of approximately 0.02, and 10 μL of the diluted culture was added to 96-well flat-bottom tissue culture plates containing 200 μL of LB diluted 1:50. Each strain was added to six wells (blank control wells contained medium only), and the plates were incubated as static cultures at 37 ℃ for 48 h. Biofilms were washed with normal saline, dried at room temperature, and stained with crystal violet (0.1% in water, 150 μL/well) for 20 min at room temperature. The stained biofilms were washed three times with 1 mL of normal saline, and the dye was solubilized with 150 μL 95% ethanol and measured by absorbance at 570 nm (OD_{570}) using a microplate reader (KHB ST-360, Shanghai, China). The biofilm formation rate (OD_{570})/(mg·mL) was calculated as the average OD_{570} value of three measured wells minus the average of three blank wells.

Swimming motility assay

The flagellum-mediated motility of *P. aeruginosa* was assessed using plates containing 0.3% LB agar as a motility medium. A 1-μL aliquot of overnight LB cultures was inoculated in the agar, and after 16-h incubation at 37 ℃, the diameter of the swim zone was measured. All assays were performed in triplicate (11).

Twitching motility assay

Plates containing 3-mm deep 1% LB agar were dried briefly, inoculated with *P. aeruginosa* isolates using a needle placed at the bottom of the plate, and incubated at 37 ℃ for 48 h (except when noted otherwise). After the incubation period, a zone between the agar and the plate bottom, referred to as the twitch zone, was measured. All assays were performed in triplicate (12).

Table 1 General clinical data

	Sex		Source of specimens		Infection	
	Male	Female	Intubation	Deep sputum	Clearance	No clearance
Number	30 (62.5%)	18 (37.5%)	32 (66.7%)	16 (33.3%)	6 (26.3%)	42 (73.7%)

Swarming motility assay

The isolates were tested for swimming motility on the plates containing 0.2% glucose, 0.05% monosodium glutamate, and 0.5% agar. A 1-μL aliquot of overnight LB cultures was placed on the agar surface, and the diameter of the swarm zone was measured after 48-h incubation at 37 ℃. All assays were performed in triplicate (13).

Pyocyanin production assay

P. aeruginosa isolates were grown overnight in LB medium at 37 ℃. The cultures were subsequently diluted with TB to OD_{600} of approximately 0.06, and 2.0 mL of the dilution was added to 24-well flat-bottom culture plates at 37 ℃ for 24 h. Cultures were extracted with 3 mL of chloroform and then reextracted into 1 mL of 0.2 N HCl to give a pink to deep red solution. The absorbance of this solution was measured at 520 nm. All assays were performed in triplicate (14).

RNA extraction and quantitative reverse transcription PCR (qRT-PCR)

Bacteria were grown in LB broth at 37 ℃ to the mid-exponential phase (OD_{600} 1.0-1.4) for 24 h. Total RNA was isolated using the RNeasy Mini Kit (SBS, Takara, Kyoto, Japan) according to the manufacturer's instructions. Differential gene expression was examined by real-time quantitative reverse transcription PCR (qRT-PCR) using the SYBR RT-PCR platform (Takara) according to the manufacturer's instructions. Primer pairs were designed using the Primer Express software package (Takara):

LasI, sense 5'-GCCCCTACATGCTGAAGAACA-3', antisense 5'-GTCCAGAGTTGATGGCGAAA-3';

LasR, sense 5'-ACGCTCAAGTGGAAAATTGGA-3', antisense 5'-GGGTAGTTGCCGACGATGAA-3';

RhlI, sense p, 5'-AGCTTCTCGATGAAGACCTGATG-3', antisense 5'-TGCTCTCTGAATCGCTGGAA-3';

RhlR, sense 5'-TCGCTCCAGACCACCATTTC-3', antisense 5'-CCACACGATTCCCTTCACC-3'.

Prior to comparative analysis, the relative efficiency of each primer pair was tested and compared to that of the primer pair for ribosomal *RplU* (sense, 5'-TCGTGTCGGATGTTGGGTTA-3'; antisense, 5'-GGTTTCGCTGCCCTTTGTATTGT-3') to ensure that the threshold cycle (C_t) data analysis could be used. The absolute value of the slope of the log input amount versus the ΔC_t was less than 0.1 for all comparisons, allowing us to use the $\Delta\Delta C_t$ calculation to compare gene expression in the experimental cultures to that of the controls (15). The experiments were repeated at least three times.

Grouping according to bacterial clearance

The patients were grouped according to bacterial clearance after antibiotic treatments. The clearance was determined as the absence of *P. aeruginosa* in two consecutive lower respiratory tract specimens during the 14-day observation period. No-clearance was scored if *P. aeruginosa* was cultured from all lower respiratory tract specimens after the 14-day observation period.

Statistical analysis

The data were expressed as the mean ± standard deviation or as percentage (for invariable parameters). Correlation analysis was performed using the Spearman method; comparison between two groups was performed using the Kolmogorov-Smirnov Z rank-sum test. P values of <0.05 were considered statistically significant.

Results

General clinical data

The study population included 48 hospitalized patients, 30 men (62.5%) and 18 women (37.5%), with an average age of 68.18±15.08 years. There were 32 cases of ventilator-associated pneumonia (66.7%). After the antibiotic treatment, six cases with *P. aeruginosa* clearance (26.3%) and 42 cases without clearance (73.7%) were detected (*Table 1*). All 48 patients demonstrated hospital-acquired pneumonia complications, including 20 cases of neurological disease (41.7%), 17 of cardiovascular disease (35.4%), eight

Table 2 Relationship between *Las* and *Rhl* gene expression and swimming motility

Time after treatment	Gene	Spearman r	P
Day 1	*LasI*	0. 442	<0.01
	LasR	0.440	<0.01
	RhlI	0.474	<0.01
	RhlR		>0.05
Day 7	*LasI*	0.365	<0.05
	LasR	0.366	<0.05
	RhlI	0.473	<0.01
	RhlR		>0.05
Day 14	*LasI*	0.405	<0.01
	LasR	0.462	<0.01
	RhlI	0.330	<0.05
	RhlR		>0.05
Clearance	*ΔLasI*		>0.05
	ΔLasI		>0.05
	ΔRhlI		>0.05
	ΔRhlR		>0.05
No clearance	*ΔLasI*	0.371	<0.05
	ΔLasR	0.383	<0.05
	ΔRhlI		>0.05
	ΔRhlR		>0.05

Table 3 Relationship between *Las* and *Rhl* gene expression and twitching motility

Time after treatment	Gene	Spearman r	P
Day 1	*LasI*	0.402	<0.01
	LasR	0.372	<0.01
	RhlI	0.408	<0.01
	RhlR		>0.05
Day 7	*LasI*	0.328	<0.05
	LasR	0.420	<0.05
	RhlI	0.401	<0.01
	RhlR		>0.05
Day 14	*LasI*	0.380	<0.05
	LasR	0.416	<0.01
	RhlI	0.345	<0.05
	RhlR		>0.05
Clearance	*ΔLasI*		>0.05
	ΔLasR		>0.05
	ΔRhlI		>0.05
	ΔRhlR		>0.05
No clearance	*ΔLasI*	0.380	<0.05
	ΔLasR	0.438	<0.05
	ΔRhlI	0.379	<0.05
	ΔRhlR		>0.05

of lung disease (16.7%), seven of malignant kidney disease (14.6%), seven of diabetes (14.6%), five of acute pancreatitis (10.4%), and four of blood diseases (8.3%). Antibiotic therapy to clear *P. aeruginosa* infection included carbapenems (40 cases, 83.3%), β-lactam/β-lactamase inhibitors (15 cases, 31.3%), cephalosporins (9 cases, 18.8%), and fluoroquinolones (5 cases, 10.4%).

Relationship between QS gene expression and swimming motility

In total, 138 *P. aeruginosa* isolates were analyzed. At all time points (days 1, 7, and 14), *P. aeruginosa* clinical isolates demonstrated a significant positive correlation between swimming behavior and the expression of *LasI*, *LasR* (P<0.01 for days 1 and 14, P<0.05 for day 7), and *RhlI* genes (P<0.01 for days 1 and 7, P<0.05 for day 14). However, no correlation was observed between swimming motility and *RhlR* expression (P>0.05) (*Table 2*).

In the patient group demonstrating bacterial clearance after antibiotic treatment, the differences in the relative expression of *LasI*, *LasR*, *RhlI*, and *RhlR* before and after therapy (Δ*LasI*, Δ*LasI*, Δ*RhlI*, and Δ*RhlR*) were not correlated with the changes in swimming behavior (P>0.05). However, in the no-clearance group, the changes in swimming motility positively correlated with Δ*LasI* (r=0.371, P<0.05) and Δ*LasR* (r=0.383, P<0.05), whereas no correlation was observed for Δ*RhlI* and Δ*RhlR* (P>0.05).

Relationship between QS gene expression and twitching motility

Similar to swimming behavior, twitching motility of *P. aeruginosa* clinical isolates at all time points showed a significant positive correlation with the relative expression of *LasI* (P<0.01 for day 1 and 14, P<0.05 for days 7 and 14), *LasR* (P<0.01 for days 1 and 14, P<0.05 for day 7), and *RhlI* (P<0.01 for days 1 and 7, P<0.05 for day 14), whereas no correlation was detected for *RhlR* expression (P>0.05) (*Table 3*).

In the patient group demonstrating bacterial clearance after antibiotic treatment, the changes in twitching motility did not correlate with Δ*LasI*, Δ*LasI*, Δ*RhlI*, or Δ*RhlR*

Table 4 Relationship between *Las* and *Rhl* gene expression and swarming motility

Time after treatment	Gene	Spearman r	P
Day 1	*LasI*	0.422	<0.01
	LasR	0.448	<0.01
	RhlI	0.457	<0.01
	RhlR		>0.05
Day 7	*LasI*	0.401	<0.05
	LasR	0.502	<0.01
	RhlI	0.473	<0.01
	RhlR		>0.05
Day 14	*LasI*	0.385	<0.05
	LasR	0.458	<0.01
	RhlI	0.339	<0.05
	RhlR		>0.05
Clearance	*ΔLasI*		>0.05
	ΔLasR		>0.05
	ΔRhlI		>0.05
	ΔRhlR		>0.05
No clearance	*ΔLasI*	0.440	<0.05
	ΔLasR	0.443	<0.05
	ΔRhlI		>0.05
	ΔRhlR		>0.05

Table 5 Relationship between *Las* and *Rhl* gene expression and biofilm formation

Time after treatment	Gene	Spearman r	P
Day 1	*LasI*	0.366	<0.01
	LasR	0.385	<0.01
	RhlI	0.282	<0.05
	RhlR		>0.05
Day 7	*LasI*	0.733	<0.01
	LasR	0.592	<0.01
	RhlI	0.758	<0.01
	RhlR		>0.05
Day 14	*LasI*	0.477	<0.01
	LasR	0.502	<0.01
	RhlI	0.412	<0.01
	RhlR	0.367	<0.05
Clearance	*ΔLasI*		>0.05
	ΔLasR		>0.05
	ΔRhlI		>0.05
	ΔRhlR		>0.05
No clearance	*ΔLasI*	0.679	<0.01
	ΔLasR	0.659	<0.01
	ΔRhlI	0.671	<0.01
	ΔRhlR	0.564	<0.01

(P>0.05), whereas in no-clearance group, they positively correlated with Δ*LasI* (r=0.380, P<0.05), Δ*LasR* (r=0.438, P<0.05), and Δ*RhlI* (r=0.379, P<0.05). However, no such correlation was observed between *P. aeruginosa* twitching and Δ*RhlR* (P>0.05).

Relationship between QS gene expression and swarming motility

Swarming motility of *P. aeruginosa* clinical isolates at all time points showed a significant positive correlation with the relative expression of *LasI* (P<0.01 for day 1, P<0.05 for days 7 and 14), *LasR* (P<0.01), and *RhlI* (P<0.01 for days 1 and 7, P<0.05 for day 14), but not with that of *RhlR* (P>0.05) (*Table 4*).

Similar to the trend detected in other motility assays, in the patient group demonstrating infection clearance after antibiotic treatment, the differences in swarming motility did not correlate with Δ*LasI*, Δ*LasI*, Δ*RhlI*, or Δ*RhlR* (P>0.05); however, in the no-clearance group, they showed a significant positive association with Δ*LasI* (r=0.440, P<0.05) and Δ*LasR* (r=0.443, P<0.05). No such link was detected for the Rhl genes: Δ*RhlI* and Δ*RhlR* showed no correlation with swarming motility (P>0.05).

Relationship between QS gene expression and biofilm formation

At all tested time points, a significant positive correlation was found between biofilm formation and the expression of *LasI*, *LasR* (P<0.01), and *RhlI* (P<0.05 for day 1, P<0.01 for days 7 and 14), whereas *RhlR* expression positively correlated with biofilm formation only on day 14 (P<0.05) (*Table 5*).

No correlation was observed between changes in biofilm formation and differential expression of QS genes in the patient group demonstrating bacterial clearance after antibiotic treatment (P>0.05). However, in patients with persistent *P. aeruginosa* infection (no-clearance group), a significant positive association was detected between altered biofilm formation and differential expression of *Las* and *Rhl* genes: Δ*LasI* (r=0.679, P<0.01), Δ*LasR* (r=0.659, P<0.01),

Table 6 Relationship between *Las* and *Rhl* gene expression and pyocyanin production

Time after treatment	Gene	Spearman r	P
Day 1	*LasI*	0.355	<0.05
	LasR		>0.05
	RhlI	0.379	<0.05
	RhlR		>0.05
Day 7	*LasI*	0.503	<0.05
	LasR		>0.05
	RhlI	0.556	<0.05
	RhlR		>0.05
Day 14	*LasI*		>0.05
	LasR		>0.05
	RhlI		>0.05
	RhlR		>0.05
Clearance	*ΔLasI*		>0.05
	ΔLasR		>0.05
	ΔRhlI		>0.05
	ΔRhlR		>0.05
No clearance	*ΔLasI*		>0.05
	ΔLasR		>0.05
	ΔRhlI		>0.05
	ΔRhlR		>0.05

Δ*RhlI* (r=0.671, P<0.01), and Δ*RhlR* (r=0.564, P<0.01).

Relationship between QS gene expression and pyocyanin production

P. aeruginosa clinical isolates from specimens collected on days 1 and 7 showed positive correlation between pyocyanin production and the expression of *LasI* and *RhlI* (P<0.05) but not with that of *LasR* and *RhlR* (P>0.05). For *P. aeruginosa* isolates from day 14, no correlation was observed between the pigment production and QS gene expression (P>0.05) (*Table 6*). For both clearance and no-clearance patient groups, the differences in the relative expression of the QS genes before and after antibiotic treatment did not correlate with changes in pyocyanin production (P>0.05).

Comparison between patients with cleared and persistent P. aeruginosa infection

P. aeruginosa swimming motility was significantly different between the clearance (0.210±0.075) and no-clearance (–0.140±0.070) patient groups (P<0.05, *Z*=1.379). In contrast, there was no statistical difference between these patients in regard to *P. aeruginosa* twitching motility (0.060±0.028 and -0.060±0.033, respectively) and swarming motility (0.090±0.030 and -0.080±0.043, respectively) (P>0.05). However, a significant difference was observed between the clearance and no-clearance patients in *P. aeruginosa* biofilm formation (0.146±0.035 and 0.137±0.047, respectively) (P<0.01, *Z*=2.385) and pyocyanin production (0.064±0.044 and –0.066±0.028, respectively) (P<0.01, *Z*=1.938).

Discussion

In *P. aeruginosa*, quorum sensing systems play key roles in colonization and pathogenesis. An important phenotype regulated by QS is the communal movement of bacterial population. In this study of *P. aeruginosa* isolated from patients with hospital-acquired infection, we observed a strong positive correlation between the expression of QS genes *LasI*, *LasR*, and *RhlI* and all types of bacterial motility (swimming, twitching, and swarming), although no such correlation was detected for *RhlR* expression. These findings are consistent with previous data showing that *P. aeruginosa* QS *Rhl* genes regulated swimming, twitching, and swarming (16). Using genetic analysis, Caiazza *et al*. (17) showed that the regulation of *P. aeruginosa* swimming, twitching, and swarming motility depends on rhamnolipid biosynthesis controlled by the QS Rhl system (18). In *P. aeruginosa*, QS regulates the expression of several loci, including flagella and pili required for bacterial swarming movement, a phenotype important for community formation and host colonization.

In our study, a significant positive correlation was observed between *P. aeruginosa* biofilm formation and the expression of the Las signaling genes. However, a mixed pattern was detected for the Rhl system: *RhlI* was consistently associated with biofilm production, whereas for *RhlR*, such association was observed only on day 14. These data suggest that in *P. aeruginosa* virulent isolates, QS, especially the Las system, is critical for biofilm formation and thus for the bacterial parasitism of human hosts. Our data are in agreement with previous findings. O'Toole and Kolter (19) found that *LasI* mutation in *P. aeruginosa* resulted in a formation of defective flat, uniform undifferentiated biofilms lacking mature three-dimensional structure. Another study showed that in a *P. aeruginosa RhlI* (C4-HSL-deficient) mutant, the biofilm

volume was reduced by 70%; the phenotype was rescued by the addition of exogenous C4-HSL, suggesting that the *RhlI* gene plays an important role in the formation of biofilms by *P. aeruginosa* (20,21). Xie *et al*. (22) observed that LasR and RhlR proteins induced biofilm formation by *P. aeruginosa*, indicating a direct involvement of the *LasR* and *RhlR* QC genes in the pathogen colonization of the host.

The secretion of *P. aeruginosa* virulence factor pyocyanin is under the control of the QS systems. In this study, we found that *LasI* and *RhlI* expression positively correlated with pyocyanin production on days 1 and 7, although no association was found on day 14. Another study suggested that *LasR* and *RhlR* mutations could be related to the spread of a drug-resistant strain of *P. aeruginosa* (23), although this mechanism requires additional investigation.

In the *P. aeruginosa* clearance patient group, no correlation was observed between pathogen communal behavior (motility, biofilm formation, and pyocyanin production) and the difference in the expression of the Las and Rhl signaling systems before and after antibiotic therapy. However, in the patients with persistent *P. aeruginosa* infection (no-clearance group), the differential expression of *LasI*, *LasR*, and *RhlI* was found to positively correlate with the changes in bacterial movement and biofilm formation, although no such link was detected for *RhlR*. Given that *P. aeruginosa* isolated from no-clearance patients exhibited increased biofilm formation, motility, and pyocyanin secretion compared to those isolated from clearance group, these results indicate that in the persistent clinical isolates, the Las and Rhl QS systems are directly associated with the pathogen communal behavior, suggesting QS involvement in *P. aeruginosa* drug resistance.

We believe that the investigation of the Las and Rhl signaling systems as potential targets in the control and treatment of *P. aeruginosa* infection represents a new research direction. In the present study, we collected samples from patients with hospital-acquired pneumonia at different time points and found that *P. aeruginosa* isolates reflected the patients' conditions in terms of clinical development during treatment. Thus, we observed that the *Las* and *Rhl* genes of the QS systems were closely related to biofilm formation and other important parameters of *P. aeruginosa* group behavior, although previous data in this respect are controversial. Given that the current standard strains have been selected based on previous research, the results of this study may have a significant clinical impact.

However, this study also had several limitations. First, because of a small sample size, the results may not be representative of a larger population. In future investigations, we plan to expand the number of patients in order to increase the statistical power of association between the QS-related genes and *P. aeruginosa* communal behavior. In addition, mechanistic links between the QS genes should be addressed to better clarify their functions *in vivo*. Second, although we found the difference in QS gene expression between bacterial isolates from samples obtained before and after antibiotic treatment, we did not compare antibiotic type and dose and thus did not analyze specific factors influencing the regulation of QS genes. *P. aeruginosa* group behavior (biofilm formation, motility, and virulence factor secretion) is associated with clinically important chronic refractory infections. Therefore, the validation of the QS systems as potential drug targets for the control and treatment of *P. aeruginosa* infection requires further investigation.

Conclusions

In conclusion, our results indicate that the expression of QS genes, especially of the Las signaling system, in clinical isolates of *P. aeruginosa* is strongly associated with the pathogen communal behavior (motility, biofilm formation, and pyocyanin production) and resistance to antibiotic treatment, indicating the involvement of QS signaling in the clearance of *P. aeruginosa* infection.

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Authors' contribution: Jia-Lin Liu designed the study and performed the experiments. Yong Li performed the experiments, analyzed the data, and wrote the manuscript. Hong-Ping Qu and Huan-Ying Wan reviewed the data and participated in quality control. All authors read and approved the final version of the manuscript.

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References

1. American Thoracic Society; Infectious Diseases Society of America. Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. Am J Respir Crit Care Med 2005;171:388-416.

2. Fuqua C, Parsek MR, Greenberg EP. Regulation of gene expression by cell-to-cell communication: acyl-homoserine lactone quorum sensing. Annu Rev Genet 2001;35:439-68.
3. González JE, Keshavan ND. Messing with bacterial quorum sensing. Microbiol Mol Biol Rev 2006;70:859-75.
4. Williams P, Winzer K, Chan WC, et al. Look who's talking: communication and quorum sensing in the bacterial world. Philos Trans R Soc Lond B Biol Sci 2007;362:1119-34.
5. von Bodman SB, Willey JM, Diggle SP. Cell-cell communication in bacteria: united we stand. J Bacteriol 2008;190:4377-91.
6. Pearson JP, Gray KM, Passador L, et al. Structure of the autoinducer required for expression of Pseudomonas aeruginosa virulence genes. Proc Natl Acad Sci U S A 1994;91:197-201.
7. Latifi A, Winson MK, Foglino M, et al. Multiple homologues of LuxR and LuxI control expression of virulence determinants and secondary metabolites through quorum sensing in Pseudomonas aeruginosa PAO1. Mol Microbiol 1995;17:333-43.
8. Whiteley M, Lee KM, Greenberg EP. Identification of genes controlled by quorum sensing in Pseudomonas aeruginosa. Proc Natl Acad Sci U S A 1999;96:13904-9.
9. Schuster M, Lostroh CP, Ogi T, et al. Identification, timing, and signal specificity of Pseudomonas aeruginosa quorum-controlled genes: a transcriptome analysis. J Bacteriol 2003;185:2066-79.
10. Wagner VE, Bushnell D, Passador L, et al. Microarray analysis of Pseudomonas aeruginosa quorum-sensing regulons: effects of growth phase and environment. J Bacteriol 2003;185:2080-95.
11. Schaber JA, Carty NL, McDonald NA, et al. Analysis of quorum sensing-deficient clinical isolates of Pseudomonas aeruginosa. J Med Microbiol 2004;53:841-53.
12. Darzins A. The pilG gene product, required for Pseudomonas aeruginosa pilus production and twitching motility, is homologous to the enteric, single-domain response regulator CheY. J Bacteriol 1993;175:5934-44.
13. Glessner A, Smith RS, Iglewski BH, et al. Roles of Pseudomonas aeruginosa las and rhl quorum-sensing systems in control of twitching motility. J Bacteriol 1999;181:1623-9.
14. Essar DW, Eberly L, Hadero A, et al. Identification and characterization of genes for a second anthranilate synthase in Pseudomonas aeruginosa: interchangeability of the two anthranilate synthases and evolutionary implications. J Bacteriol 1990;172:884-900.
15. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001;25:402-8.
16. O'Toole GA, Kolter R. Flagellar and twitching motility are necessary for Pseudomonas aeruginosa biofilm development. Mol Microbiol 1998;30:295-304.
17. Caiazza NC, Shanks RM, O'Toole GA. Rhamnolipids modulate swarming motility patterns of Pseudomonas aeruginosa. J Bacteriol 2005;187:7351-61.
18. Pamp SJ, Tolker-Nielsen T. Multiple roles of biosurfactants in structural biofilm development by Pseudomonas aeruginosa. J Bacteriol 2007;189:2531-9.
19. O'Toole GA, Kolter R. Initiation of biofilm formation in Pseudomonas fluorescens WCS365 proceeds via multiple, convergent signalling pathways: a genetic analysis. Mol Microbiol 1998;28:449-61.
20. Christensen LD, Moser C, Jensen PØ, et al. Impact of Pseudomonas aeruginosa quorum sensing on biofilm persistence in an in vivo intraperitoneal foreign-body infection model. Microbiology 2007;153:2312-20.
21. Kirisits MJ, Parsek MR. Does Pseudomonas aeruginosa use intercellular signalling to build biofilm communities? Cell Microbiol 2006;8:1841-9.
22. Xie Y, Zeng W, Jia WX, et al. Functional effects of LasR/RhlR on Pseudomonas aeruginosa biofilm development and lung infections in mice. Prog Biochem Biophys 2006;33:31-8.
23. Fothergill JL, Panagea S, Hart CA, et al. Widespread pyocyanin over-production among isolates of a cystic fibrosis epidemic strain. BMC Microbiol 2007;7:45.

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Original Article

Remodeling of rat pulmonary artery induced by chronic smoking exposure

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Objective: To evaluate the dominant role in rat pulmonary artery (PA) remodeling induced by chronic smoking exposure (CSE).

Methods: Thirty-five male Sprague-Dawley (SD) rats were exposed to 36 cigarettes per day, 6 days per week, for 1, 3, or 5 months. Another 35 SD rats were sham-exposed during the same period. Hemodynamic measurement, evaluation of the right ventricular hypertrophy index (RVHI) plus right ventricle-to-weight ratio, and hematoxylin eosin staining was performed. Wall thickness, artery radius, luminal area, and total area were measured morphometrically. Western blotting assessed expression of PPAR-γ BMP4, BMPR2, and TRPC1/4/6 in the artery and lung. Store-operated calcium entry (SOCE) and $[Ca^{2+}]i$ were measured using Fura-2 as dye.

Results: Mean right ventricular pressure increased after 3 months of smoking exposure and continued to increase through 5 months. Right ventricular systolic pressure (RVSP) increased after 3 months of exposure and then stabilized. RVHI increased after 5 months; right ventricle-to-weight ratio was elevated after 3 months and further increased after 5 months. Wall thickness-to-radius ratio does-dependently increased after 3 months through 5 months, in parallel with the decreased luminal area/total area ratio after 5 months. Other changes included the development of inflammatory responses, enlargement of the alveolar spaces, and reductions in the endothelial lining of PAs, proliferative smooth muscle cells, fibroblasts, and adventitia. Moreover, BMP4 and TRPC1/4/6 expression increased to varying degrees in the arteries and lungs of smoking-exposed animals, whereas BMPR expression and SOCE increased only in the arteries, and PPAR-γ was downregulated in both the arteries and lungs.

Conclusions: In SD rats, smoking exposure induces pulmonary vascular remodeling. The consequences of increased SOCE include increase in TRPC1/4/6, probably via augmented BMP4 expression, which also contribute to inflammatory responses in the lung. Moreover, interactions between BMP4 and PPAR-γ may play a role in preventing inflammation under normal physiological conditions.

Keywords: Pulmonary artery hypertension (PAH); smoking; remodeling

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Introduction

Pulmonary artery hypertension (PAH) is a disease affecting the precapillary pulmonary arterial bed, and it results from abnormal interactions between endothelial and smooth muscle cells, leading to a progressive narrowing of the pulmonary arteries (PAs) and their branches (1-3). PAH is a severe complication of smoking-induced chronic obstructive pulmonary disease (COPD) (4), in which pulmonary arterial remodeling and vasoconstriction play crucial roles, but the underlying pathogenic mechanisms are not fully understood.

BMP4 is a recently discovered vascular pro-inflammatory biomarker; its levels are enhanced in endothelial cells by disturbed blood flow, and it activates inflammation along with the generation of reactive oxygen species (ROS) in an endothelium-dependent manner (5), leading to endothelial cell malfunction (6,7). BMP4 was showed to induce and stimulate NADPH oxidases in endothelial cells *in vitro*, and therefore to produce superoxide, which in turn causes inflammatory responses (6). Although BMPs implement various functions, their signaling is transduced by binding with two types of serine/threonine kinase receptors, BMPRI and BMPRII (8-11).

According to Takano *et al*., modulation of the interactive BMP system and TNF-α receptor signaling essential for bone metabolism is related to the functional activities of PPAR (12). Moreover, BMP4 was previously found to augment the expression of TRPC1, TRPC4, and TRPC6 in cultured rat pulmonary arterial smooth muscle cells (PASMCs) (13).

In addition, TRPC1 and TRPC4 are likely to constitute a store-operated calcium channel (SOCC) (14-17), participating primarily in store-operated calcium entry (SOCE), while TRPC6 is a receptor-operated calcium channel (ROCC) involved in the regulation of vascular contractility (18-21). TRPC4 may contribute to the regulation of SOCE-mediated and agonist-stimulated cell proliferation and contraction of PASMCs (21-23). According to Liu *et al*., enhanced expression of TRPC1/4/6 and SOCE was observed in monocrotaline (MCT)-induced PAH (24), implying that TRPC-dependent SOCE plays a crucial role in the pathogenesis of PAH.

We, therefore, further investigated the pathogenesis of PAH by using a smoking-exposed rat model, and performed western blotting and calcium imaging to test the hypothesis that enhanced SOCE in smoking-treated arteries is involved in PPAR-γ signaling, which also plays a part in stimulating inflammatory responses that are followed by vascular remodeling.

Methods

Animals

Male SD rats (180-200 g) from the Guangdong Medical Laboratory Animal Centre were maintained in a specefic pathogen free (SPF) room providing a 12/12 light/dark cycle. The rats were acclimatized for one week before smoking exposure (25).

Smoking-exposed rat model

The smoke-exposure group consisted of 35 SD rats that were exposed to 36 commercially available non-filtered cigarettes every 12 hours (10:00-12:00 in the morning and 16:00-18:00 in the afternoon), 6 days per week, for 5 months. A further 35 SD rats in the control group were exposed to air (26,27). Rats were anesthetized with 3% pentobarbital sodium (45 mg/kg, intraperitoneal) after 1, 3, or 5 months of smoking exposure. Right ventricular systolic pressure (RVSP), right ventricular diastolic pressure (RVDP), and heart rate was measured by catheterizing the right ventricle directly with polyethylene catheters connected to pressure transducers (MP150; BIOPAC Systems. Inc., USA). After the hemodynamic measurements, the heart and lungs were removed and the ratio of the wet weight of the right ventricle to that of the left ventricular wall plus septum [RV/(LV + S)] was calculated. This is the right ventricular hypertrophy index (RVHI). The PAs were dissected as described below. All procedures were carried out according to the guidelines of the Animal Care and Use Committee of Guangzhou Medical University (24).

Isolation and culture of PASMCs

We dissected the distal (>4th generation) intrapulmonary arteries and removed the endothelium with a cotton swab. Myocytes obtained by enzymatic digestion were cultured for 4-5 days in smooth muscle growth medium (GIBCO DMEM 31600) with 10% serum in a damp atmosphere of 5% CO_2:95% air at 37 ℃. Twenty-four hours before an experiment, we exchanged the medium for some containing 0.5% serum to stop cell growth. Purity was assessed as >95% (18) by observing cell morphology under a phase-contrast microscope after immune-fluorescence staining of α-actin.

Histological staining & morphological analysis

We dissected the heart and lungs from exposed and control

rats as described above, with distal lung samples isolated for observation of remodeling in the PA and bronchus. Rats anesthetized with 3% pentobarbital sodium (45 mg/kg, intraperitoneal) were restrained in the supine position. The distal part of the left lung was removed and fixed for 24 hours in 4% paraformaldehyde, followed by embedding in paraffin wax. Six 5-μm-thick sections from each lung were stained with hematoxylin and eosin and observed and photographed using a Leica DM4000 B microscope with 20× and 40× objectives. The lumen and total area, as well as the wall thickness and arterial radii of the 51- to 150-μm (outer diameter) PAs were measured using Image Pro Plus 6.0 software (24,28).

Western blotting

PASMCs and lung specimens were lysed in radio immunoprecipitation assay lysis buffer containing 1% phenylmethanesulfonyl fluoride as a protease inhibitor, 1× PBS, 1% NP40, 0.1% SDS, 5 mM EDTA, 0.5% sodium deoxycholate, and 1 mM sodium orthovanadate. They were homogenized manually (PASMCs) or with an electric homogenizer (lung tissue). The overall protein concentration of the homogenates was quantified using bicinchoninic acid protein reagents (Bio-Rad) and bovine serum albumin standards. Protein homogenates were resolved by 10% SDS-PAGE calibrated with precision plus prestained protein molecular weight markers (Bio-Rad). Separated proteins were transferred to polyvinylidene difluoride membranes (pore size, 0.45 μm; Bio-Rad), blocked with 5% non-fat milk powder dissolved in tris-buffered saline (TBS) containing 0.2% Tween 20, and blotted with specific antibodies. Western blots were performed using rabbit anti-PPAR-ɣ (SANTA CRUZ), mouse anti-BMP4 (Millipore), mouse anti-BMPR2 (BD Transduction Laboratories), rabbit anti-TRPC1 (Alomone Labs), rabbit anti-TRPC4 (SANTA CRUZ), rabbit anti-TRPC6 (Alomone Labs), mouse anti-α-actin (SANTA CRUZ), goat anti-rabbit and goat anti-mouse IgG (KPL). The membranes were then washed five times for 10 min each and incubated with horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse IgG for 70 min. Bound antibodies were detected using an Immun-Star™ WesternC™ Chemiluminescence Kit (Bio-Rad) (29).

Measurement of intracellular Ca^{2+}

SOCE and $[Ca^{2+}]_i$ were measured by dyeing with Fura-2 (Molecular Probes, Eugene, OR, USA), as previously described (18,30). The coverslips were fixed in a polycarbonate chamber clamped to a heated aluminum platform (RC-26G; Warner Instruments, Hamden, CT, USA) on the stage of a Leica DMI4000B inverted microscope. A dual channel heater controller (TC-344B; Warner Instruments) was connected to the heat exchanger to maintain its temperature at 37 ℃. Ratiometric measurement at 340 and 380 nm was performed on the Fura-2 fluorescence of single PASMCs visualized with a 20× fluorescence objective (UApo N340; Leica).

Statistical analysis

Data are shown as means ± SEM. Statistical comparisons were performed using Student's *t*-test. Differences were considered significant when $P<0.05$ (31).

Results

There were profound symptoms of PAH in smoking-exposed rats examined at the end of the 1st, 3rd, or 5th month of smoking exposure. The control animals weighed 203±3.7 g (n=21) at the start and 596±21 g (n=11) at the end of the experiment, whereas the smoke-exposed animals weighed 199±4.2 g (n=21; values not significantly different from the control group) at the start, and 472±19 g (n=10; values significantly different from the controls) at the end. RVSP significantly increased at the end of the 3rd month [control, 18.35±0.7 mmHg, n=4; chronic smoking exposure (CSE), 22.49±1.4 mmHg, n=3, $P<0.05$; *Figure 1*]. The mean RVP, calculated from the formula 1/3(RVSP - RVDP) + RVDP, also increased after three months (control, 9.06±0.63 mmHg, n=4; CSE, 12.45±0.93 mmHg, n=3, $P<0.05$), and after five months (control, 7.5±0.4 mmHg, n=5; CSE, 11.51±1.5 mmHg, n=5, $P<0.05$; *Figure 1C-G*). The RVHI RV/(LV + S) was elevated at the end of the 5th month (control, 30.54±2.32%, n=6; CSE, 43.32%±3%, n=6, $P<0.05$; *Figure 1I*), as was the RV/weight ratio (g/kg) at the end of the 3rd month (control, 0.49±0.05, n=4; CSE, 0.63±0.02, n=4, $P<0.05$) and the 5th month (control, 0.46±0.02, n=6; CSE, 0.66±0.02, n=6, $P<0.001$; *Figure 1J*).

In the histological examinations of distal lung sections of CSE rats, we observed the medial walls of the muscular small PAs (vessel outer diameters of 50-150 μm) were significantly thickened. Morphological analysis of these vessels showed the luminal area to total area ratio had significantly diminished at the end of the 5th month (control, 0.45±0.1, n=3; CSE, 0.16±0.05, n=6, $P<0.05$; *Figure 1O-Q*), and the wall thickness to artery radius ratio was notably enhanced in CSE-treated

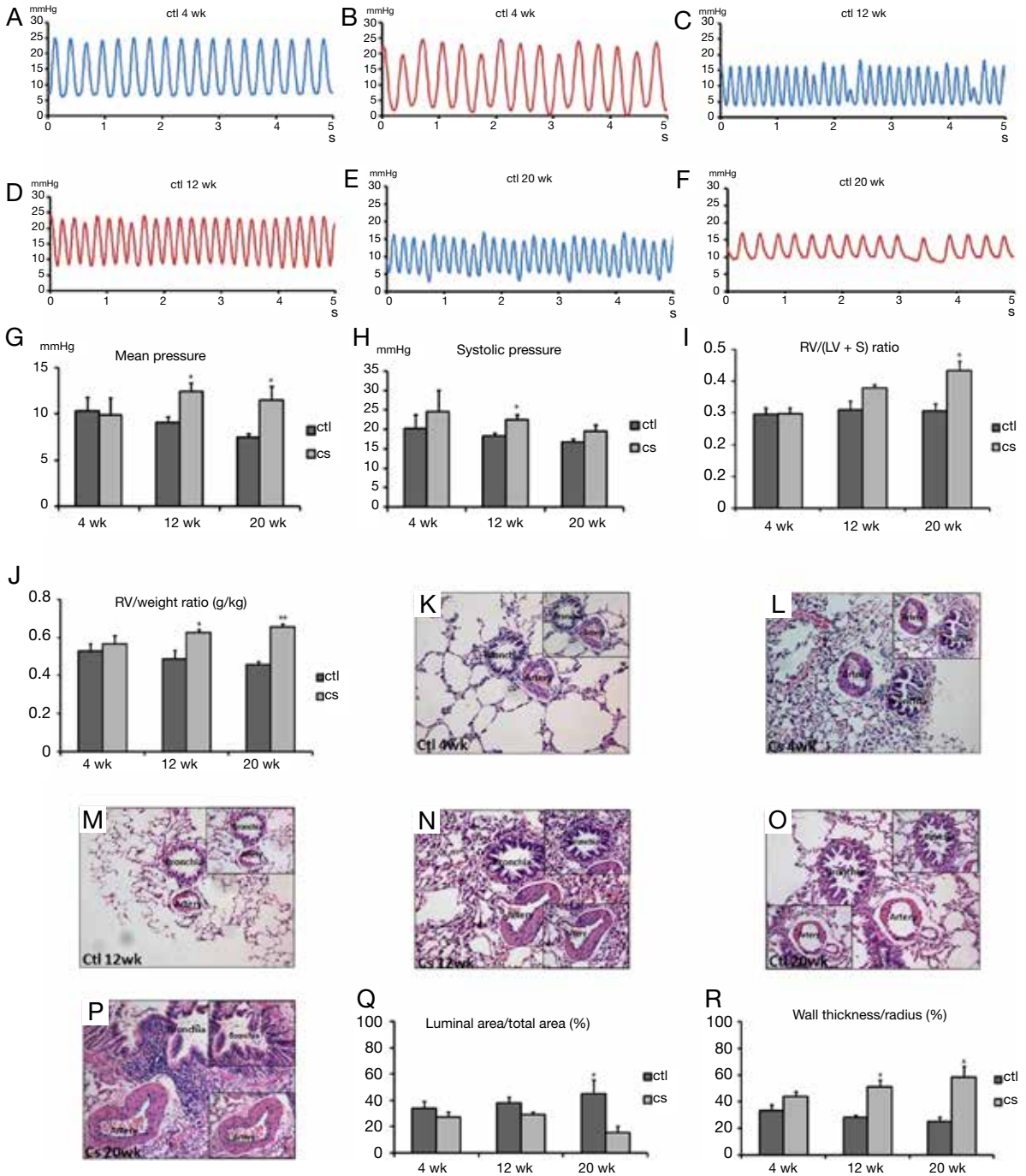

Figure 1 Verification of PAH in smoking-exposed rats. (A-F) Waveforms of representative right ventricular pressures in rats exposed to air or 4/12/20 weeks of cigarette smoke; (G,H) statistical analyses of right ventricular pressure [4-week control, n=3, 4-week chronic smoking exposure (CSE), n=5; 12-week control, n=4, 12-week CSE, n=3; 20-week control, n=5, 20-week CSE, n=5]; (I,J) RVHI, calculated as RV/(LV + S) and RV/weight (4-week control and CSE, n=4; 12-week control and CSE, n=4; 20-week control and CSE, n=6); (K-P) representative hematoxylin and eosin staining of lung slices from control and CSE rats, showing small PAs and bronchia (main photomicrographs, magnification 200×, scale bars 100 μm; small photomicrographs, magnification 400×, scale bars 50 μm); (Q,R) the ratio of luminal area to total area (%) and the ratio of wall thickness to artery radius (%) in control and CSE PAs of 51-150 μm outer diameter (4-week control and CSE, n=4; 12-week control, n=3, 12-week CSE, n=4; 20-week control, n=3, 20-week CSE, n=6). *, P value <0.05; **, P value <0.001.

rats at the end of the 3rd month (control, 0.28±0.02, n=3; CSE, 0.51±0.05, n=4, P<0.05) and the 5th month (control, 0.25±0.04, n=3; CSE, 0.58±0.08, n=6, P<0.05); these two parameters altered in a dose-dependent way (*Figure 1M-P,R*). In addition, there were the infiltration of inflammatory factors, the enlargement of the alveolar spaces, the low level of endothelial lining in the severely dilated PAs, as well as the scarcity of proliferative smooth muscle cells, fibroblasts, and adventitia (*Figure 1K-P*) (1), all of which indicate that vascular remodeling and neo-muscularization was taking place in the distal intrapulmonary arteries of rats experiencing CSE.

To further explore the molecular mechanisms underlying pulmonary vascular remodeling, SOCE and basal [Ca^{2+}] were examined at the end of five months of smoking exposure. We found that SOCE increased from 0.069±0.007 (control, n=5 in 57 cells) to 0.107±0.012 (CSE, n=5 in 87 cells; *Figure 2A,B*). The augmented SOCE in the PASMCs of rats undergoing CSE indicates that TRPC protein expression is elevated during CSE-induced PAH, as described below.

PPAR-γ protein levels (related to α-actin in endothelium-denuded PAs) declined significantly after 3 and 5 months of smoking exposure compared with the control (3 months: control, n=4; CSE, n=5, P<0.001. 5 months: control, n=4; CSE, n=4, P<0.05; *Figure 3*), with the corresponding levels in whole lung declining after exposures of 3 months (control, n=4; CSE, n=4, P<0.05) and 5 months (control, n=4; CSE, n=5, P<0.05; *Figure 3F-H*). Mature and precursor BMP4 protein levels related to α-actin in endothelium-denuded PAs were upregulated significantly after smoking exposures of 3 and 5 months compared with the control (3 months: control, n=4; CSE, n= 5; both precursor and mature BMP4, P<0.05; 5 months: control, n=4; CSE, n=4; precursor BMP4, P<0.05, mature BMP4, P<0.001; *Figure 4*). The same trends were seen in whole lung (3 months: control, n=4; CSE, n=4; precursor BMP4, P<0.001, mature BMP4, P<0.05; 5 months: control, n=4; CSE, n=5; both precursor and mature BMP4, P<0.001; *Figure 4G-J*). In addition, the expression of BMPRII was markedly enhanced in the PAs of rats subjected to CSE for 5 months (control, n=4; CSE, n=4, P<0.05; *Figure 5*), while there was no significant alteration of BMPRII expression in whole lung (*Figure 5E-H*). In summary, CSE had opposite effects on BMP4 and PPAR-γ, with the former being elevated and the latter lowered. The augmented expression of BMP4 might contribute to the upregulation of BMPRII.

Smoking exposures of 3 and 5 months markedly enhanced TRPC1 expression in the PA (3 months: control n=4; CSE n=5, P<0.05. 5 months: control n=4; CSE n=5, P<0.05; *Figure 6*); smoking exposure of 1, 3 and 5 months significantly increased TRPC1 expression in whole lung (1 month: control n=4; CSE n=4, P<0.05. 3 months: control n=4; CSE n=4, P<0.05. 5 months: control n=4; CSE n=5, P<0.05; *Figure 6E-H*). TRPC4 protein levels in the PA were upregulated after smoking exposure of 1 month (control, n=4; CSE, n=4, P<0.05), 3 months (control, n=4; CSE, n=5, P<0.05) and 5 months (control, n=4; CSE, n=4, P<0.05; *Figure 7*), although TRPC4 expression in whole lung was significantly enhanced only after smoking exposure of 5 months (control, n=4; CSE, n=4, P<0.001; *Figure 7G-H*). TRPC6 expression in the PA increased considerably after smoking exposure of 3 and 5 months (3 months: control, n=4; CSE, n=5, P<0.05. 5 months: control, n=4; CSE, n=5, P<0.001; *Figure 8*), and the corresponding levels in whole lung were enhanced after smoking exposure of 1 month (control, n=4: CSE, n=4, P<0.05), 3 months (control, n=4; CSE, n=5, P<0.05), and 5 months (control, n=4; CSE, n=5, P<0.001; *Figure 8E-H*).

Discussion

PAH is an important complication of COPD and an independent risk factor that affects the course of COPD. Studies on smoking patients with mild COPD have demonstrated that 25% have slow-progressive increases in pulmonary arterial pressure (32,33). Smoking is one of the main causes of COPD and PAH, but the specific mechanism by which CSE causes chronic PAH is still unclear.

This research presents evidence suggesting that PPAR-γ and BMP4 function as upstream [Ca^{2+}]i regulators in the PAs of rats exposed to cigarette smoke. First, PPAR-γ expression was found to be inhibited in the PAs and whole lungs of CSE rats, suggesting that during cigarette-smoke-induced PAH, this anti-inflammatory biomarker became dysfunctional, and the defect is at the level of PPAR-γ gene expression. Second, we found that (1) cigarette smoke exposure upregulated BMP4 and TRPC expression not only in PAs but also in the whole lung and (2) cigarette smoke induced inflammatory responses in whole lung as consequences of vascular remodeling. According to Floyd *et al*. (30), there was no doubt that persistent vasoconstriction was induced by increased [Ca^{2+}]i. Moreover, Lu *et al*. (13) demonstrated that calcium signaling in PASMCs is regulated by BMP4, probably via upregulated TRPC expression; the latter increases SOCE and basal [Ca^{2+}]i in PASMCs, and thus promotes pulmonary

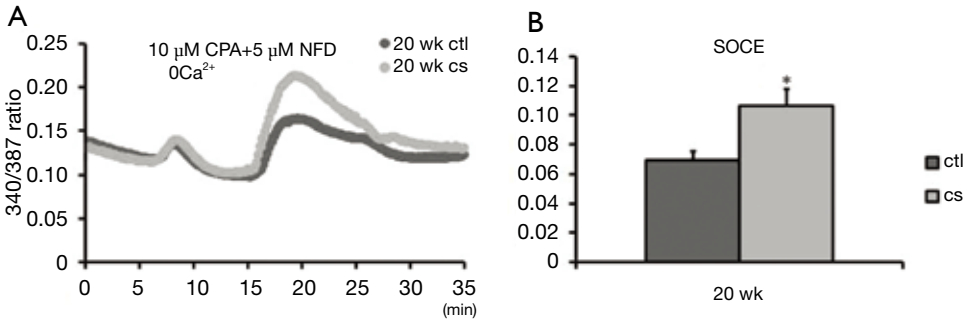

Figure 2 Alterations in $[Ca^{2+}]i$ expression in distal PASMCs of rats exposed to air or cigarette smoke for 20 weeks. (A) Time courses of $[Ca^{2+}]i$ measured by 340/387 ratio before and after restoration of extracellular Ca^{2+} in distal PASMCs perfused with Ca^{2+}-free Krebs-Ringer bicarbonate (KRB) solution containing 10 μM cyclopiazonic acid (CPA), 0.5 mM EGTA, and 5 μM nifedipine (NFD; control, n=5 experiments in 57 cells; CSE, n=5 experiments in 87 cells); (B) statistical analysis of SOCE in (A). *, P value <0.05.

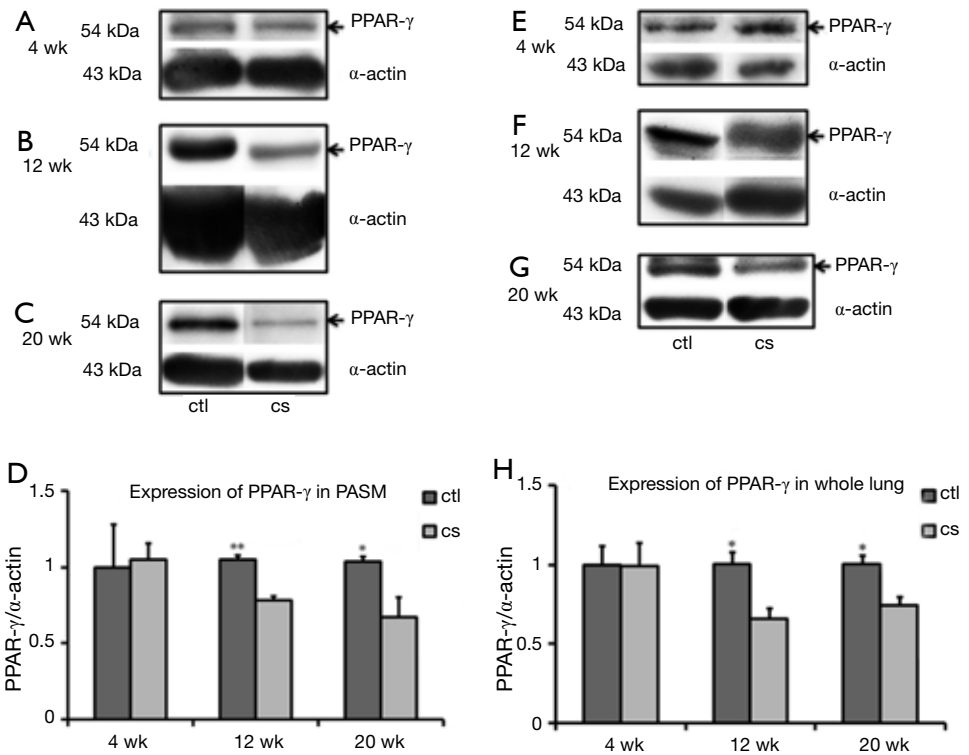

Figure 3 Alterations in PPAR-γ expression in PAs and whole lungs from rats exposed to air or 4/12/20 weeks of cigarette smoke. (A-C) Representative western blots of PPAR-γ proteins in PAs of control and CSE rats; (D) quantitative analysis of PPAR-γ proteins in PAs (4-week control, n=4, 4-week CSE, n=5; 12-week control, n=4, 12-week CSE, n=5; 20-week control and CSE, n=4); (E-G) representative western blots of PPAR-γ proteins in whole lungs from control and CSE rats; (H) quantitative analysis of PPAR-γ proteins in whole lungs (4-week control, n=4, 4-week CSE, n=5; 12-week control, n=4, 12-week CSE, n=4; 20-week control, n=4, 20-week CSE, n=5). Equal protein loading was confirmed using α-actin. PA indicates pulmonary artery. *, P value <0.05; **, P value <0.001.

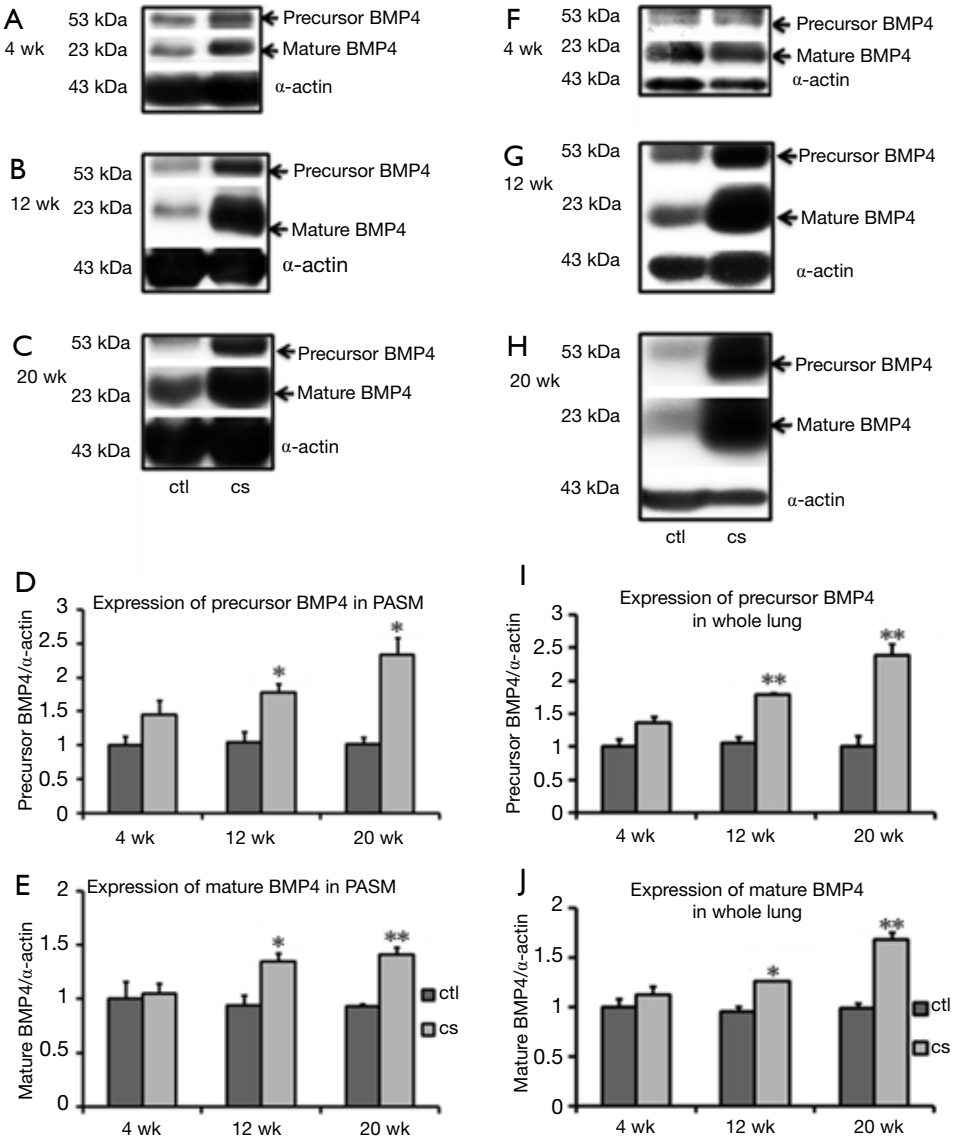

Figure 4 Alterations in BMP4 expression in PAs and whole lungs from rats exposed to air or 4/12/20 weeks cigarette smoke. (A-C) Representative western blots of BMP4 proteins in the PAs of control and CSE rats; (D,E) quantitative analyses of precursor and mature BMP4 proteins in PAs (4-week control, n=4, 4-week CSE, n=5; 12-week control, n=4, 12-week CSE, n=5; 20-week control and CSE, n=4); (F-H) representative western blots of BMP4 proteins in whole lungs of control and CSE rats; (I,J) quantitative analyses of precursor and mature BMP4 proteins in whole lungs (4-week control, n=3, 4-week CSE, n=4; 12-week control and CSE, n=4; 20-week control, n=4, 20-week CSE, n=5). *, P value <0.05; **, P value <0.001.

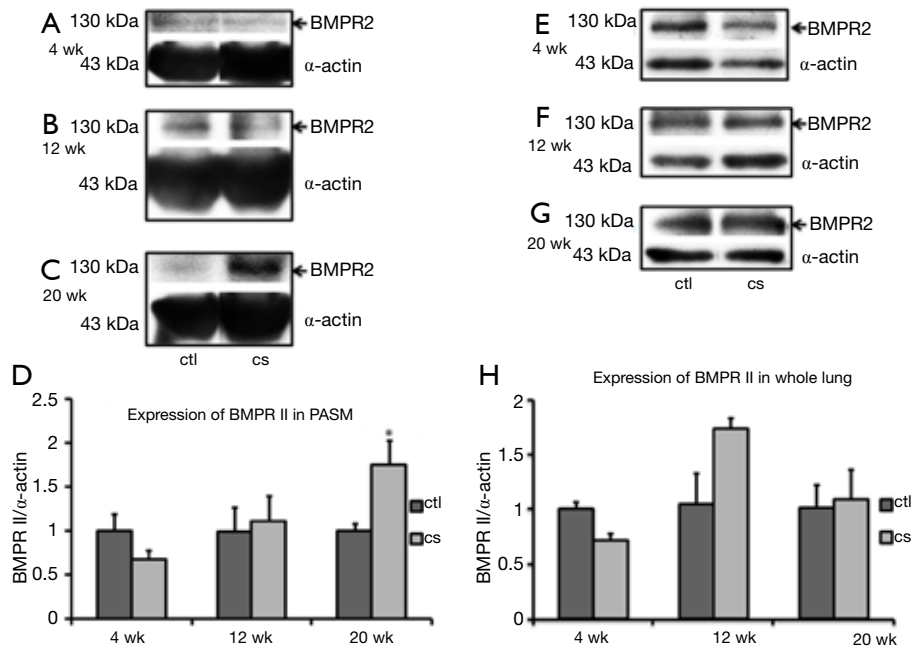

Figure 5 Alterations in BMPR2 expression in PAs and whole lungs from rats exposed to air or 4/12/20 weeks of cigarette smoke. (A-C) Representative western blots of BMPR2 proteins in PAs of control and CSE rats; (D) quantitative analysis of BMPR2 proteins in PAs (4-week control and CSE, n=4; 12-week control, n=4, 12-week CSE, n=5; 20-week control and CSE, n=4); (E-G) representative western blots of BMPR2 proteins in whole lungs of control and CSE rats; (H) quantitative analysis of BMPR2 proteins in whole lungs (4-week control, n=3, 4-week CSE, n=4; 12-week control, n=4, 12-week CSE, n=3; 20-week control, n=4, 20-week CSE, n=5). *, P value <0.05.

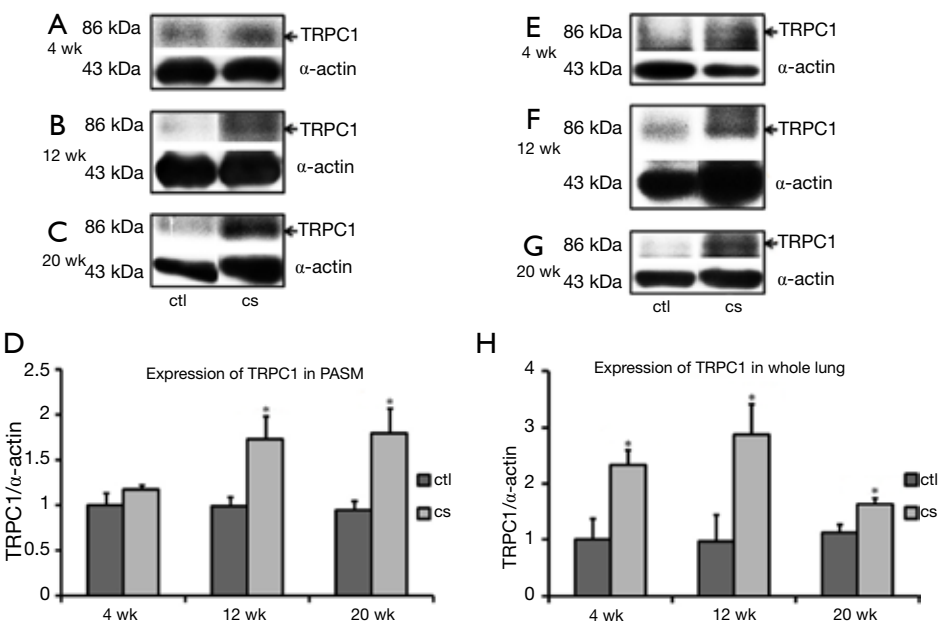

Figure 6 Alterations in TRPC1 expression in PAs and whole lungs from rats exposed to air or 4/12/20 weeks cigarette smoke. (A-C) Representative western blots of TRPC1 proteins in PAs of control and CSE rats; (D) quantitative analysis of TRPC1 proteins in PAs (4-week control, n=4, 4-week CSE, n=5; 12-week control, n=4, 12-week CSE, n=5; 20-week control, n=4, 20-week CSE, n=5); (E-G) representative western blots of TRPC1 proteins in whole lungs of control and CSE rats; (H) quantitative analysis of TRPC1 proteins in whole lungs (4-week control and CSE, n=4; 12-week control and CSE, n=4; 20-week control, n=4, 20-week CSE, n=5). *, P value <0.05.

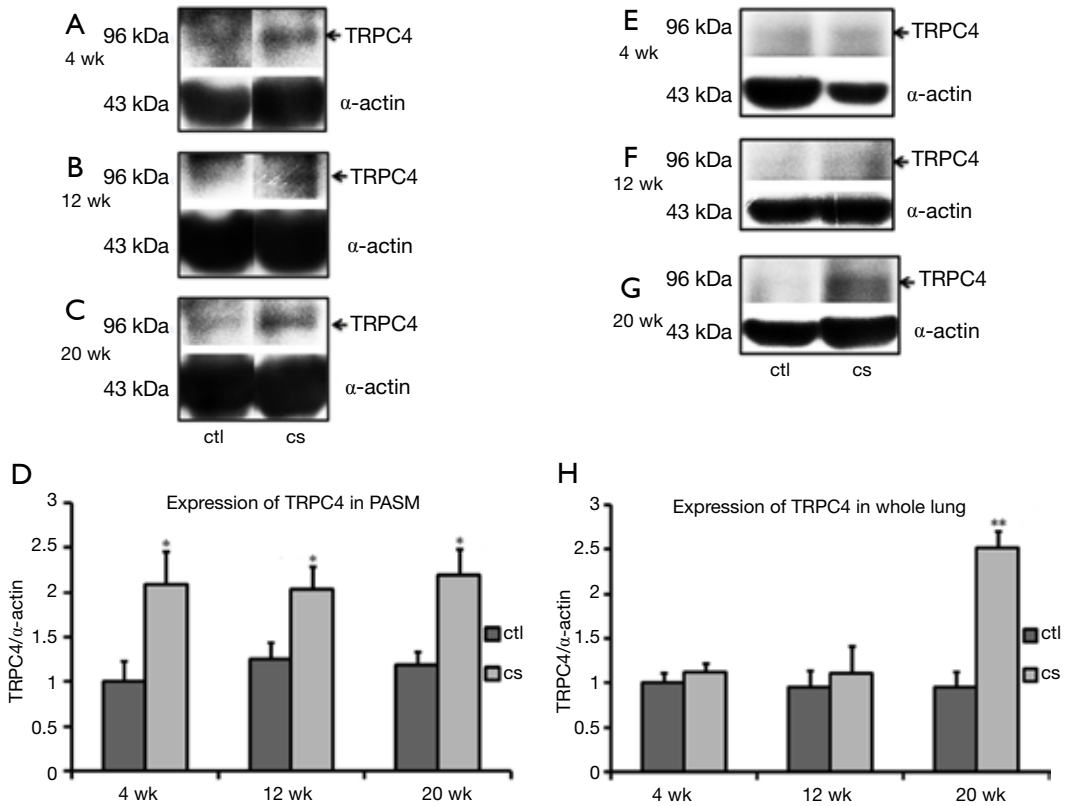

Figure 7 Alterations in TRPC4 expression in PAs and whole lungs from rats exposed to air or 4/12/20 weeks cigarette smoke. (A-C) Representative western blots of TRPC4 proteins in PAs from control and CSE rats; (D) quantitative analysis of TRPC4 proteins in PAs (4-week control and CSE, n=4; 12-week control, n=4, 12-week CSE, n=5; 20-week control and CSE, n=4); (E-G) representative western blots of TRPC4 proteins in whole lungs of control and CSE rats; (H) quantitative analysis of TRPC4 proteins in whole lungs (4-week control, n=4, 4-week CSE, n=5; 12-week control and CSE, n=4; 20-week control and CSE, n=4). *, P value <0.05; **, P value <0.001.

vascular remodeling during PAH. Last, our findings suggest that augmented BMP4 expression may contribute to the enhanced expression of BMPR2.

In conclusion, we found that cigarette smoke upregulates TRPC1, TRPC4, and TRPC6 expression in pulmonary arteries, probably by promoting BMP4 expression. This leads to increased SOCE, which plays a prominent role in dose-dependent vascular remodeling. Augmented expression of BMP4 in the whole lung contributes to inflammatory responses, and BMP4 may also interact with PPAR-γ under normal physiological conditions, thus establishing a barrier to inflammatory responses.

We propose that the downregulation of PPAR-γ and upregulation of BMP4 is crucial in enhanced SOCE, following the upregulation of TRPC1/4/6 in the PAs and lungs of smoking-exposed rats. These changes in PPAR-γ and BMP4 expression also play a part in stimulating inflammatory responses that lead to vascular remodeling. PPAR-γ, expressed in both alveolar macrophages and neutrophils, plays an anti-inflammatory role, and is involved in macrophage activation (34-36).

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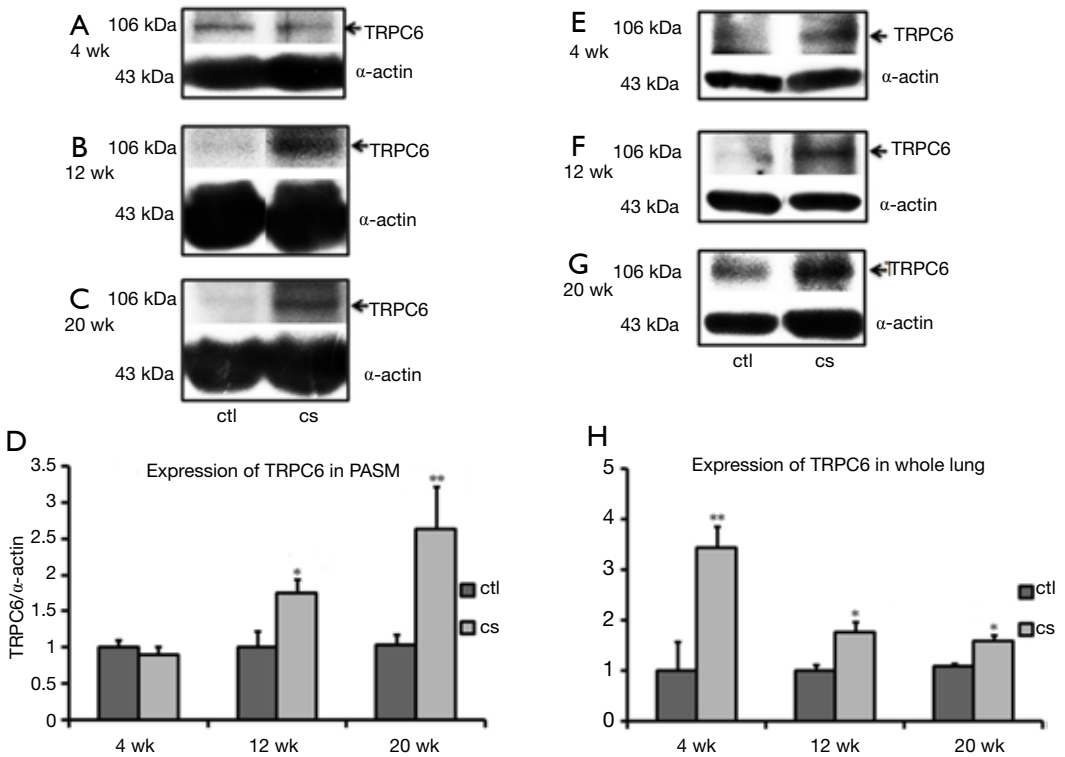

Figure 8 Alterations in TRPC6 expression in PAs and whole lungs from rats exposed to air or 4/12/20 weeks cigarette smoke. (A-C) Representative western blots of TRPC6 proteins in PAs from control and CSE rats; (D) quantitative analysis of TRPC6 proteins in PAs (4-week control, n=4, 4-week CSE, n=5; 12-week control, n=4, 12-week CSE, n=5; 20-week control and CSE, n=4); (E-G) representative western blots of TRPC6 proteins in whole lungs from control and CSE rats; (H) quantitative analysis of TRPC6 proteins in whole lungs (4-week control and CSE, n=4; 12-week control, n=4, 12-week CSE, n=5; 20-week control, n=4, 20-week CSE, n=5). *, P value <0.05; **, P value <0.001.

experiments.

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References

1. Essop MR. Contemporary insights into the pathogenesis, diagnosis and therapy of pulmonary arterial hypertension. Cardiovasc J Afr 2010;21:334-7.
2. Gomberg-Maitland M. Learning to pair therapies and the expanding matrix for pulmonary arterial hypertension: Is more better? Eur Respir J 2006;28:683-6.
3. Chaouat A, Naeije R, Weitzenblum E. Pulmonary hypertension in COPD. Eur Respir J 2008;32:1371-85.
4. Wright JL, Tai H, Wang R, et al. Cigarette smoke upregulates pulmonary vascular matrix metalloproteinases via TNF-alpha signaling. Am J Physiol Lung Cell Mol Physiol 2007;292:L125-33.
5. Miriyala S, Gongora Nieto MC, Mingone C, et al. Bone morphogenic protein-4 induces hypertension in mice: role of noggin, vascular NADPH oxidases, and impaired vasorelaxation. Circulation 2006;113:2818-25.
6. Tian XY, Yung LH, Wong WT, et al. Bone morphogenic protein-4 induces endothelial cell apoptosis through oxidative stress-dependent p38MAPK and JNK pathway. J Mol Cell Cardiol 2012;52:237-44.
7. Chang K, Weiss D, Suo J, et al. Bone morphogenic protein antagonists are coexpressed with bone morphogenic protein 4 in endothelial cells exposed to unstable flow in vitro in mouse aortas and in human coronary arteries: role of bone morphogenic protein antagonists in inflammation and atherosclerosis. Circulation 2007;116:1258-66.
8. Canalis E, Pash J, Varghese S. Skeletal growth factors. Crit Rev Eukaryot Gene Expr 1993;3:155-66.
9. Reddi AH. Role of morphogenetic proteins in skeletal tissue engineering and regeneration. Nat Biotechnol 1998;16:247-52.
10. Wozney JM, Rosen V, Celeste AJ, et al. Novel regulators of bone formation: molecular clones and activities. Science 1988;242:1528-34.
11. Yamashita H, Ten Dijke P, Heldin CH, et al. Bone

morphogenetic protein receptors. Bone 1996;19:569-74.
12. Takano M, Otsuka F, Matsumoto Y, et al. Peroxisome proliferator-activated receptor activity is involved in the osteoblastic differentiation regulated by bone morphogenetic proteins and tumor necrosis factor-α. Mol Cell Endocrinol 2012;348:224-32.
13. Lu W, Ran P, Zhang D, et al. Bone morphogenetic protein 4 enhances canonical transient receptor potential expression, store-operated Ca2+ entry, and basal [Ca2+]i in rat distal pulmonary arterial smooth muscle cells. Am J Physiol Cell Physiol 2010;299:C1370-8.
14. Xu SZ, Beech DJ. TrpC1 is a membrane-spanning subunit of store-operated Ca(2+) channels in native vascular smooth muscle cells. Circ Res 2001;88:84-7.
15. Beech DJ, Xu SZ, McHugh D, et al. TRPC1 store-operated cationic channel subunit. Cell Calcium 2003;33:433-40.
16. Bergdahl A, Gomez MF, Dreja K, et al. Cholesterol depletion impairs vascular reactivity to endothelin-1 by reducing store-operated Ca2+ entry dependent on TRPC1. Circ Res 2003;93:839-47.
17. Dietrich A, Chubanov V, Kalwa H, et al. Cation channels of the transient receptor potential superfamily: their role in physiological and pathophysiological processes of smooth muscle cells. Pharmacol Ther 2006;112:744-60.
18. Wang J, Shimoda LA, Sylvester JT. Capacitative calcium entry and TRPC channel proteins are expressed in rat distal pulmonary arterial smooth muscle. Am J Physiol Lung Cell Mol Physiol 2004;286:L848-58.
19. Dietrich A, Mederos y Schnitzler M, Kalwa H, et al. Functional characterization and physiological relevance of the TRPC3/6/7 subfamily of cation channels. Naunyn Schmiedebergs Arch Pharmacol 2005;371:257-65.
20. Dietrich A, Mederos Y, Schnitzler M, et al. Increased vascular smooth muscle contractility in TRPC6-/- mice. Mol Cell Biol 2005;25:6980-9.
21. Firth AL, Remillard CV, Yuan JX. TRP channels in hypertension. Biochim Biophys Acta 2007;1772:895-906.
22. Zhang S, Remillard CV, Fantozzi I, et al. ATP-induced mitogenesis is mediated by cyclic AMP response element-binding protein-enhanced TRPC4 expression and activity in human pulmonary artery smooth muscle cells. Am J Physiol Cell Physiol 2004;287:C1192-201.
23. Ng LC, Gurney AM. Store-operated channels mediate Ca(2+) influx and contraction in rat pulmonary artery. Circ Res 2001;89:923-9.
24. Liu XR, Zhang MF, Yang N, et al. Enhanced store-operated Ca^{2}+ entry and TRPC channel expression in pulmonary arteries of monocrotaline-induced pulmonary hypertensive rats. Am J Physiol Cell Physiol 2012;302:C77-87.
25. Nadziejko C, Fang K, Bravo A, et al. Susceptibility to pulmonary hypertension in inbred strains of mice exposed to cigarette smoke. J Appl Physiol (1985) 2007;102:1780-5.
26. Yamato H, Sun JP, Churg A, et al. Guinea pig pulmonary hypertension caused by cigarette smoke cannot be explained by capillary bed destruction. J Appl Physiol (1985) 1997;82:1644-53.
27. Wright JL, Tai H, Churg A. Vasoactive mediators and pulmonary hypertension after cigarette smoke exposure in the guinea pig. J Appl Physiol (1985) 2006;100:672-8.
28. Satoh K, Matoba T, Suzuki J, et al. Cyclophilin A mediates vascular remodeling by promoting inflammation and vascular smooth muscle cell proliferation. Circulation 2008;117:3088-98.
29. Lu W, Wang J, Peng G, et al. Knockdown of stromal interaction molecule 1 attenuates store-operated Ca2+ entry and Ca2+ responses to acute hypoxia in pulmonary arterial smooth muscle. Am J Physiol Lung Cell Mol Physiol 2009;297:L17-25.
30. Floyd R, Wray S. Calcium transporters and signalling in smooth muscles. Cell Calcium 2007;42:467-76.
31. Wang J, Weigand L, Wang W, et al. Chronic hypoxia inhibits Kv channel gene expression in rat distal pulmonary artery. Am J Physiol Lung Cell Mol Physiol 2005;288:L1049-58.
32. Kessler R, Faller M, Weitzenblum E, et al. "Natural history" of pulmonary hypertension in a series of 131 patients with chronic obstructive lung disease. Am J Respir Crit Care Med 2001;164:219-24.
33. Scharf SM, Iqbal M, Keller C, et al. Hemodynamic characterization of patients with severe emphysema. Am J Respir Crit Care Med 2002;166:314-22.
34. Szatmari I, Nagy L. Nuclear receptor signalling in dendritic cells connects lipids, the genome and immune function. EMBO J 2008;27:2353-62.
35. Penyige A, Poliska S, Csanky E, et al. Analyses of association between PPAR gamma and EPHX1 polymorphisms and susceptibility to COPD in a Hungarian cohort, a case-control study. BMC Med Genet 2010;11:152.
36. Standiford TJ, Keshamouni VG, Reddy RC. Peroxisome proliferator-activated receptor-{gamma} as a regulator of lung inflammation and repair. Proc Am Thorac Soc 2005;2:226-31.

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Original Article

Comparison of mammosphere formation from breast cancer cell lines and primary breast tumors

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Background: Breast cancer stem cells (BCSCs) can be enriched by culturing of cells in non-adherent non-differentiating conditions. However, culturing mammospheres from primary breast tumors are costly and difficult to control. In order to overcome problems associated with using primary human tissues, continuous breast cancer cell lines have been developed from various sources.

Methods: In this study, a luminal subtype breast cancer cell line MCF-7 and a basal subtype cell line MDA-MB-231 were chosen. We explored the optimal culturing system for BCSCs from the two cell lines and primary breast tumors. Then, mammosphere formation efficiency (MFE), $CD44^+/CD24^{-/low}ESA^+Lin^-$ cell proportion in mammospheres, and tumorigenecity of mammospheres generated from the two breast cancer cell lines and primary breast tumors were compared.

Results: Enzymatic digestion of 60 mins and the addition of B27 to the culture medium were optimal for mammosphere culturing. Mammospheres could be formed in all the three cells, in which MCF-7 had the highest MFE. After 3 weeks culture, $CD44^+/CD24^{-/low}ESA^+Lin^-$ cell proportion in mammospheres from MCF-7, MDA-MB-231 cells and primary breast tumors was 95.0%±2.5%, 82%±22% and 21.5%±1.0%, respectively. A total of 1,000 cells from MCF-7, MDA-MB-231 mammospheres but not primary mammospheres were tumorigenic.

Conclusions: This study validates the use of breast cancer cell lines as models to elucidate the nature of BCSCs.

Keywords: Breast cancer stem cells (BCSCs); mammosphere; MCF-7; MDA-MB-231; primary breast tumor; flow cytometry; tumorigenicity

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Introduction

Recent years, increasing evidence indicates that cancer originates from a small fraction of tumor initiating cells with the abilities of self-renewal, unlimited propagation, multipotent differentiation and giving rise to phenotypically distinct cells found within the tumor population. Such capacities share similarity with normal stem cells. Thus, these cells are also called cancer stem cells (CSCs) (1,2). The existence of CSCs had been successfully approved in variety of tumors such as leukemia, breast cancer, prostate cancer, ovarian cancer, glioma, and gastrointestinal cancer, and had been successfully isolated and cultured *in vitro* (2,3). CSCs are resistant to standard chemotherapy and radiotherapy (4,5). It is believed that CSCs are not only the source of the tumor, but also may be responsible for tumor progression, metastasis, resistance to therapy, and subsequent tumor recurrence. Therefore, a better understanding of the biology of CSCs in each tumor may be a critical step toward the development of treatments to

eventual cure of cancer (6,7).

Breast cancer stem cells (BCSCs) were first identified by Al-Hajj and colleagues (3). They inoculated human breast cancer cells to the mammary fat pad of severe combined immunodeficiency disease (SCID) mice, and found that only a minority of breast cancer cells had the ability to form new tumors. These cells were $CD44^+/CD24^{-/low}/lineage^-$. Dontu and colleagues (8) developed an *in vitro* culture system that allows for propagation of human mammary epithelial cells (HMECs) in non-adherent non-differentiated culture conditions. Cells capable of surviving and proliferating in such conditions formed discrete clusters of cells termed "mammospheres". Such spheroids were enriched in progenitor cells capable of differentiating along multiple lineages including luminal, myoepithelial and alveolar. Ponti and colleagues (9) found that 95% to 96% of cells in mammospheres cultured from cell lines and primary breast tumors were $CD44^+/CD24^{-/low}$.

Generally stated, BCSCs can be isolated or enriched by sorting breast cancer cells for $CD44^+/CD24^{-/low}$ cells by selection for side-population (10), or by culturing of cells in non-adherent non-differentiating conditions to form mammospheres (8,9). In breast cancer, the mammosphere culture system has been widely used to identify and enrich for putative CSCs from breast cancer cell lines or primary breast tumors. Serum-free culture has been proven to be an efficient way to enrich tumor stem cells, but culturing mammospheres from primary breast tumors still remains an obstacle to many researchers (11).

Use of primary breast tumor cells is considered to be the best means to study tumor repopulation (12). However, experiments with primary tumor cells are costly and difficult to control because of the heterogeneous nature of the cellular, genetic, and epigenetic composition among patient tissue samples. In order to overcome problems associated with using primary human tissues, continuous breast cancer cell lines have been developed from various sources. Despite their acquired ability to grow *in vitro*, cell lines continue to share many of the molecular and genetic features of the primary breast cancers from which they were derived. In this study, a luminal subtype cell line MCF-7 and a basal subtype cell line MDA-MB-231 both derived from pleural effusion of breast cancer patients (13-16) were chosen. We explored the optimal culturing system for BCSCs from breast cancer cell lines and primary breast tumors. Then, the mammosphere formation efficiency (MFE), the $CD44^+/CD24^{-/low}ESA^+Lin^-$ cell proportion in mammospheres, and the tumorigenecity of mammospheres generated from the two breast cancer cell lines and primary breast tumors were compared. This study validates the use of breast cancer cell lines as models to elucidate the nature of BCSCs.

Materials and methods

Culture of MCF-7 and MDA-MB-231 cell lines

Human breast epithelial adenocarcinoma cells MCF-7 and MDA-MB-231 were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). As monolayer culture, cells were routinely maintained in Dulbecco's modified Eagle's medium (DMEM, Hyclone, Logan, UT) supplemented with 10% fetal bovine serum (FBS, Hyclone), 100 units/mL penicillin and 100 μg/mL streptomycin (Invitrogen, Carlsbad, CA) at 37 ℃ in a humidified atmosphere with 5% CO_2.

Mammosphere culture from cell lines

For mammosphere culture from MCF-7 and MDA-MB-231 cells, cells were suspended at 1×10^5 cells/mL and seeded into ultralow attachment plates (Corning, NY, USA) in serum free DMEM/F12 (1:1) supplemented with 10 ng/mL basic fibroblast growth factor (b-FGF, peprotech, St. Louis, MO, USA), 20 ng/mL epidermal growth factor (EGF, peprotech), ITS (insulin + transferrin + selenium, Sigma), with or without B27 (GIBCO). Two milliliters of fresh media was added to each well every two days (without removing the old media). Cells grown in these conditions as nonadherent spherical clusters of cells (usually named "mammospheres") were collected every seven days by gentle centrifugation and dissociated to single cell suspensions using the method of Dontu *et al.* (8).

Mammosphere culture from breast tumors

From April 2011 to December 2011, 39 fresh breast invasive ductal carcinoma tissue samples were obtained from the Department of Thyroid and Breast Surgery, West China Hospital, Sichuan University. All patients were female which did not receive any treatment before operation.

Mammosphere culture was performed according to the method of Dontu *et al.* (8) with modifications. After histologic assessment, the tumor lesions were sent to the laboratory within 30 mins of surgery. The tissue were disaggregated mechanically into pieces around 1 mm^3 and digested enzymatically for 40-60 mins at 37 ℃ in a

1:1 solution of collagenase/hyaluronidase (Sigma). After filtration through a 70 μm pore filter, single cells were plated in DMEM/F-12 (Hyclone) supplemented with 10 ng/mL bFGF, 20 ng/mL EGF, ITS with our without B27, incubated in 37 ℃ incubator containing 5% CO_2. Mammospheres were enzymatically dissociated every 7 days by incubation in a 0.5% trypsin-EDTA solution (Invitrogen) for about 5-10 mins at 37 ℃ and plated at 1×10^5 cells/mL in the growth media described above.

Mammosphere formation assays

Mammospheres from MCF-7, MDA-MB-231 cells and primary breast tumors were plated at 1×10^5 single cells/mL into ultralow attachment plates. The number of spheres (diameter >50 μm) for each well was evaluated under microscope on days 7, 14 and 21, respectively. MFE was calculated as the number of spheres divided by the original number of cells seeded and expressed as percentage means (± SD).

Flow cytometry

Mammoshperes from MCF-7, MDA-MB-231 cells and primary breast tumors were collected, washed with phosphate-buffered saline (PBS) and then enzymatically dissociated with 0.05% trypsin/0.25% EDTA into single cell suspension. Combinations of monoclonal antibodies against human CD44-PE, CD24-FITC (BD Biosciences, San Diego, CA, USA), lineage makers (CD2, CD3, CD10, CD16,CD18, CD31, CD64,CD140b-PE-cy7, all from Pharmingen) and ESA-PE-Cy 5.5 (BD) were added to the cell suspension at concentrations recommended by the manufacturer and incubated at 4 ℃ in dark for 30 to 40 mins. For all mammospheres, labeled cells were washed in PBS to eliminate unbounded antibody, and then analyzed on a FACSAria (MountainView, CA, USA).

Tumorigenicity assay

Nude mice purchased from Beijing HFK Bioscience CO were maintained in laminar flow rooms under constant temperature and humidity. Mammospheres were collected, enzymatically dissociated, washed in PBS, and kept at 4 ℃ until 1,000 cells were suspended in 50-100 μL matrigel and slowly injected into mammary fat pad of 6-week-old female nude mice. Mice were inspected for tumor formation by observation and palpation for 10 weeks after injection. After this time interval, all mice were sacrificed by cervical dislocation. At sacrifice, the xenograft tumors were immediately removed, fixed in 10% neutral buffered formalin solution (Sigma), and embedded in paraffin.

Statistical analysis

The results are presented as the mean ± SD for at least three individual. P values of 0.05 or less, calculated using a paired two-sided Student's *t*-test were considered to indicate statistically significant differences.

Results

Optimize mammosphere culture system

Serum-free culture has been proven to be an efficient way to enrich tumor stem cells, but culturing mammospheres from primary breast tumors still remain difficult to many researchers (11). We carried out a series of exploration on culture conditions, such as different digestion time and medium composition.

For BCSCs primary culture from breast tumors, collagenase/hyaluronidase (1:1) is used for digestion of tumor tissue into single cells. In order to get enough number of cells with optimal cell viability, we tried different digestion time (*Table 1*). We found out digestion time around 60 mins gave rise to enough single cells with the highest cell viability.

According to the literature (9), the culture medium for mammosphere contains serum-free DMEM/F12 (1:1) supplemented with 10 ng/mL b-FGF, 20 ng/mL EGF, 5 μg/mL insulin and selenium 0.4% bovine serum albumin. However, in our experiment, MCF-7 and MDA-MB-231 cells grown in this condition tended to attach to the dish and could hardly form spheres. B27 was used for culturing embryonic neurons (17), and also for mammosphere culturing (8,11). We added B27 as a supplement to promote mammosphere formation. With the addition of B27, MFE of both cell lines and primary breast tumor cells improved significantly (*Table 2*).

Thus, enzymatic digestion of 60 min and the addition of B27 to the culture medium were optimal for mammosphere culturing.

Mammosphere formation from cell lines and primary breast tumors

MCF-7 cells were capable of forming mammospheres

Table 1 The effect of different digestion time on the yield of single cells and cell viability

Digestion time (mins)	Cells/mL	Cell viability (%)
30	2×10^5	>95
60	6×10^5	>95
120	8×10^5	>70
240	1×10^6	<65

Table 2 Mammosphere formation efficiency (MFE) of MCF-7, MDA-MB-231 cell lines and primary breast tumor cells after 2 weeks culture with or without the addition of B27

	MFE (%)		P value
	Without B27	With B27	
MCF-7	0	1.9±0.5	0.008
MDA-MB-231	0.05±0.01	0.73±0.18	<0.001
Primary tumor cells	3.00±0.25	3.15±1.3	0.017

of 50 μm in diameter under mammosphere culture condition for 2 weeks. After 3 weeks of culture, more mammospheres appeared and the diameter increased to around 100 μm. While in MDA-MB-231 cells and primary breast tumor cells, mammospheres appeared after 1 week culture under mammosphere culture condition. Cultured for 3 weeks, mammospheres enlarged to 100-200 μm in diameter (*Figure 1*). After three week culture, mammospheres from MDA-MB-231 cells tended to attach. Thus, both breast cancer cell lines and primary breast tumor cells were capable of forming mammospheres under mammosphere culture condition. However, mammospheres from different origins showed different morphology.

Mammosphere formation efficiency (MFE)

After one week culture under mammosphere culture condition, MFE of MCF-7, MDA-MB-231 cell lines,

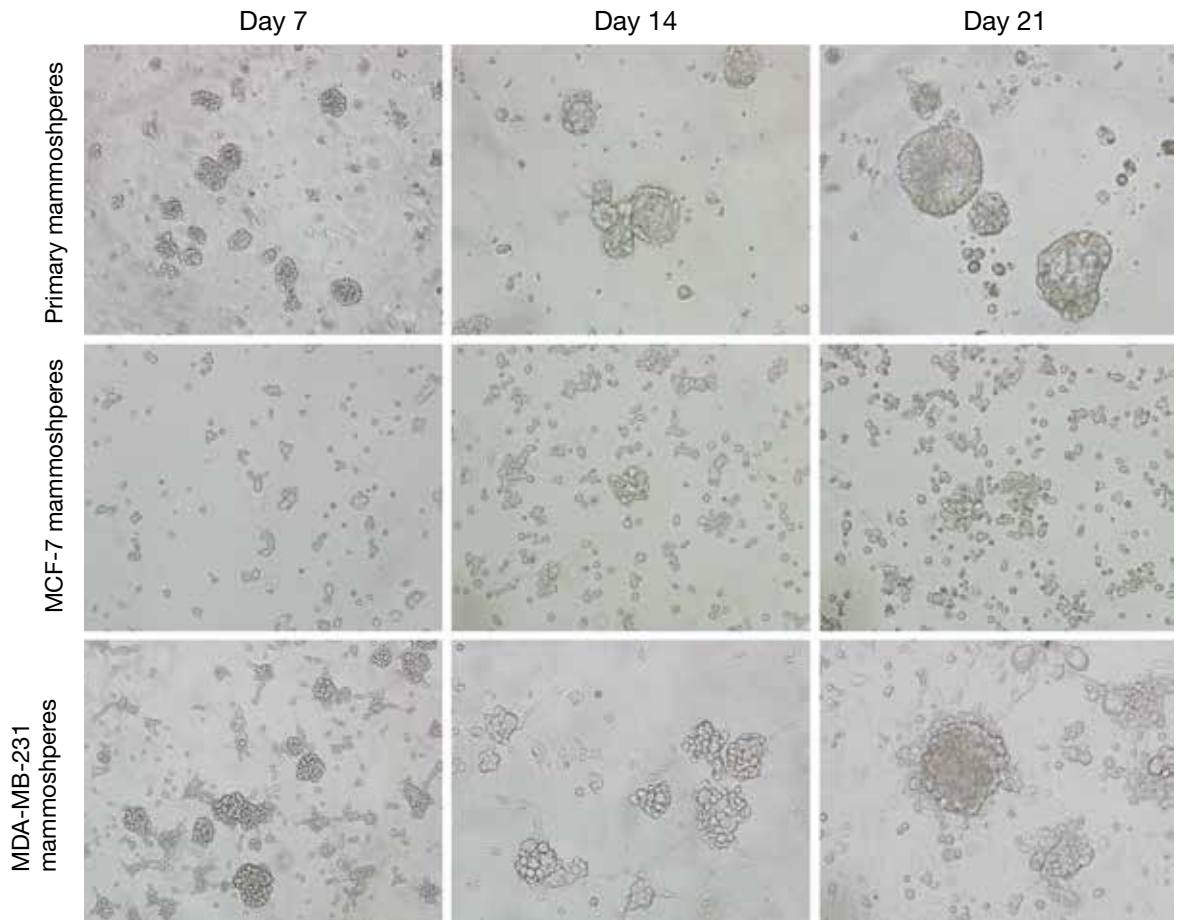

Figure 1 Morphology of mammospheres from MCF-7, MDA-MB-231cells, and primary breast tumors at 7, 14, and 21 days of culture (×400).

Table 3 Mammosphere formation efficiency (MFE) in mammospheres from MCF-7, MDA-MB-231, and primary breast tumors

Mammosphere	MFE (%)				P value
	0 d	7 d	14 d	21 d	
MCF-7	0	0.07±0.1	1.90±0.5	5.30±1.32	0.001
MDA-MB-231	0	0.36±0.14	0.73±0.18	0.35±0.12	1.0
Primary	0	0.65±0.09	3.15±1.3	3.49±0.8	<0.001

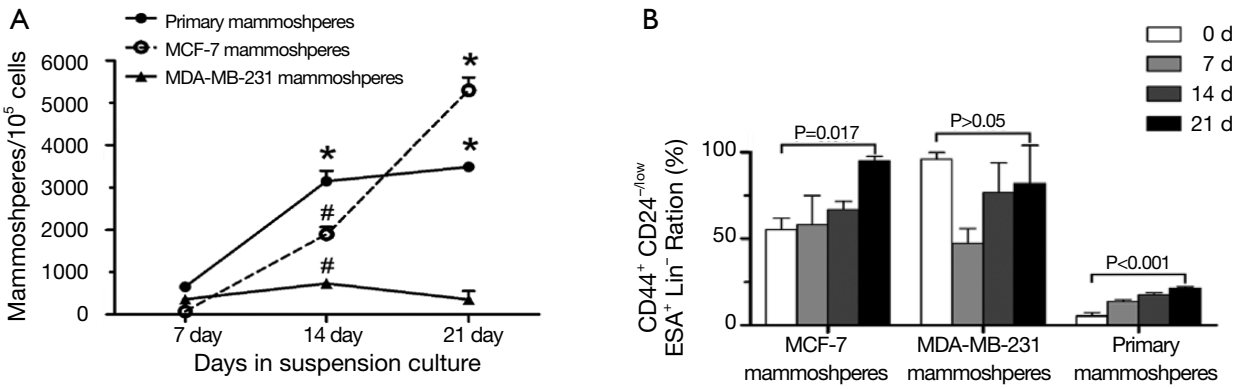

Figure 2 MFE (A) and $CD44^+/CD24^{-/low}ESA^+Lin^-$ cell proportion (B) in mammospheres from MCF-7, MDA-MB-231cells, and primary breast tumors. MFE, mammosphere formation efficiency.

and primary breast tumors were 0.07%±0.036%, 0.356%±0.083%, and 0.654%±0.059%, respectively. After three week culture, MFE of the above cells increased to 5.30%±0.307%, 0.35%±0.204%, 3.492%±0.11%, respectively (*Table 3*, *Figure 2A*). Thus, MCF-7 had the highest MFE among these three cells.

Mammoshperes enriched for CD44+/CD24-/lowESA+Lin- cells

The proportion of $CD44^+/CD24^{-/low}ESA^+Lin^-$ cells in the three mammospheres was detected at 0, 7, 14, 21 days after culture by flow cytometry (*Figures 2B,3*). Before culturing under mammosphere culture condition, proportion of $CD44^+/CD24^{-/low}ESA^+Lin^-$ cells in MCF-7, MDA-MB-231 cell lines, and primary breast tumors were 55.3%±6.5%, 96.2%±3.6%, and 5.3%±2.1%, respectively. After 3 weeks culture under mammosphere culture condition, $CD44^+/CD24^{-/low}$ ESA^+Lin^- cell proportion in mammospheres from MCF-7 cells and primary breast tumors significantly increased to 95.0%±2.5% and 21.5%±1.0%, respectively (*Table 4*, *Figure 2B*). However, $CD44^+/CD24^{-/low}ESA^+Lin^-$ cell proportion in MDA-MB-231 cells didn't increase under mammosphere culture condition. Thus, BCSCs can be enriched by culturing of MCF-7 cells and primary breast tumor cells in non-adherent non-differentiating conditions to form mammospheres.

Tumorigenicity of mammospheres from MCF-7, MDA-MB-231 cell lines, and primary breast tumors

Ponti *et al.* (9) were the first to report that cells initiated from MCF-7 mammospheres could form tumors when as few as 1,000 cells were injected to SCID mice. Accordingly, in our study, Mammospheres from MCF-7, MDA-MB-231 cell lines, and primary breast tumors were enzymatically digested into single cells, 1,000 cells were injected into mammary fat pad of nude mice. After 4 weeks, cells from MCF-7 and MDA-MB-231 mammospheres could form palpable tumors in 3 of 4 and 4 of 4 nude mice, respectively. The tumors became obvious at 10 weeks (*Figure 4A*). However original MCF-7 and MDA-MB-231 cells and cells from primary mammosphere failed to form tumors. H&E staining of tumors grown in mice after injection of tumor initiating cells revealed the presence of malignant cells, with large nuclei and active mitosis; In some area, erythrocytes were visible within tumor cell lined cavities (*Figure 4B*). These data show that cells from MCF-7 and MDA-MB-231 mammospheres have tumor-initiating capacity *in vivo*, when compared with original MCF-7 and MDA-MB-231 cells.

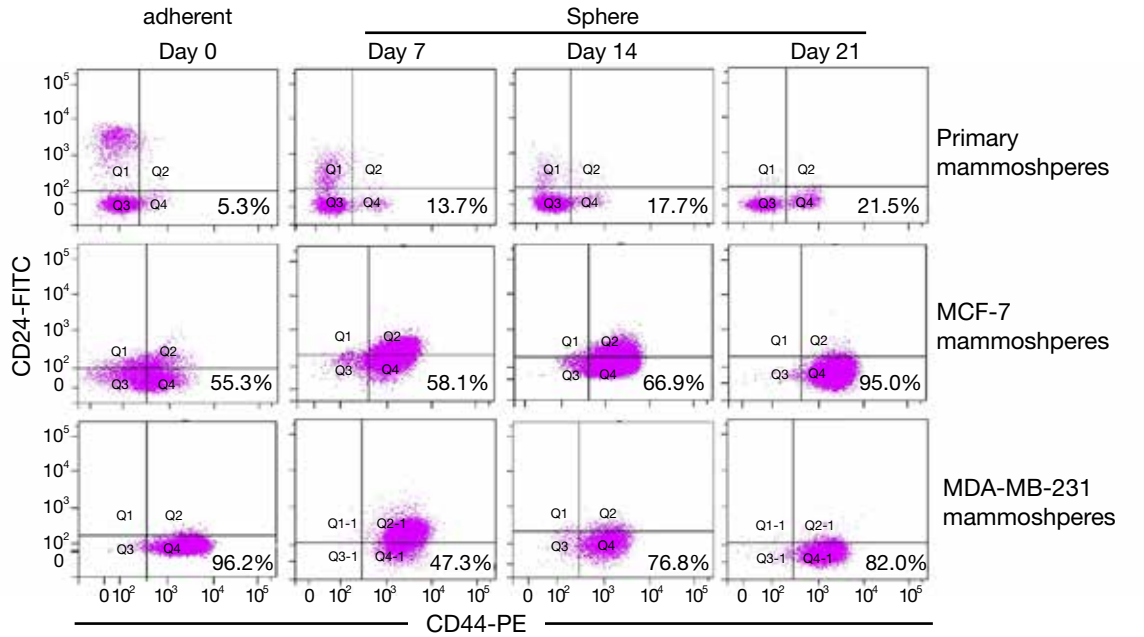

Figure 3 Flow cytometry of mammospheres from MCF-7, MDA-MB-231 cells, and primary breast tumors.

Table 4 $CD44^+/CD24^{-/low}ESA^+Lin^-$ cell proportion in mammospheres from MCF-7, MDA-MB-231, and primary breast tumors

Mammosphere	$CD44^+/CD24^{-/low}ESA^+Lin^-$ cell proportion				P value
	0 d	7 d	14 d	21 d	
MCF-7	55.3±6.5	58.1±16.8	66.9±4.8	95.0±2.5	0.017
MDA-MB-231	96.2±3.6	47.3±8.6	76.8±17.0	82.0±22.0	1.0
Primary	5.3±2.1	13.7±1.2	17.7±1.3	21.5±1.0	<0.001

Discussion

Although growing evidence has revealed the existence of CSC in a variety of human cancers (3,18-21). Culturing mammospheres from breast cancer cell lines and primary breast tumors still remains an obstacle to many researchers (11). In this study, we tried to optimize the culture condition of BCSCs from MCF-7 cells, MDA-MB-231 cells and primary breast tumors. Meanwhile, we compared their MFE, $CD44^+/CD24^{-/low}ESA^+Lin^-$ cell proportion in mammopheres, and tumorigenicity.

For mammosphere culture from primary breast tumors, we found enzymatic digestion time around 60 min gave rise to enough single cells with the highest cell viability (*Table 1*). What we need to notice is to adjust the digestion time according to pathological characters of tumors. For example, breast adenocarcinoma is usually hard to digest. While, mucinous carcinoma is relatively fragile, which needs shorter digestion time.

Without the addition of B27, low MFE always presented in mammospheres from primary breast tumors. At the same time, mammospheres from breast cancer cell lines were easy to attach. Although B27 was initially used for the culture of progenitor or stem cells from central or peripheral nervous system (17), many researchers found B27 was an essential component of culture medium necessary for non-adherent cells suspension (11,22). With the addition of B27 to the culture medium, MFE was significantly increased (*Table 2*), owing to the role of B27 in increased survival of tumor spheres (8,23,24) and prevention of adherences (11). Thus, enzymatic digestion of 60 min and the addition of B27 to non-adherent non-differentiated culture medium were optimal for mammosphere culturing.

Mammospheres from different origins showed different morphology. Mammoshperes from primary breast tumors tended to develop into regular round shape. Whereas, mammoshperes from MCF-7 cells were relatively loose.

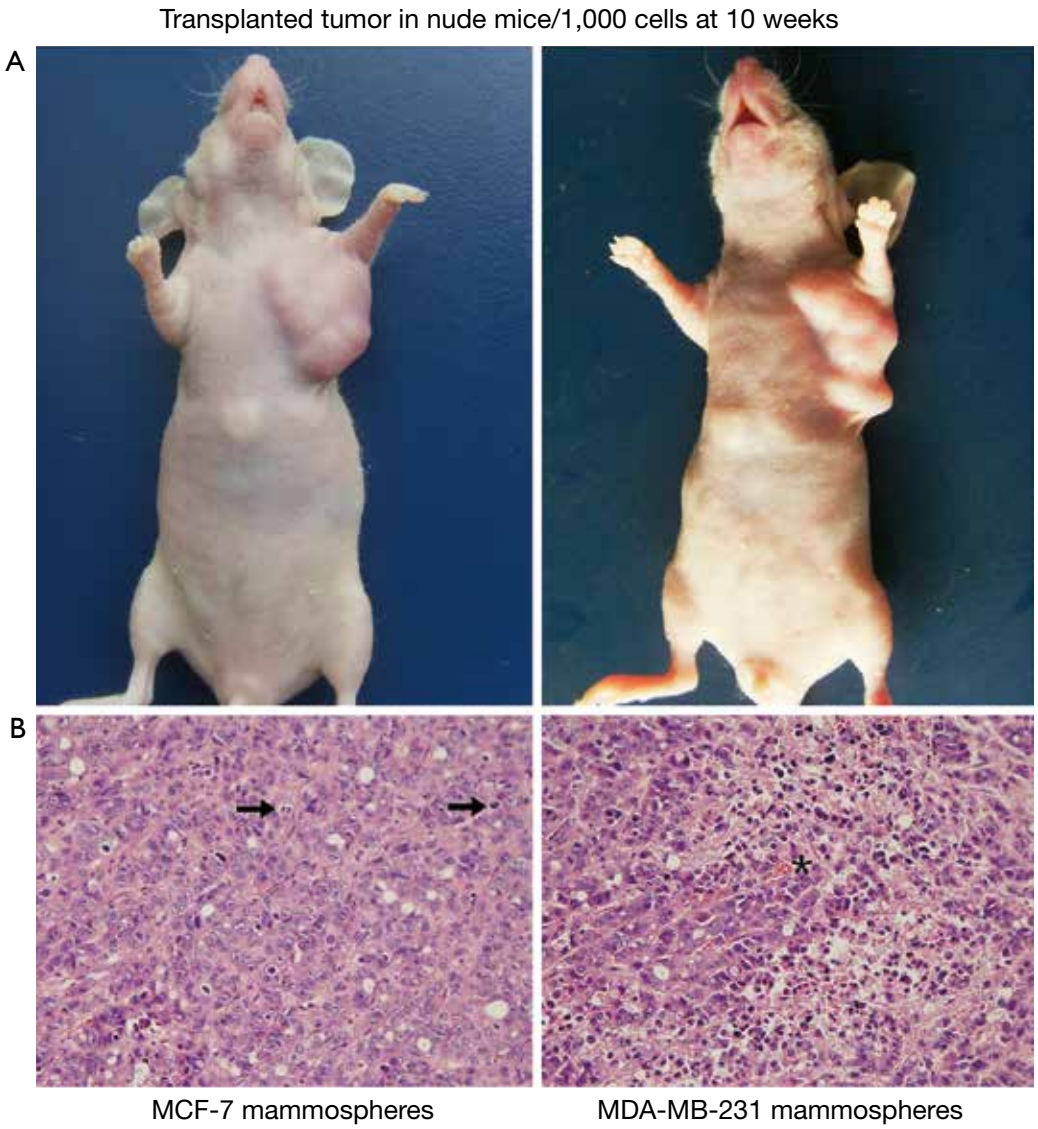

Figure 4 Macroscopical appearance (A) and H&E staining (B) of tumor grown in nude mice when 1,000 cells from MCF-7 and MDA-MB-231 mammospheres were injected into their mammary fat pad. Malignant cells with big nuclei and mitosis (arrows) could be observed. In some area, erythrocytes are visible within tumor cell lined cavities (*) (×400).

Mammoshperes from MDA-MB-231 cells grew bigger than 200 μm in diameter, which tended to attach after 3-week culture (*Figure 1*).

Fillmore CM *et al*. reported basal type cell line MDA-MB-231 consisted more than 90% $CD44^+/CD24^{-/low}$ cells (7). Moreover, cell lines with high $CD44^+/CD24^-$ cell numbers express basal/mesenchymal or myoepithelial but not luminal markers (25). Consisted with these results, our data showed before culturing under mammosphere culture condition, proportion of $CD44^+/CD24^{-/low}ESA^+Lin^-$ cells in MCF-7, MDA-MB-231 cell lines, and primary breast tumors were 55.3%±6.5%, 96.2%±3.6%, and 5.3%±2.1%, respectively. Rosen *et al.* reported more than 90% of cells in mammospheres cultured from MCF-7 cells and primary breast tumors had $CD44^+/CD24^{-/low}/lineage^-$ phenotype (26). After 3 weeks culture under mammosphere culture condition, we found $CD44^+/CD24^{-/low}ESA^+Lin^-$ cell proportion in MCF-7 cells and primary breast tumors significantly increased to 95.0%±2.5% and 21.5%±1.0%, respectively (*Table 4*, *Figure 2B*). However, under

mammosphere culture condition, $CD44^+/CD24^{-/low}$ ESA^+Lin^- cell proportion in MDA-MB-231 cell line did not increase (*Table 4*, *Figure 2B*), probably due to the already high stem cell proportion in MDA-MB-231 cell line.

In our study, MFE of MCF-7 cells and primary breast tumors is higher than MDA-MB-231 cells (*Table 3*). Ponti D reported long-term cultures which could be expanded as floating mammospheres for more than 40 passages *in vitro* derived only from estrogen receptor—positive lesions and estrogen receptor—positive MCF-7 cells (9). These data indicates a correlation between estrogen receptor expression and mammosphere formation that needs to be further investigated.

Ponti *et al.* (9) reported that cells initiated from MCF-7 mammospheres could form tumors when as few as 1,000 cells were injected to SCID mice. Cell lines with high proportion of $CD44^+/CD24^-$ subpopulation were more invasive than other cell lines (25). Our study found that 1,000 cells from MCF-7 and MDA-MB-231 mammospheres with high proportion of $CD44^+/CD24^-$ cell proportion detected by flow cytometry could form tumors in nude mice in 4 weeks after subcutaneous injection. Nevertheless, the primary mammopheres and the two cell lines failed to form tumors. Primary mammospheres especially the first generation consists of cells of heterogenous origins, in which myoepithelial cells and luminal epithelial cells both could form mammospheres (8). The percentage of breast cancer stem cells was 68% in the first generation mammospheres, and increased to 98% in second and later generation mamospheres (8). Therefore, mammospheres from purified cell lines could be more tumorigenic than primary mammospheres.

Conclusions

Under non-adherent and non-differentiated culture condition, mammospheres could be formed in breast cancer cell lines MCF-7, MDA-MB-231, and primary breast tumors. MCF-7 cells showed highest MFE and stem cell proportion after three week culture. Cells from both MCF-7 and MDA-MB-231 mammospheres were tumorigenic. This study validates the use of breast cancer cell lines as well as primary breast tumors for the research of breast cancer stem cells.

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References

1. Schulenburg A, Ulrich-Pur H, Thurnher D, et al. Neoplastic stem cells: a novel therapeutic target in clinical oncology. Cancer 2006;107:2512-20.
2. Jordan CT, Guzman ML, Noble M. Cancer stem cells. N Engl J Med 2006;355:1253-61.
3. Al-Hajj M, Wicha MS, Benito-Hernandez A, et al. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci U S A 2003;100:3983-8.
4. Li X, Lewis MT, Huang J, et al. Intrinsic Resistance of Tumorigenic Breast Cancer Cells to Chemotherapy. J Natl Cancer Inst 2008;100:672-9.
5. Phillips TM, McBride WH, Pajonk F. The Response of CD24–/low/CD44+ Breast Cancer–Initiating Cells to Radiation. J Natl Cancer Inst 2006;98:1777-85.
6. Al-Hajj M, Becker MW, Wicha M, et al. Therapeutic implications of cancer stem cells. Current Opinion in Genetics Development 2004;14:43-7.
7. Fillmore CM, Kuperwasser C. Human breast cancer cell lines contain stem-like cells that self-renew, give rise to phenotypically diverse progeny and survive chemotherapy. Breast Cancer Res 2008;10:R25.
8. Dontu G, Abdallah WM, Foley JM, et al. In vitro propagation and transcriptional profiling of human mammary stem/progenitor cells. Genes Dev 2003;17:1253-70.
9. Ponti D, Costa A, Zaffaroni N, et al. Isolation and In vitro Propagation of Tumorigenic Breast Cancer Cells with Stem/Progenitor Cell Properties. Cancer Res. 2005;65:5506-11.
10. Patrawala L, Calhoun T, Schneider-Broussard R, et al. Side population is enriched in tumorigenic, stem-like cancer cells, whereas ABCG2+ and ABCG2- cancer cells are similarly tumorigenic. Cancer Res 2005;65:6207-19.
11. Huang MZ, Zhang FC, Zhang YY. Influence factors on the formation of mammospheres from breast cancer stem cells. Beijing Da Xue Xue Bao 2008;40:500-4.

12. Clarke MF, Dick JE, Dirks PB, et al. Cancer stem cells--perspectives on current status and future directions: AACR Workshop on cancer stem cells. Cancer Res 2006;66:9339-44.
13. Neve RM, Chin K, Fridlyand J, et al. A collection of breast cancer cell lines for the study of functionally distinct cancer subtypes. Cancer Cell 2006;10:515-27.
14. Lacroix M, Leclercq G. Relevance of breast cancer cell lines as models for breast tumours: an update. Breast Cancer Res Treat 2004;83:249-89.
15. Levenson AS, Jordan VC. MCF-7: the first hormone-responsive breast cancer cell line. Cancer Res 1997;57:3071-8.
16. Subik K, Lee JF, Baxter L, The Expression Patterns of ER, PR, HER2, CK5/6, EGFR, Ki-67 and AR by Immunohistochemical Analysis in Breast Cancer Cell Lines. Breast Cancer (Auckl) 2010;4:35-41.
17. Brewer GJ. Isolation and culture of adult rat hippocampal neurons. J Neurosci Methods 1997;71:143-55.
18. Singh SK, Clarke ID, Hide T, et al. Cancer stem cells in nervous system tumors.Oncogene 2004;23:7267-73.
19. Fang D, Nguyen TK, Leishear K, et al. A tumorigenic subpopulation with stem cell properties in melanomas. Cancer Res 2005;65:9328-37.
20. O'Brien CA, Pollett A, Gallinger S, Dick JE. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. Nature 2007;445:106-10.
21. Ricci-Vitiani L, Lombardi DG, Pilozzi E, et al. Identification and expansion of human colon-cancer-initiating cells. Nature 2007;445:111-5.
22. Chen H. The effect of B27 supplement on promoting in vitro propagation of Her2/neu-transformed mammary tumorspheres. J Biotech Res 2011;3:7-18.
23. Svendsen CN, Fawcett JW, Bentlage C, et al. Increased survival of rat EGF-generated CNS precursor cells using B27 supplemented medium. Exp Brain Res 1995;102:407-14.
24. Brewer GJ, Torricelli JR, Evege EK, et al. Optimized survival of hippocampal neurons in B27-supplemented Neurobasal, a new serum-free medium combination. J Neurosci Res 1993;35:567-76.
25. Sheridan C, Kishimoto H, Fuchs RK, et al. CD44+/CD24- breast cancer cells exhibit enhanced invasive properties: an early step necessary for metastasis. Breast Cancer Res 2006;8:R59.
26. Rosen JM, Jordan CT. The increasing complexity of the cancer stem cell paradigm. Science 2009;324:1670-3.

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Original Article

A propensity score analysis on the effect of on-pump versus off-pump coronary artery bypass grafting for patients with coronary artery disease

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Aim: The aim of this retrospective observational study was to investigate the effect of on-pump versus off-pump coronary artery bypass grafting (CABG) for patients with coronary artery diseases (CAD).

Methods: A retrospective observational study was performed using a propensity score analysis in 290 consecutive patients undergoing CABG between April 2009 and March 2014, of them, 54 patients undergoing off-pump CABG (OPCABG) were matched with 54 patients undergoing on-pump CABG (ONCABG) by propensity score. The perioperative complications and hospital mortality were documented.

Results: Preoperative characteristics were comparable in both groups following propensity matching. Postoperative myocardial infarction (MI) incidence was lower in OPCABG group than in ONCABG group (3.7% *vs.* 14.8%, P=0.046); both hospital mortality and the major complications rates were similar in the two groups after propensity adjustment for preoperative characteristics.

Conclusions: The perioperative complications are similar in both off-pump and on pump CABG groups, the short-term effect of OPCABG is similar to that of ONCABG.

Keywords: Coronary artery disease (CAD); coronary artery bypass grafting (CABG); cardiopulmonary bypass (CPB); propensity score analysis

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Introduction

Coronary artery diseases (CAD) are very common (1,2), and cause a significant morbidity and mortality in patients with severe stenosis of coronary artery (1-4). Medical therapy confers a poor survival advantage relative to surgical revascularization in some severe CAD patients. Coronary artery bypass grafting (CABG) is indicated for patients with severe stenosis of coronary artery (2), and can be finished by conventional on pump CABG (ONCABG) with cardiopulmonary bypass or off pump CABG (OPCABG) (1,5,6).

OPCABG is now an established procedure, recent studies show OPCABG reduced early operative mortality and the incidences of major complications in redo CABG (7), but it appears not to increase mid-term major adverse cardiovascular and cerebrovascular events over ONCABG (1). There is no conclusion on which option is better than other. Moreover, the studies on OPCABG have reported on relative small cohorts of patients and have lacked statistical adjustment to reduce the differences in selection bias (8). In order to reduce the selection bias, we used a propensity score matching analysis to evaluate the early outcomes of OPCABG versus ONCABG in a consecutive cohort of CAD patients during four years periods.

Materials and methods

Patient selection

This was a retrospective, observational cohort study of data

from consecutive CAD patients who underwent CABG at the China-Japan Friendship Hospital between April 2010 and March 2014. The study followed the China-Japan Friendship Hospital ethical and legal requirements, and individual written consent was obtained for surgical management and related medical study.

Inclusion conditions were patients who underwent CABG with complete medical document, the exclusion conditions were those who underwent concomitant percutaneous coronary intervention or carotid thromboendarterectomy (2-4). Surgical procedure selection was at discretion of the operating surgeon. The resulting base sample contained detailed clinical information on 290 patients, including 192 (66.2%) undergoing OPCABG and 98 (33.8%) undergoing ONCABG. A propensity score matching analysis was used to minimize the impact of treatment selection bias and potential confounding, 54 patients who underwent OPCABG were matched with 54 patients who underwent ONCABG.

Definitions

Critical CAD disease was defined as a stenosis of greater than 50% of lumen based on a preoperative coronary angiogram. In-hospital mortality was all deaths after surgery occurring in hospital regardless of time after surgery. A postoperative myocardial infarction (MI) was diagnosed if new Q waves longer than 0.04 ms or a reduction in R waves greater than 25% in at least two continuous leads appear on electrocardiography. Stroke was defined as new onset of global or focal brain injury that persisted for over 72 hours. Acute postoperative renal failure was defined as new requirement of hemodialysis or an elevated creatinine level 50% or greater over baseline preoperative value or >200 mmol/L. Infection was defined septicemia, sternal or leg wound infections after harvesting of great saphenous veins or sternotomy with a positive culture and requiring antibiotics (9).

Surgical management

The heart was exposed via a median sternotomy, proximal aortic atheromathous disorder was palpated manually, and the anastomotic site was tailored to avoid atherosclerotic part. Cardiopulmonary bypass (CPB) was established in a standardized manner with mild hypothermia and a roller pump. For patients undergoing ONCABG, CBP was instituted with the use of ascending aortic cannulation and 2-stage venous cannulation of the right atrium. The proximal anastomosis to the ascending aorta was constructed during a single cross clamp period. Myocardial protection was achieved with intermittent hyperkalemic antegrade warm blood cardioplegia. For OPCABG surgery, the up-to-date stabilizing retractor was used. Distal anastomosis was routinely constructed after proximal anastomosis. During surgery, heparin at 300 IU/kg for the ONCABG and 150 IU/Kg for the OPCABG were used. Activated clotting time was maintained over 480 s for ONCABG and over 300 s for OPCABG. The effect of heparin was reversed with protamine sulphate at 1:1 ratio. At the end of surgery, patients were transferred to the intensive care unit (ICU), a standardized protocol for immediate postoperative care was followed in ICU (10). Outcomes of care observed include reoperation for bleeding, hospital death, stroke, renal failure, and new MI.

Statistical analysis

A nonparsimonious multiple logistic regression analysis was used to determine the propensity for CABG. A propensity score was estimated from the logistic equation for each patient, and was used to match OPCABG patients with those undergoing ONCABG (1:1 match). Independent risk factors for in-hospital mortality were identified by a stepwise, multivariable logistic regression modeling. *Table 1* shows all baseline characteristics of covariates under consideration for models. All P values are reported as 2-sided. All statistical analyses were performed. A SPSS statistical software (version 13.0; IBM Corporation, Armonk, NY, USA) and R statistical software were used for study. The Kolmogorov-Smirnov test was used to check for normality of data in the two groups initially. Continuous data were expressed as mean ± SD, and categoric data were expressed as percentages.

Differences between groups were compared with the chi-square statistic test for categoric variables and students' *t*-tests for continuous variables.

Results

Table 1 shows the baseline characteristics of patients with CAD, 192 patients (66.2%) with CAD underwent OPCABG; 98 patients (33.8%) underwent ONCABG. Compared with the OPCABG group, patients in ONCABG group had a higher prevalence of smoking and alcohol consumption, and lower ejection fraction less than 40% (P<0.05). In addition, patients undergoing ONCABG were more likely received urgent operation (P=0.038). There was no difference in the use of bilateral internal mammary artery between the two groups (P>0.05).

Table 1 Baseline characteristics of the entire cohort

Variable	OPCABG (n=192)	ONCABG (n=98)	P value
Age (years)	62.91±12.19	61.05±12.34	0.222
Female, n (%)	47 (24.5)	23 (23.5)	0.998
Creatinine (mol/L)	105.11±37.87	113.18±46.16	0.112
HPT, n (%)	126 (65.6)	68 (69.4)	0.52
DM, n (%)	26 (13.5)	16 (16.3)	0.524
Dyslipedimia, n (%)	43 (22.4)	23 (23.5)	0.837
Current smoker, n (%)	39 (20.3)	41 (41.8)	<0.001
Alcoholics, n (%)	25 (13.0)	22 (22.4)	0.039
COPD, n (%)	17 (8.9)	8 (8.2)	0.843
Stroke or TIA, n (%)	26 (13.5)	20 (20.4)	0.13
EF <40%, n (%)	33 (17.2)	31 (31.6)	0.005
No. of distal grafts	1.69±0.71	1.71±0.70	0.852
LIMA, n (%)	43 (22.4)	23 (23.5)	0.837
IABP, n (%)	117 (60.9)	64 (65.3)	0.468
Urgent surgery, n (%)	9 (4.7)	11 (11.2)	0.038

Data are presented as means ± SD; OPCABG, off-pump coronary artery bypass grafting; ONCABG, on-pump coronary artery bypass grafting; HPT, hypertension; DM, diabetes mellitus; COPD, chronic obstructive pulmonary disease; TIA, transient ischemia attack; EF, ejection fraction; LIMA, left internal mammary artery; IABP, intra-artery balloon pumping.

After propensity score matching, 54 pairs of patients were matched (*Table 2*). In the matched cohorts, no significant difference between the two groups for any covariate was observed. Patients undergoing OPCABG had a lower needs for postoperative intra-aortic balloon pump, but it did not reach a significant difference (P>0.05, *Table 2*).

Table 3 indicates that the incidence of postoperative MI in OPCABG group was lower than ONCABG group(3.7% *vs.* 14.8%, P=0.046), and other clinical outcomes of propensity matched patients were similar in both groups, including rates of blood transfusion, ICU stay and postoperative hospital stay, reoperation rate and hospital mortality. The incidence of wound infection in OPCABG group was higher than in ONCABG group (13% *vs.* 7.4%, P=0.34). One of the most common organisms cultured from the infected chest wounds was Staphylococcus aureus. Patients with wound infection had a poor healing of the sternal wound and wound dehiscence (*Figure 1*). The multivariate analysis revealed that a postoperative MI was an independent risk factor for surgical revascularization (odds ratio, 3.4; 95% CI: 0.45-1.09, P=0.046).

Discussion

Our propensity score analysis study demonstrates that OPCABG is safe for patients with coronary artery disease and is associated with lower postoperative MI incidence and similar perioperative complications and hospital mortality with respect to ONCABG.

There are controversies about the possible benefits of OPCABG compared to ONCABG. Some institutes still use the ONCABG technique to treat severe CAD patients currently, the main reason is the hemodynamic instability that may occur in performing revascularization on a beating heart. Recent studies show that OPCABG resulted in significantly lower patency rate for arterial and saphenous vein graft conduits, and less effective revascularization than ONCABG. At one year after surgery, patients with less effective revascularization had higher adverse event rates (5). A meta analysis suggest that OPCABG may increase late all-cause mortality by a factor of 1.37 over ONCABG (11). Randomized controlled trials did not find, except for atrial fibrillation, the statistically significant reductions in short-term mortality and morbidity (12). Moreover, one systematic review did not demonstrate any significant benefit of OPCABG compared with ONCABG regarding mortality, stroke, or MI. In contrast, patients in ONCABG group had a better long-term survival (13). In another randomized study, a total of 2539 patients 75 years of age or older were randomly assigned for elective first-time CABG to undergo OPCABG or ONCABG, there was no significant difference between two groups with

Table 2 Baseline features of propensity-matched patients

Variable	OPCABG (n=54)	ONCABG (n=54)	P value
Age (years)	61.19±11.28	59.33±14.33	0.457
Female, n (%)	12 (22.2)	15 (27.8)	0.505
Creatinine (mol/L)	106.76±27.0	116.75±45.38	0.168
HPT, n (%)	36 (66.7)	35 (64.8)	0.839
DM, n (%)	20 (37.0)	12 (22.2)	0.092
Dyslipedimia, n (%)	14 (25.9)	10 (18.5)	0.355
Current smoker, n (%)	20 (37.0)	23 (42.6)	0.555
Alcoholics, n (%)	15 (27.8)	18 (33.3)	0.531
COPD, n (%)	11 (20.4)	5 (9.3)	0.104
Stroke or TIA, n (%)	16 (29.6)	14 (25.9)	0.667
EF <40%, n (%)	15 (27.8)	20 (37.0)	0.304
No. of distal grafts	1.69±0.72	1.72±0.71	0.789
LIMA, n (%)	12 (22.2)	15 (27.8)	0.505
IABP, n (%)	24 (44.4)	33 (61.1)	0.123
Urgent surgery, n (%)	3 (5.6)	9 (16.7)	0.066

Plus-minus values are means ± SD; OPCABG, off-pump coronary artery bypass grafting; ONCABG, on-pump coronary artery bypass grafting; HPT, hypertension; DM, diabetes mellitus; COPD, chronic obstructive pulmonary disease; TIA, transient ischemia attack; EF, ejection fraction; LIMA, left internal mammary artery; IABP, intra-artery balloon pumping.

Table 3 Postoperative outcomes of propensity score matched patients

Variable	OPCABG (n=54)	ONCABG (n=54)	P value
Transfusion rate			
Plasma, n (%)	30 (55.6)	33 (61.1)	0.558
Platelet, n (%)	3 (5.6)	9 (16.7)	0.066
Intensive care unit stay	1.69±0.72	1.72±0.71	0.789
Postoperative length of stay	16 [12, 22.5]	15 [12, 19]	0.568
Tracheostomy, n (%)	9 (16.7)	5 (9.3)	0.252
Wound infection, n (%)	7 (13.0)	4 (7.4)	0.340
Reoperation, n (%)	6 (11.1)	5 (9.3)	0.750
Postoperative MI, n (%)	2 (3.7)	8 (14.8)	0.046
Stroke/TIA, n (%)	1 (1.9)	4 (7.4)	0.169
Mortality, n (%)	2 (3.7)	1 (1.9)	0.558

Data are expressed as means ± SD or median (25th percentile-75th percentile); OPCABG, off-pump coronary artery bypass grafting; ONCABG, on-pump coronary artery bypass grafting; MI, myocardial infarction; TIA, transient ischemic attack.

regard to the composite outcome of death, stroke, MI, repeat revascularization, or new renal-replacement therapy within 30 days and within 12 months after surgery (14).

In order to reduce the patient selection bias, we used propensity score analysis in this study. Our study results were consistent with the data reported (6,9,15-17). The randomized trial and observational study show OPCABG now becomes an established procedure with results comparable to ONCABG, both procedures were associated with similar early and late graft patency, incidence of recurrent or residual myocardial ischemia, need for reintervention, long-term survival, and the similar late mortality (6,9,15-17). Moreover, in comparison to ONCABG,

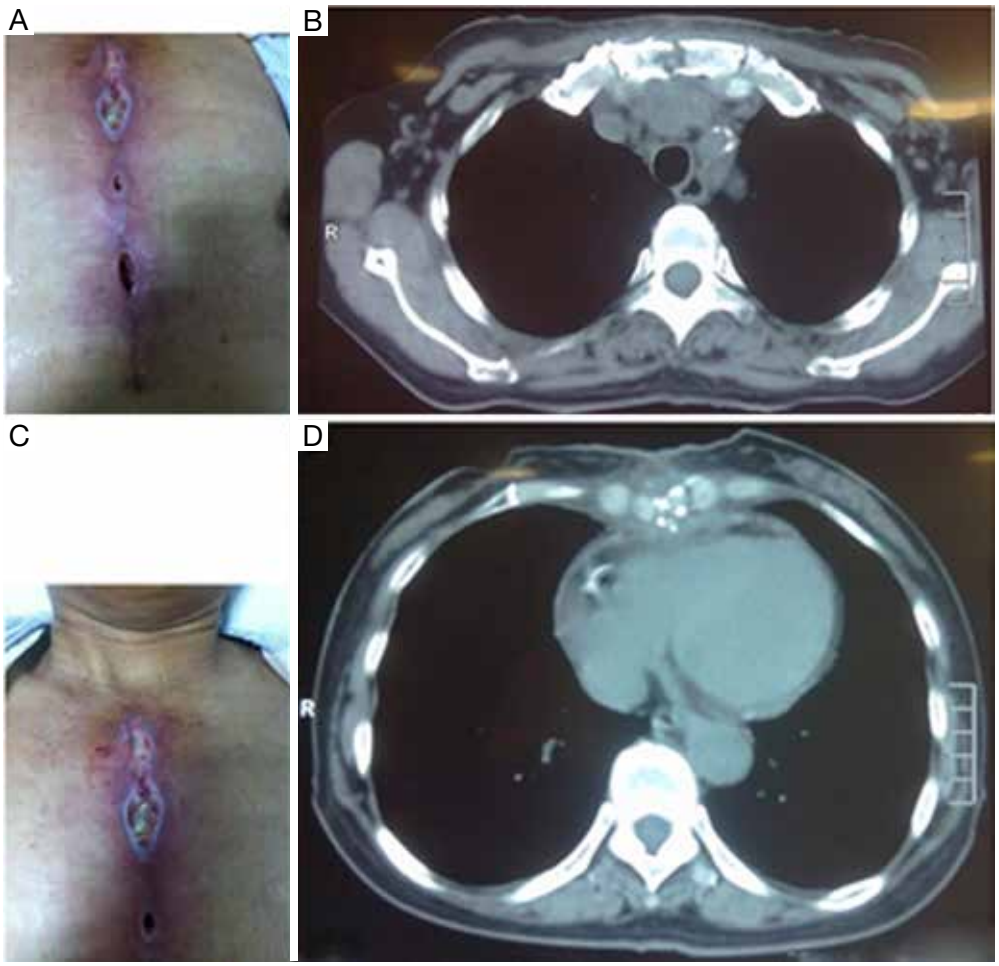

Figure 1 A female patient underwent OPCABG procedure 2.5 months earlier had chest wound dehiscence and infection (A,C), no healing of sterna wound (B) and pericardial effusion (D) demonstrated on CT scan. OPCABG, off-pump coronary artery bypass grafting.

Meta analysis demonstrates that OPCABG reduces the incidence of post-operative stroke and has no substantial effect on mortality or MI (18). Furthermore, other studies showed that OPCABG significantly reduces perioperative mortality with long-term good outcome (19-21). Use of CPB is an independent predictor of in-hospital mortality (14).

Complete revascularization remains the gold standard of CABG. The main goal of surgical revascularization is to reestablish the blood supply to the ischemic cardiac region and to prevent MI (22). One of the great concerns is the early graft patency with OPCABG did not match the excellent outcomes of conventional ONCABG (5,11). The postoperative MI in OPCABG group was higher than in OCCPB group, which might be induced by the blocked graft, as coronary angiography could not be performed in all patients with postoperative MI to verify the presence of blocked graft, it is difficult to assure the graft patency rate in CABG patients. Another concern regarding OPCABG is possible reduced quality of anastomosis on beating heart. As a patient with complete revascularization is more likely free from severe angina than one with incomplete revascularization (22).

The surgical options for ischemic heart disease should be tailed individually to optimize the benefits and minimize the risk of adverse effects, the surgeon should consider bypass options and bypass graft conduit to maximize the long-term benefits of coronary revascularization while minimizing the risks (6,23).

The cause of wound infection in our study is most likely due to the jeopardized immunity of patients leading to the growth of bacteria such as staphylococcus in surgical area, and poor healing of sternal wound and surgical wounds (24) (*Figure 1*). The preventive approach is to observe the sterilization rule carefully to sterilize the surgical wound area and use antibiotics

prophylactically (24).

Study limitations

This is an observational retrospective study rather than a randomized controlled trial; even though a propensity score matched analysis was used in this study, it still has some limitations. Only perioperative outcomes but not long-term follow up results are reported, and data on surgical revascularization rates and graft patency following surgery are not available. There is no report on the degree of stenosis of carotid artery, which is associated with stroke before or after CABG. Long-term follow-up of patients from current study will offer additional evidence.

Overall, our propensity score matching showed that patients in OPCABG group had lower postoperative MI incidence and similar other clinical outcomes in comparison with those in ONCABG group. The short-term effect of OPCABG was similar to that of ONCABG. Based on the current evidence, ONCABG should continue to be the standard surgical option. Yet, OPCABG may be an alternative when there are contraindications for cannulation of the aorta and CPB. Further long-term follow up and randomised clinical trials are warranted to address the optimal treatment.

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References

1. Takagi H, Watanabe T, Mizuno Y, et al. A meta-analysis of large randomized trials for mid-term major cardio- and cerebrovascular events following off-pump versus on-pump coronary artery bypass grafting. Interact Cardiovasc Thorac Surg 2014;18:522-4.
2. Ren S, Liu P, Ma G, et al. Long-term outcomes of synchronous carotid endarterectomy and coronary artery bypass grafting versus solely carotid endarterectomy. Ann Thorac Cardiovasc Surg 2012;18:228-35.
3. Ren S, Fan X, Peng L, et al. Expression of NF-κB, CD68 and CD105 in carotid atherosclerotic plaque. J Thorac Dis 2013;5:771-6.
4. Ren S, Li X, Wen J, et al. Systematic review of randomized controlled trials of different types of patch materials during carotid endarterectomy. PLoS One 2013;8:e55050.
5. Hattler B, Messenger JC, Shroyer AL, et al. Off-Pump coronary artery bypass surgery is associated with worse arterial and saphenous vein graft patency and less effective revascularization: Results from the Veterans Affairs Randomized On/Off Bypass (ROOBY) trial. Circulation 2012;125:2827-35.
6. Lu JC, Grayson AD, Pullan DM. On-pump versus off-pump surgical revascularization for left main stem stenosis: risk adjusted outcomes. Ann Thorac Surg 2005;80:136-42.
7. Dohi M, Miyata H, Doi K, et al. The off-pump technique in redo coronary artery bypass grafting reduces mortality and major morbidities: propensity score analysis of data from the Japan Cardiovascular Surgery Database. Eur J Cardiothorac Surg 2014. [Epub ahead of print].
8. Börgermann J, Hakim K, Renner A, et al. Clampless off-pump versus conventional coronary artery revascularization: a propensity score analysis of 788 patients. Circulation 2012;126:S176-82.
9. Yeatman M, Caputo M, Ascione R, et al. Off-pump coronary artery bypass surgery for critical left main stem disease: safety, efficacy and outcome. Eur J Cardiothorac Surg 2001;19:239-44.
10. Yeatman M, Caputo M, Narayan P, et al. Magnesium-supplemented warm blood cardioplegia in patients undergoing coronary artery revascularization. Ann Thorac Surg 2002;73:112-8.
11. Takagi H, Matsui M, Umemoto T. Off-pump coronary artery bypass may increase late mortality: a meta-analysis of randomized trials. Ann Thorac Surg 2010;89:1881-8.
12. Wijeysundera DN, Beattie WS, Djaiani G, et al. Off-pump coronary artery surgery for reducing mortality and morbidity: meta-analysis of randomized and observational studies. J Am Coll Cardiol 2005;46:872-82.
13. Møller CH, Penninga L, Wetterslev J, et al. Off-pump versus on-pump coronary artery bypass grafting for ischaemic heart disease. Cochrane Database Syst Rev 2012;3:CD007224.
14. Diegeler A, Börgermann J, Kappert U, et al. Off-pump versus on-pump coronary-artery bypass grafting in elderly patients. N Engl J Med 2013;368:1189-98.
15. Puskas JD, Williams WH, O'Donnell R, et al. Off-pump and on-pump coronary artery bypass grafting are

associated with similar graft patency, myocardial ischemia, and freedom from reintervention: long-term follow-up of a randomized trial. Ann Thorac Surg 2011;91:1836-42; discussion 1842-3.
16. Fattouch K, Runza G, Moscarelli M, et al. Graft patency and late outcomes for patients with ST-segment elevation myocardial infarction who underwent coronary surgery. Perfusion 2011;26:401-8.
17. Virani SS, Lombardi P, Tehrani H, et al. Off-pump coronary artery grafting in patients with left main coronary artery disease. J Card Surg 2005;20:537-41.
18. Sá MP, Ferraz PE, Escobar RR, et al. Off-pump versus on-pump coronary artery bypass surgery: meta-analysis and meta-regression of 13,524 patients from randomized trials. Rev Bras Cir Cardiovasc 2012;27:631-41.
19. Hong S, Youn YN, Yi G, et al. Long term results of ST-segment elevation myocardial infarction versus non-ST-segment elevation myocardial infarction after off-pump coronary artery bypass grafting: propensity score matching analysis. J Korean Med Sci 2012;27:153-9.
20. Mack MJ, Brown P, Houser F, et al. On-pump versus off-pump coronary artery bypass surgery in a matched sample of women: a comparison of outcomes. Circulation 2004;110:II1-6.
21. Godinho AS, Alves AS, Pereira AJ, et al. On-pump versus off-pump coronary-artery bypass surgery: a meta-analysis. Arq Bras Cardiol 2012;98:87-94.
22. Yi G, Youn YN, Joo HC, et al. Association of incomplete revascularization with long-term survival after off-pump coronary artery bypass grafting. J Surg Res 2013;185:166-73.
23. Tranbaugh RF, Dimitrova KR, Lucido DJ, et al. The second best arterial graft: a propensity analysis of the radial artery versus the free right internal thoracic artery to bypass the circumflex coronary artery. J Thorac Cardiovasc Surg 2014;147:133-40.
24. Ren S, Sun G, Yang Y, et al. Management of concomitant large aortic aneurysm and severe stenosis of aortic arc. Ann Thorac Cardiovasc Surg 2014;20:84-7.

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Original Article

The carina is approximately 1-2 cm above the pericardial reflection among Chinese patients

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Background: Central venous catheters (CVCs) and central venous pressure (CVP) monitor is essential in fluid resuscitation and management for critically ill patients. Accuracy of the CVP is mainly dependent on the proper position of the catheter tip. Although the X-ray visible carina was generally recommended as the alternative of pericardial reflection (PR) to guide the placement of CVCs, few data was available with respect to the distance between the carina and PR among Chinese patients. The purpose of this study was to explore the topographic relationship between the trachea carina and PR among Chinese patients by using computed tomography (CT) images.

Methods: CT images of 172 patients who underwent CT pulmonary angiogram or CT angiogram for aorta from January 1, 2013 to November 30, 2013 were retrospectively reviewed. Distances between upper margin of the right clavicular notch, trachea carina, PR and atriocaval junction (ACJ) were calculated using the table positions on axial images.

Results: The mean length of extrapericardial superior vena cava (SVC) was 2.5 cm. For all patients, the PR was lower than the carina by average 1.6 cm.

Conclusions: Given the PR was average 1.6 cm lower than the carina among Chinese patients, placing the CVCs tip approximate 1.6 cm lower the carina among Chinese patients would be more likely to result in a satisfactory placement.

Keywords: Central venous catheter (CVC); carina; pericardial reflection (PR)

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Introduction

Central venous catheters (CVCs) and central venous pressure (CVP) monitor is essential in fluid resuscitation and management for critically ill patients. Accuracy of the CVP is mainly dependent on the proper position of the catheter. It is generally recommended that the CVC tip should lie in the superior vena cava (SVC) and outside the pericardial sac, so as to guarantee the catheter performance and spare the patient from life-threatening complications such as arrhythmia and pericardial tamponade.

However, it is very difficult to put the CVCs tips to the recommended position in clinical practice because the recommended pericardial reflection (PR) is not palpable and can not be seen on chest X-ray. Therefore, the carina, easily visualized on the chest X-ray, is alternatively used as the radiographic landmark to guide of the placement of the CVCs (1-4). Nevertheless, few data is available so far as to whether the recommended carina is also a good landmark for PR among Chinese patients. Thus, this study was designed to explore the topographic relationship between the trachea carina and PR among Chinese patients by using computed tomography (CT) images.

Methods

The study was a retrospective review of CT of all patients underwent CT pulmonary angiogram or CT angiogram

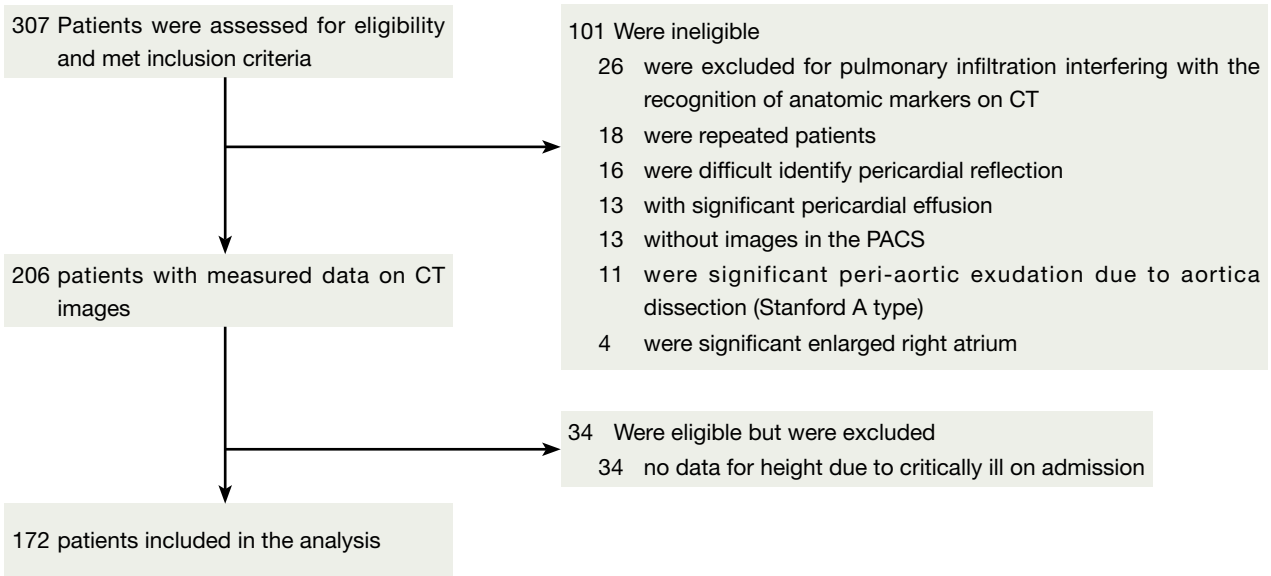

Figure 1 Schematic chart of the enrollment of the study patients.

for aorta from January 1, 2013 to November 30, 2013, to examine the distance between the carina and PR among Chinese patients. The institutional review board of Sir Run Run Shaw hospital approved the study protocol and waived from the need for a consent form. All CT examinations were performed for clinical indications. Patients with unrecognizable anatomic markers, significant pericardial effusion, peri-aortic exudation or significant enlarged right atrium were excluded (*Figure 1*).

All examinations were performed on routine clinical CT scanners (SENSATION 16, SIEMENS, Germany). In all patients, the imaging was performed at end-inspiration. A tube current of 120 kV and rotation time of 0.5 s was used. Slice thickness varied between 0.75 and 2 mm, depending on the clinical protocol used. Images were evaluated on a standard clinical picture archiving and communication system workstation, and the reader of CT images was blinded to patient data. The preselected radiographic landmarks, most often used in our clinical practice, included the upper margin of the right clavicular notch (RSCJ), carina, PR, and atriocaval junction (ACJ). First of all, the ACJ was determined by the method described by Ridge and colleagues (5), i.e., localizing on the sagittal reformatted CT image as the midpoint of an oblique line drawn from the crista terminalis anteriorly to the crista dividens posteriorly. This point was then cross-referenced with axial CT images and the calibrated anteroposterior scout topogram to determine its horizontal level (5). After recognition of the above landmarks on CT images (*Figure 2*), likewise, the horizontal level of the RSCJ, carina and PR were also marked on the scout topogram. Thereafter, distances (in centimeters) between RSCJ, trachea carina, PR and ACJ were calculated using the table positions on axial images. The distance between PR and lower level of the junction of left and right innominate veins was referred as the length of extrapericardial SVC.

Statistical analysis

Descriptive data were reported as mean, standard deviation, and range. The relationship between RSCJ-ACJ (distance between upper margin of the right clavicular notch and atriocaval junction), RSCJ-Carina (distance between upper margin of the right clavicular notch and carina), RSCJ-PR (distance between upper margin of the right clavicular notch and pericardial reflection), Carina-PR (distance between carina and pericardial reflection) and patients' height were examined, using a linear regression model. Statistical analysis was performed, using SPSS 16.0 (Chicago, Ill, USA). Significance was defined as a P value <0.05.

Results

From January 2013 to November 2013, 172 patients were enrolled in this study (*Figure 1*). Of them, 122 were men

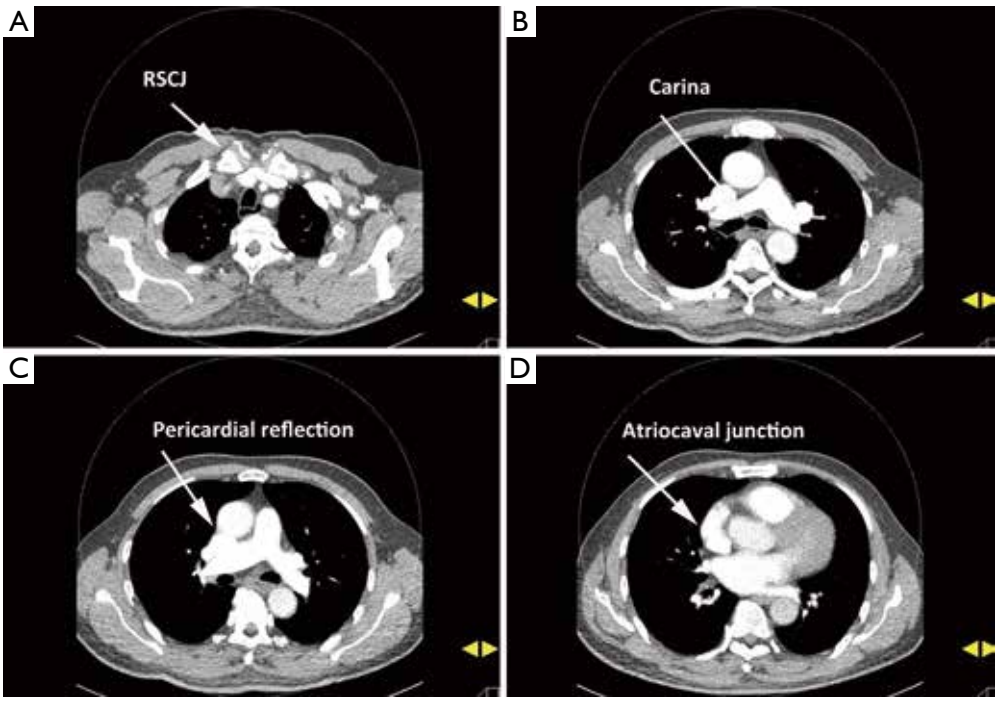

Figure 2 Demonstration of the anatomical landmarks used on CT for measurements. (A) RSCJ (upper margin of the right clavicular notch); (B) carina; (C) pericardial reflection; (D) atriocaval junction. CT, computed tomography.

Table 1 Summary of distance measurements between anatomical landmarks in CT

Distance (cm)	Mean ±SD (cm)	Range (cm)
RSCJ-Carina	6.1±1.5	2.9-10.1
Carina-PR	1.6±0.5	0.4-2.8
PR-ACJ	3.0±0.9	1.3-5.4
Extrapericardial SVC	2.5±1.0	0.3-4.8
RSCJ-PR	7.7±1.5	4.3-11.3
RSCJ-ACJ	10.7±1.8	6.6-15.9

CT, computed tomography; RSCJ, upper margin of the right clavicular notch; PR, pericardial reflection; ACJ, atriocaval junction; SVC, superior vena cava; SD, standard deviation.

and 50 were women, with a mean age of 53 years old (range, 18-80 years old).

Anatomical presentation in CT

The extrapericardial SVC, target segment of recommended CVCs tips placement, had a mean length of 2.5 cm, ranging from 0.3 to 4.8 cm. The average distances between upper margin of the right clavicular notch and carina and PR were 6.1 cm (range, 2.9-10.1 cm) and 7.7 cm (range, 4.3-11.3 cm) respectively. For all patients, the PR was lower than the carina. On average, the PR was 1.6 cm below the carina (*Table 1*).

Correlation between the carina-pericardial reflection distance and height

As expected, the distances between upper margin of the right clavicular notch and carina, PR and ACJ were linear correlated with height (*Figure 3*). Consequently, distance between carina and PR was equivalent to –1.714+0.02× height (cm) (*Figure 3*).

Discussion

Our *in vivo* CT measurements demonstrated that the mean length of extrapericardial SVC was 2.5 cm and carina was averagely 1.6 cm higher than the PR among Chinese patients. Given this, placing the tips of CVCs averagely 1.6 cm lower the carina would be more reasonable among Chinese patients.

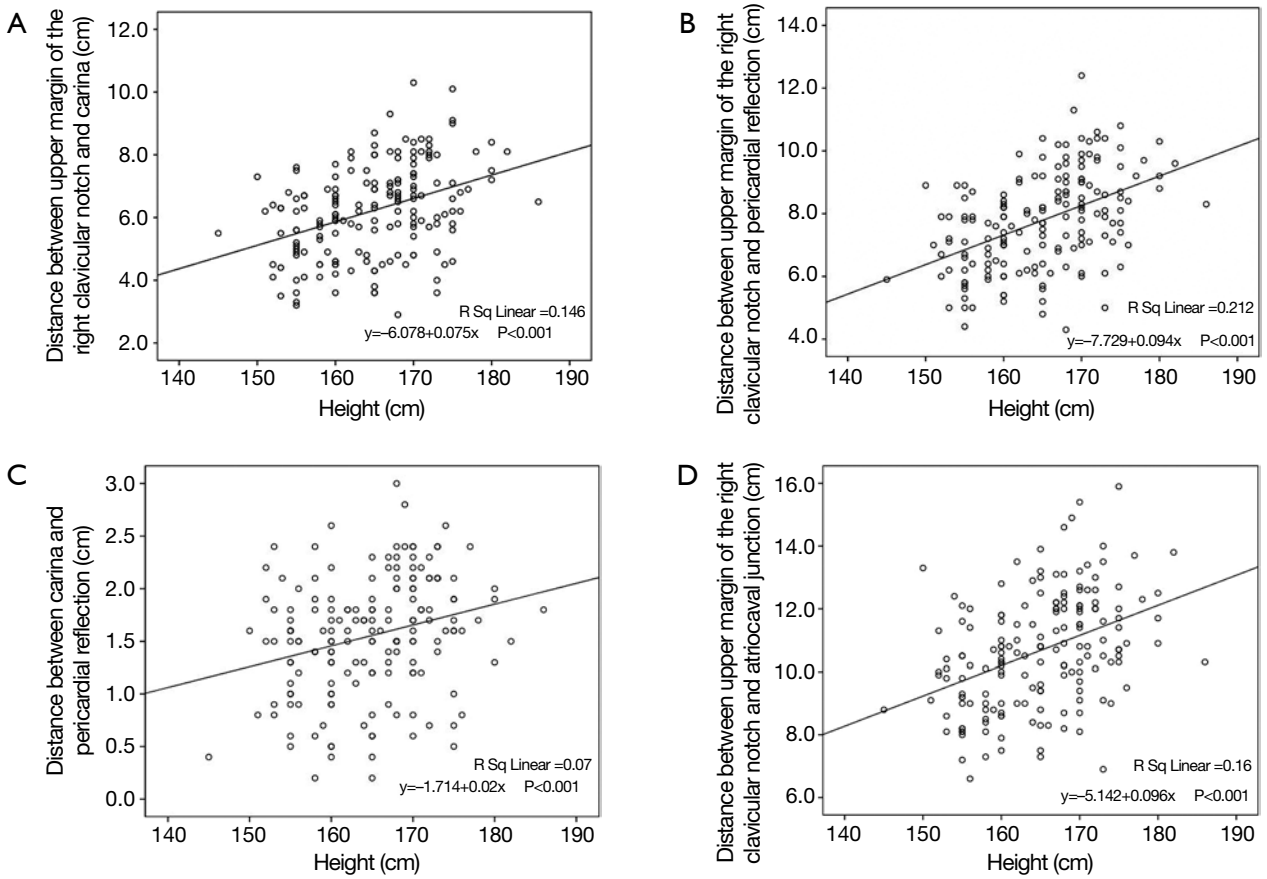

Figure 3 Correlation between RSCJ-Carina (A), RSCJ-PR (B), Carina-PR (C), RSCJ-ACJ (D), and patients' height. RSCJ-Carina, distance between upper margin of the right clavicular notch and carina; RSCJ-PR, distance between upper margin of the right clavicular notch and pericardial reflection; Carina-PR, distance between carina and pericardial reflection; RSCJ-ACJ, distance between upper margin of the right clavicular notch and atriocaval junction.

The incidence of intraatrial CVC tip position after conventional placement techniques ranges from 8% to 47% (6,7). Whilst incidence of vessel perforation associated with CVC placement ranged from approximately 0.25% to 0.4%. Vascular perforation or cardiac tamponade is rare but is one of the most serious complications in relation to CVCs. Perforation of the SVC is probably more likely with left-sided than with right-sided CVCs (8). This is influenced by the steep angle the left innominate vein makes with the SVC. Here the catheter will abut the wall of the SVC unless the tip is advanced around the curve into the lower SVC or right atrium. It has been shown in the laboratory that an angle of the CVC tip to vessel wall of greater than 40° is more likely to lead to vessel wall perforation (8). To prevent such complications, many suggestions have been made for assessing correct placement of CVCs, most based on clinical investigations and analysis of chest X-rays. Besides parallel to the vessel wall, the carina was firstly proposed by Schuster *et al*. (1) as a reliable landmark for the tip of CVCs. Nevertheless, optimal position of the CVC tip remains a subject of debate.

In the present study, the PR was always below the carina, with a mean distance of 1.6 cm. This was consistent with previous studies in ethanol-formalin-fixed (1) or fresh cadavers (2), in those the carina was 0.4 and 0.8 cm mean above the PR respectively. The gap of the distances between various studies may be explained by that tissue shrinkage in cadavers. Although in no case in this study and some other studies (1,2) was the carina located below the PR, a recent study of topographic analysis based on CT demonstrated 30% of the patients had a pericardium ending above the carina, with a maximum distance of 2.5 cm (9). The exact

reasons for the variance were not elucidated. This may be because Chinese patients are somewhat anatomically different from other races with respect to the relation of carina and PR.

The influence of possible confounders such as age, weight, height, or gender on the distance between carina and PR is also of interest. The study demonstrated the distance between carina and pericardial reflection was equivalent to –1.714+0.02× height (cm). Given vast majority Chinese patients' height was approximate 160 to 170 cm, using the carina as a surrogate for the PR to guide the tip position of CVCs may underestimate the depth of catheter insertion by around 1.6 cm.

Nevertheless, to place a CVC tip in the optimal position is still challenging. Uchida and colleagues (10) demonstrated the appropriate length of CVC inserted through the right internal jugular vein or right subclavian vein could be estimated by the "calculated measurement" of adding half the length of the right clavicle and the vertical length between the sternal head of the right clavicle and the carina on the previous X-ray. Meanwhile, Dulce and colleagues (9) suggested that a higher percentage of extrapericardial placements of the CVC would be achieved by using 85% of the SCJ-to-carina distance for orientation, with similar correct placement in 86% of case in CT. Taken ours and above studies together, using the calculated measurement of the distance from insertion point and carina on previous chest X-ray and a fixed distance approximate 1.6 cm below the carina appears to be the most practicable method to optimize the CVCs placement among Chinese patients.

Conclusions

In summary, the mean length of extrapericardial SVC was 2.5 cm and the PR was average 1.6 cm lower than the carina among Chinese patients.

Acknowledgements

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References

1. Schuster M, Nave H, Piepenbrock S, et al. The carina as a landmark in central venous catheter placement. Br J Anaesth 2000;85:192-4.
2. Albrecht K, Nave H, Breitmeier D, et al. Applied anatomy of the superior vena cava-the carina as a landmark to guide central venous catheter placement. Br J Anaesth 2004;92:75-7.
3. Kim MC, Kim KS, Choi YK, et al. An estimation of right- and left-sided central venous catheter insertion depth using measurement of surface landmarks along the course of central veins. Anesth Analg 2011;112:1371-4.
4. Ryu HG, Bahk JH, Kim JT, et al. Bedside prediction of the central venous catheter insertion depth. Br J Anaesth 2007;98:225-7.
5. Ridge CA, Litmanovich D, Molinari F, et al. Radiographic evaluation of central venous catheter position: anatomic correlation using gated coronary computed tomographic angiography. J Thorac Imaging 2013;28:129-33.
6. Wirsing M, Schummer C, Neumann R, et al. Is traditional reading of the bedside chest radiograph appropriate to detect intraatrial central venous catheter position? Chest 2008;134:527-33.
7. Gebhard RE, Szmuk P, Pivalizza EG, et al. The accuracy of electrocardiogram-controlled central line placement. Anesth Analg 2007;104:65-70.
8. Stonelake PA, Bodenham AR. The carina as a radiological landmark for central venous catheter tip position. Br J Anaesth 2006;96:335-40.
9. Dulce M, Steffen IG, Preuss A, et al. Topographic analysis and evaluation of anatomical landmarks for placement of central venous catheters based on conventional chest X-ray and computed tomography. Br J Anaesth 2014;112:265-71.
10. Uchida Y, Sakamoto M, Takahashi H, et al. Optimal prediction of the central venous catheter insertion depth on a routine chest x-ray. Nutrition 2011;27:557-60.

Original Article

Meis1 regulates proliferation of non-small-cell lung cancer cells

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Abstract: As one of the most common cancers, lung cancer remains to be a major public health problem. Non-small-cell lung adenocarcinoma cancer exhibits higher resistance to chemotherapy than small cell lung cancer, which requires novel strategies. To further understand underlying mechanisms for non-small-cell lung adenocarcinoma cancer cell proliferation, we explored the role of Meis1 in non-small-cell lung adenocarcinoma cancer cells. The results show that Meis1 inhibits non-small-cell lung cancer (NSCLC) cell proliferation. Specific knockdown of Meis1 resulted in strengthened proliferative ability of non-small-cell lung adenocarcinoma cancer cells. Cell cycle analysis indicated that DNA synthesis was increased when Meis1 was down-regulated specifically. As well as histone H3 phosphorylation, which is indicative of mitosis. More importantly, forced Meis1 expression repressed the proliferation of non-small-cell lung adenocarcinoma cancer cell. These data demonstrated Meis1 limits the proliferation of non-small-cell lung adenocarcinoma cancer cell and could potentially represent a therapeutic strategy that may control non-small-cell lung adenocarcinoma cancer cell proliferation.

Keywords: Meis1; non-small-cell lung cancer (NSCLC); proliferation; cell cycle

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Introduction

Meis1 is a member of TALE (3-amino-acid loop extension) family of homeodomain transcription factors, which interacts with Hox transcription factors and promotes their target genes expression (1). Previous study has indicated that Meis1 activates HIF1α and HIF2α to inhibit ROS generation in hematopoietic stem cells (2,3). Meis1 deficiency resulted in underdeveloped hematopoietic stem cells compartment and embryonic vascular patterning (4-6). The roles of Meis1 in cancer were revealed by research on myeloid leukemia cells (7,8). These studies have proved a proliferative role of Meis1 in myeloid leukemia cells (9,10). Furthermore, Meis1 was also accumulated in neuroblastoma (11-13). Therefore, a putative oncogenic role of Meis1 has been postulated (14). However, Meis1 also inhibits neonatal and adult cardiomyocytes proliferation by modulating cell cycle progression (15). These data suggest complicated roles of Meis1 in cell proliferation.

Lung and bronchus is one of the three most common cancers in both men and women. It has been reported that more than 20% death was caused by lung cancer in all Americans which are expected to die of cancer (16). Briefly, lung cancer is subgrouped into small cell lung cancer and non-small-cell lung cancer (NSCLC). Although chemotherapy, radiotherapy and surgical resection have some benefits for NSCLC, these traditional strategies remain inefficient. To shed new lights on NSCLC treatment, mechanistic insight into NSCLC development and progression are urgently demanded, which also implies novel molecular and cellular targets for treatment.

In the current search, we demonstrated an inhibitory role of Meis1 in NSCLC cell proliferation. Down-regulation of Meis1 resulted in induced proliferation in NSCLC cells. Cell cycle analysis indicated enhanced cell cycle progression after Meis1 expression was compromised. Accordingly, ectopic expression of Meis1 plasmid repressed the proliferation of NSCLC cells. Taken together, NSCLC cell proliferation is limited by Meis1.

Methods

Plasmids, cell culture and stable cell line construction

NSCLC adenocarcinoma cancer cell line A549 was purchased from ATCC and cultured in Dulbecco's modified Eagle's medium (DMEM) (Hyclone) containing 10% FBS (Hyclone). SPC-A1 was purchased from cell bank of Chinese Academic of Sciences. Full length of Meis1 cDNA was obtained and then was cloned into pLNCX plasmid. After transfected into 293 packing cells, the supernatant was added directly into A549 cells. After 48 h, infected cells were selected with neomycin.

RNA interference (RNAi)

Meis1 RNAi (gcucaguagcuuaagggaaTT and uucccuuaagcuacugagcTT) was synthesized in GenePharma (Shanghai, China). Cells (5×10^5) were incubated in 35-mm plates with mixture of RNAi (final concentration, 50 nM) and Lipofectamine RNAiMAX Reagent (Invitrogen) in serum-free medium for 6 h. Cells were then grown in media with 10% FBS.

EdU

After treated as described, cells were incubated with 10 μM EdU for 8 h and then fixed by 4% paraformaldehyde. EdU was detected with Click-iT EdU cell proliferation kit following the instruction (Invitrogen). Nuclei were visualized by DAPI.

Cell proliferation assay

Approximate 8,000 cells treated were plated into each well of 96-well culture plates. The cell medium was replaced with 20 μL 3-(4,5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide (MTT) (5 mg/mL) (Molecular Probes) in DMEM. After incubation at 37 ℃ for 2 h, the MTT solution was removed. A total of 100 μL DMSO was added to dissolve precipitate for 10 min at room temperature. Absorbance was recorded at 540 nm using a Spectramax M2 microplate reader (Molecular Devices).

qRT-PCR

RNA was isolated by TRIzol (Invitrogen). cDNA synthesis was performed with the High Capacity cDNA Archive Kit (Applied Biosystems) following the manufacturer's instructions. Transcript levels were determined using the StepOne/StepOnePlus™ Real-time PCR System (Applied Biosystems) using SYBR Green PCR Master mix according to the manufacturer's instructions. The primer sequences are Meis1: F-TCCCCAGCACAGGTGACGATGAT, R-CTTCCCCCTTGCTTTGCGATTGGT; Arf: F-AAATGAGAAGAAAGAAACC, R-GTTGTAATAGGAGTGGAA and Cdkn1a: F-AGGTGGACCTGGAGACTC, R-CGGCGTTTGGAGTGGTAG.

Western blotting

Cells were collected and washed with ice-cold PBS and lyzed in lysis buffer (50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 0.25% Triton-X 100, phosphatase inhibitors cocktail and protein inhibitor cocktail) for 30 min on ice and then centrifuged (13,000 g, 10 min, 4 ℃). After centrifugation, the supernatants were collected. The blots were reacted with antibodies for Meis1 (Santa Cruz Biotechnology) followed by horseradish peroxidase (HRP)-conjugated secondary antibody (Jackson ImmunoResearch Laboratories). Chemiluminescence was detected with ECL western blot detection kits (Cell Signaling Technology).

Immunofluorescence

Cells were fixed with 4% paraformaldehyde, permeabilized with PBST (PBS plus 0.1% Triton X-100) and blocked with 2% BSA plus 1% goat or donkey serum in PBT. Slides were immunostained with primary antibody in blocking solution as indicated. The species-specific fluorescent secondary IgG (Invitrogen) were also diluted in blocking solution. After counterstained with DAPI, the images were captured by florescence microscope (Leica).

Statistical analysis

A total of 3 to 5 replicates were performed for all of the experiments. The data are presented as the mean and standard error of the mean (SD). Comparisons within groups were conducted using a *t*-test with repeated measures; the P values indicated in the figures are <0.05 (*), <0.01 (**) and <0.001 (***).

Results

Specific down-regulation of mesi1 in NSCLC cancer cells

The roles of Meis1 in NSCLC adenocarcinoma remain poorly understood. In order to explore this, we utilized RNAi to repress the expression of endogenous Meis1

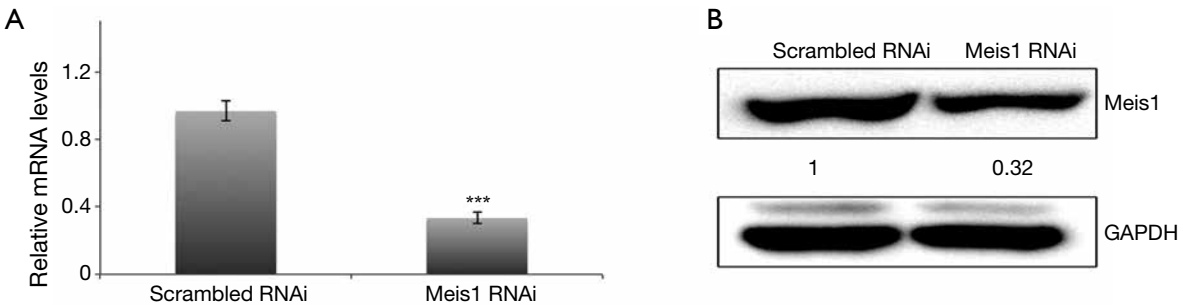

Figure 1 Efficiency of Meis1 RNAi sequence. (A) A549 cells were transfected with RNAi as indicated for 72 h and then the mRNA levels were determined with specific primers (mean ± SD, n=3; ***, P<0.001); (B) cells were treated as in (A) and then subjected to western blotting analysis. The experiments were performed for at least three times and representative images were shown. RNAi, RNA interference; SD, standard error of the mean.

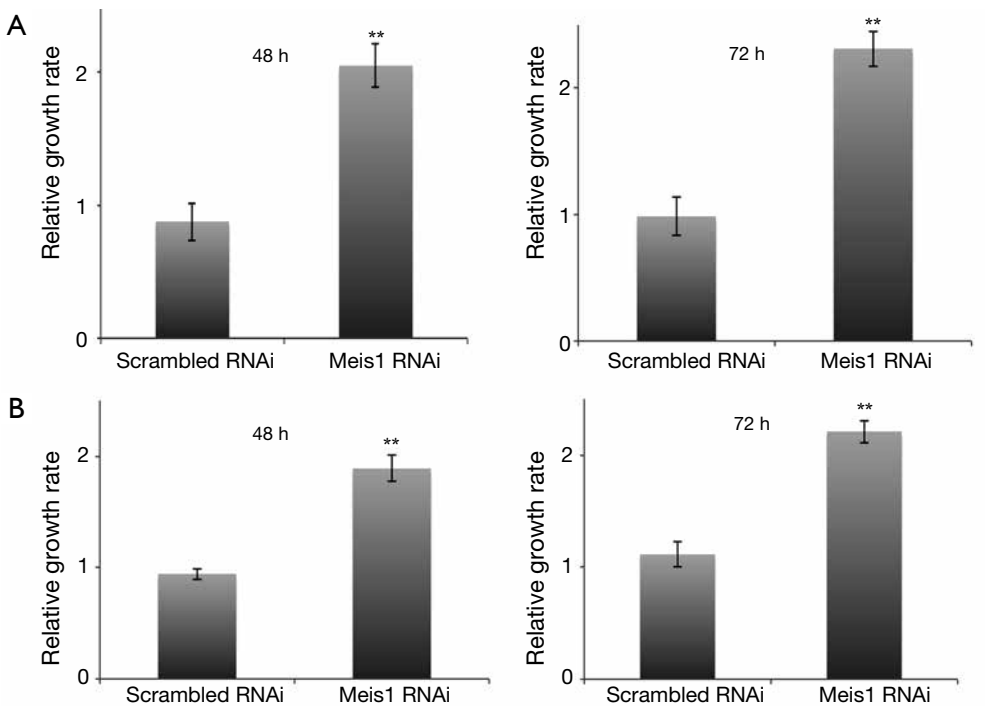

Figure 2 Meis1 RNAi inhibits NSCLC cancer cell proliferation. (A) A549 cells were transfected as indicated for 48 or 72 hr. At the time point, cells were collected for MTT assay (mean ± SD, n=4; **, P<0.01); (B) scrambled and Mesi1 RNAi were transfected into SPC-A1 cells as indicated. Cells were then collected for MTT assay (mean ± SD, n=4; **, P<0.01). RNAi, RNA interference; NSCLC, non-small-cell lung cancer; SD, standard error of the mean.

in A549 cells. An RNAi sequence was selected and then examined for its inhibitory efficiency. Compared to scrambled RNAi, Both mRNA and protein levels of Meis1 were largely decreased by RNAi (*Figure 1*).

Meis1 inhibits NSCLC cell proliferation

We further examined whether Meis1 affects NSCLC adenocarcinoma cell proliferation. We transfected Meis1 RNAi into two NSCLC cell lines A549 and SPC-A1. In A549 cells, down-regulation of Meis1 resulted in increased proliferation (*Figure 2A*). A comparable increase was also observed in SPC-A1 cells (*Figure 2B*). These data indicated that Meis1 represses NSCLC cancer cell proliferation.

Meis1 inhibit cell cycle entry of NSCLC cancer cell

If compromised Meis1 expression results in increased

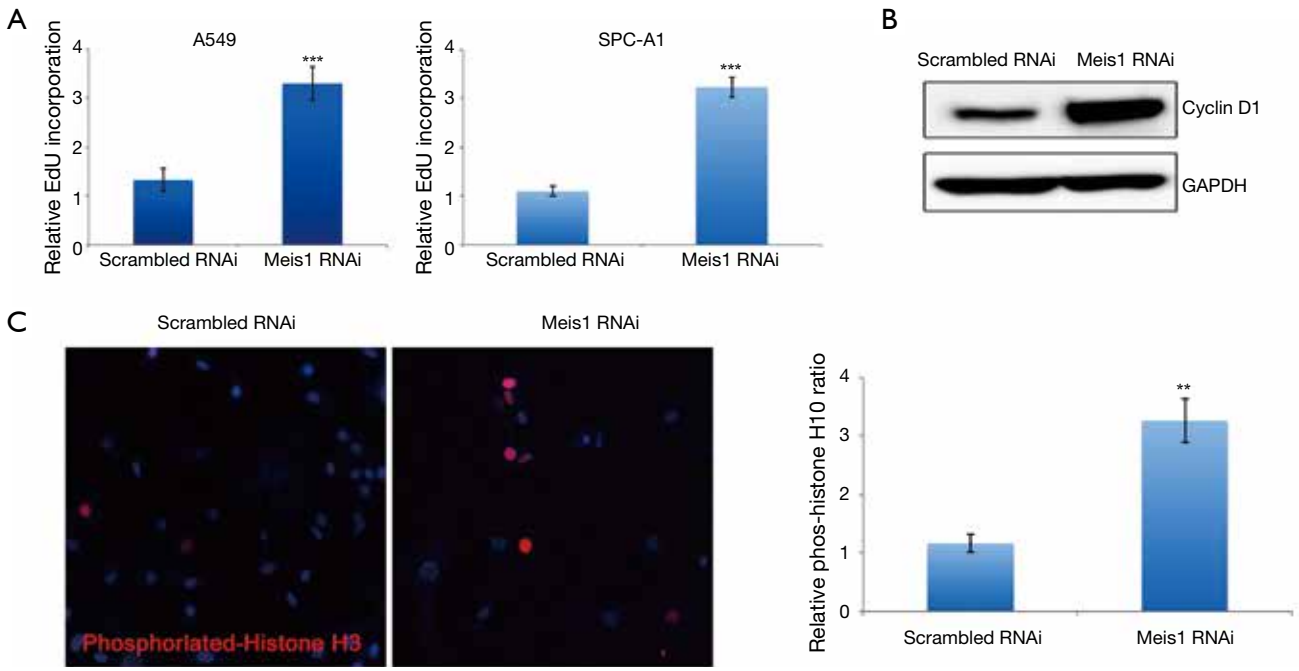

Figure 3 Meis1 regulates cell cycle in NSCLC cancer cell. (A) After RNAi transfected in A549 cells (left panel) or SPC-A1 (right panel), EdU was added into cells and cultured for 8 h before collection for EdU analysis (mean ± SD, n=3; ***, P<0.001); (B) A549 cells were transfected with RNAi as indicated. After 72 h, cells were collected and then lysed for western blotting analysis. Specific antibodies were used as indicated. Representative images from three independent experiments were shown; (C) cells were prepared described in (A) and then subjected for immunofluorescence. Phosphorylated histone H3 was stained in red and nuclei were counterstained with DAPI (Blue). Representative images from three parallel experiments were shown (mean ± SD, n=4; **, P<0.01). NSCLC, non-small-cell lung cancer; RNAi, RNA interference; SD, standard error of the mean.

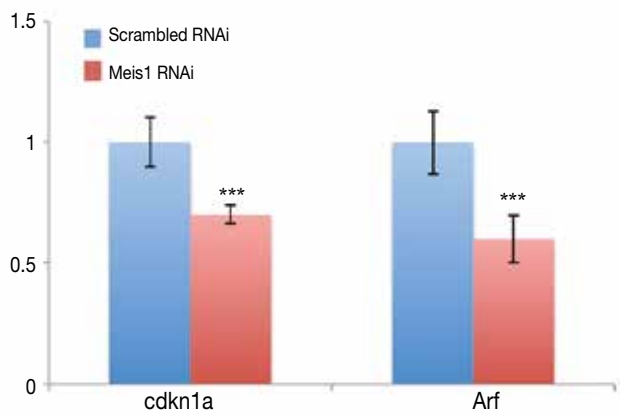

Figure 4 The expression of cdkn1a and arf in A549 cells transfected with Meis1 RNAi or scramble RNAi. Significantly decreased Arf as well as cdkn1a expression was observed in A549 cells after transfected with Meis1 RNAi as indicated for 72 h (mean ± SD, n=3; ***, P<0.001). RNAi, RNA interference; SD, standard error of the mean.

proliferation, the cell cycle progression is supposed to be accelerated. To test this hypothesis, we then examined S phase entry by monitoring DNA synthesis. EdU is a nucleoside analog that can be incorporated into newly synthesized DNA to indicate DNA synthesis. We then monitored EdU incorporation. As expected, increased EdU incorporation was observed after Meis1 expression was compromised in both A549 and SPC-A1 (*Figure 3*). Consistently, increased cyclin D1 was also detected (*Figure 3B*). Furthermore, the mRNA levels of Cdkn1a and Arf, two CDK inhibitors were significantly down-regulated after silencing the expression of Meis 1 in A549 lung cancer cells (*Figure 4*). These data indicated Meis1 inhibits the cell cycle entry of NSCLC cancer cell. To further explore the whole cell cycle progression, we examined the mitosis by detecting the phosphorylation of Histone H3 at serine10, a marker of M phase. Consistent with the increased DNA synthesis, increased mitosis was observed after Meis1 expression was inhibited, denoted by Histone H3 at

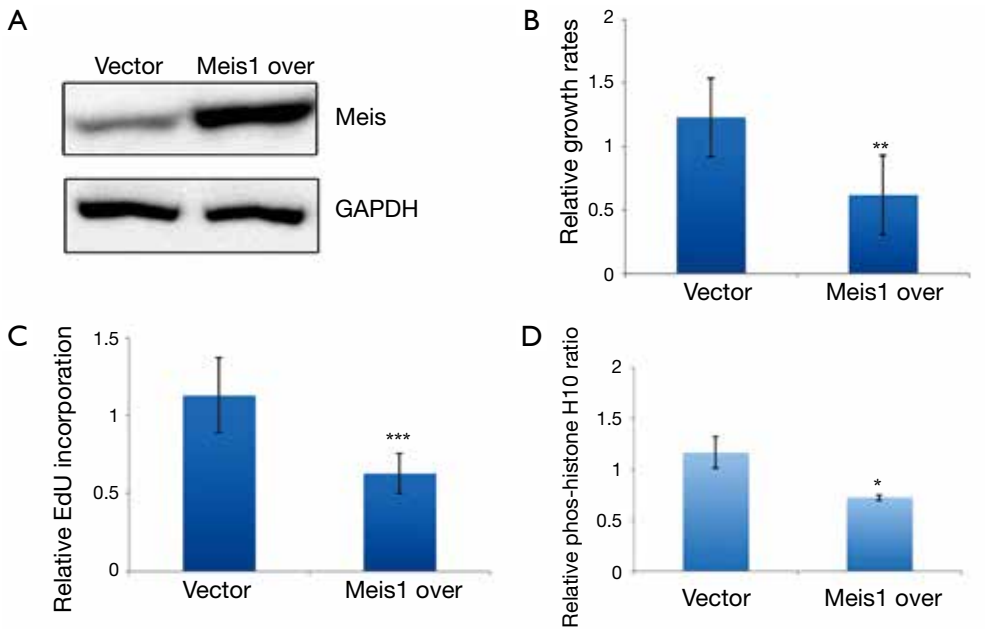

Figure 5 Accumulated Meis1 compromised NSCLC cancer cell proliferation. (A) Meis1 upregulation was confirmed by western blotting analysis in Meis1 overexpressed A549 cells. Representative images from three independent experiments were shown; (B) cell proliferation of Meis1 overexpressed A549 cells were determined by MTT assay (mean ± SD, n=3; **, P<0.01); (C) Meis1 overexpressed A549 cells were incubated with EdU for 8 h. The cells were then subjected for EdU detection (mean ± SD, n=3; ***, P<0.001); (D) quantification of phosphorylated histone H3 positive cells in control and Meis1 overexpressed A549 cells (mean ± SD, n=4; *, P<0.05). NSCLC, non-small-cell lung cancer; SD, standard error of the mean.

serine10 phosphorylation (*Figure 3C*). Collectively, these data indicated that Meis1 regulates cell cycle progression in NSCLC cancer cell.

NSCLC cancer cell proliferation was compromised by ectopic Meis1 expression

As endogenous Meis1 inhibits NSCLC cancer cell proliferation, we wondered to know whether increased Meis1 expression inhibits NSCLC cancer cell proliferation. We then established a Meis1 stably expressed A549 cells. Induced Meis1 expression was confirmed (*Figure 5A*). By using this cell line, we found that the proliferative rate was decreased compared to empty vector-transfected cells (*Figure 5B*). Similarly, DNA synthesis was down-regulated in Meis1 overexpressed A549 cells (*Figure 5C*). Consistently, reduced phosphorylation of Histone H3 at serine10 was observed in Meis1 overexpressed A549 cells (*Figure 5D*). Collectively, these data indicated increased Meis1 can inhibit NSCLC cancer cell proliferation.

Discussion

Meis1 has already been linked with proliferation in both normal and cancerous cells. In normal hematopoiesis stem cells and cardiomyocytes, Meis1 functions as an inhibitory protein to regulate these cell proliferations (15). The putative mechanisms for this regulation of Meis1 are modulation of cell cycle progression. Meis1 also limits cell cycle entry of hematopoietic stem cells, which in turn contributes to hematopoietic stem cells compartment (2). Furthermore, the expressions of cdkn1a and Ink4b-Arf-INK4a were largely compromised in Meis1 deficient cardiomyocytes, which then lead to cell cycle entry (15). In contrast, Meis1 exhibits a proliferative role in several cancerous cells such as myeloid leukemia cells and neuroblastoma (10,13). In myeloid leukemia cells, Meis1 cooperates with Hox9 and Pbx to activate the expression of Bmi1 and c-Myb, which then promote the proliferation (10). These observations suggest oncogenic roles of Meis1 in cancerous cells. However, our data indicated that Meis1 exhibits an inhibitory role for NSCLC cancer cell,

suggesting the functions of Meis1 in cancerous are also diverse. These data indicate complicated roles of Meis1 in proliferation which may largely depend on cell context.

In the present study, we examined the regulation of cell cycle progression by Meis1 in NSCLC cancer cell. The EdU and cyclin D1 analysis indicated that cell cycle entry was indeed increased when Meis1 expression was compromised. This situation is similar with the alterations in hematopoiesis stem cells and cardiomyocytes induced by Meis1 deficiency, indicating a role of Meis1 in promoting cell quiescence to suppress the cell cycle entry. As a transcriptional co-regulator, Meis1 might affect cell cycle machinery by promoting downstream genes expression. As a transcriptional factor, the regulation of proliferation by Meis1 is possibly due to its transcriptional targets. Previous studies have indicated Meis1 promotes expression of CDK inhibitors. In neonatal cardiomyocytes, Meis1 promotes the up-regulation of Cdkn1a and Arf to limit the proliferative potential. Thus, it is reasonable to hypothesize that similar mechanisms may also exist in NSCLC cancer cells. In our experiment, we therefore measured the expression of those two genes in mRNA level in A549 cells. Our results demonstrated that significantly down-regulated Cdkn1a and Arf levels were observed after silencing the expression of Meis 1 in A549 lung cancer cells, suggesting the possible roles of CDK inhibitors in Meis 1 regulated proliferation in lung cancer. However, the underlying mechanisms for those CDK inhibitors in Meis 1-mediated cell cycle entry remain to be investigated.

Conclusions

Collectively, the data presented here indicated a repressive role of Meis1 in regulating NSCLC cancer cell proliferation through modulating cell cycle progression. It is intriguing to explore whether the expression levels of Meis1 was inversely correlated with the NSCLC cancer progression. If so, our data revealed a novel mechanism for the proliferation of NSCLC cancer cells and suggest a potential target for the development of future therapeutic strategies to treat NSCLC.

Acknowledgements

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References

1. Moens CB, Selleri L. Hox cofactors in vertebrate development. Dev Biol 2006;291:193-206.
2. Kocabas F, Zheng J, Thet S, et al. Meis1 regulates the metabolic phenotype and oxidant defense of hematopoietic stem cells. Blood 2012;120:4963-72.
3. Simsek T, Kocabas F, Zheng J, et al. The distinct metabolic profile of hematopoietic stem cells reflects their location in a hypoxic niche. Cell Stem Cell 2010;7:380-90.
4. Azcoitia V, Aracil M, Martínez-A C, et al. The homeodomain protein Meis1 is essential for definitive hematopoiesis and vascular patterning in the mouse embryo. Dev Biol 2005;280:307-20.
5. Hisa T, Spence SE, Rachel RA, et al. Hematopoietic, angiogenic and eye defects in Meis1 mutant animals. EMBO J 2004;23:450-9.
6. Pillay LM, Forrester AM, Erickson T, et al. The Hox cofactors Meis1 and Pbx act upstream of gata1 to regulate primitive hematopoiesis. Dev Biol 2010;340:306-17.
7. Thorsteinsdottir U, Kroon E, Jerome L, et al. Defining roles for HOX and MEIS1 genes in induction of acute myeloid leukemia. Mol Cell Biol 2001;21:224-34.
8. Kawagoe H, Humphries RK, Blair A, et al. Expression of HOX genes, HOX cofactors, and MLL in phenotypically and functionally defined subpopulations of leukemic and normal human hematopoietic cells. Leukemia 1999;13:687-98.
9. Lawrence HJ, Rozenfeld S, Cruz C, et al. Frequent co-expression of the HOXA9 and MEIS1 homeobox genes in human myeloid leukemias. Leukemia 1999;13:1993-9.
10. Wong P, Iwasaki M, Somervaille TC, et al. Meis1 is an essential and rate-limiting regulator of MLL leukemia stem cell potential. Genes Dev 2007;21:2762-74.
11. Jones TA, Flomen RH, Senger G, et al. The homeobox gene MEIS1 is amplified in IMR-32 and highly expressed in other neuroblastoma cell lines. Eur J Cancer 2000;36:2368-74.
12. Geerts D, Schilderink N, Jorritsma G, et al. The role of the MEIS homeobox genes in neuroblastoma. Cancer Lett 2003;197:87-92.
13. Spieker N, van Sluis P, Beitsma M, et al. The MEIS1 oncogene is highly expressed in neuroblastoma and amplified in cell line IMR32. Genomics 2001;71:214-21.
14. Argiropoulos B, Yung E, Humphries RK. Unraveling the crucial roles of Meis1 in leukemogenesis and normal hematopoiesis. Genes Dev 2007;21:2845-9.
15. Mahmoud AI, Kocabas F, Muralidhar SA, et al. Meis1 regulates postnatal cardiomyocyte cell cycle arrest. Nature 2013;497:249-53.
16. Landis SH, Murray T, Bolden S, et al. Cancer statistics, 1999. CA Cancer J Clin 1999;49:8-31, 1.

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Brief Report

Retreatment with pemetrexed chemotherapy in advanced non-small cell lung cancer patient

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Objective: The aim of this study was to evaluate the feasibility and safety of retreatment the pemetrexed after the failure prior pemetrexed-based chemotherapy in non-small cell lung cancer (NSCLC) from our institute.

Patients and methods: Patients with advanced NSCLC who were admitted to Zhejiang Cancer Hospital from Dec 2009 to Dec 2012 were retrospectively analyzed. All of the patients were given pemetrexed chemotherapy after the prior pemetrexed-based treatment. Survival analysis was evaluated by Kaplan-Meier method.

Results: Twenty-five patients were included in current study. Initial pemetrexed-based therapy was given as first-line treatment in all patients. Nine patients retreated with pemetrexed as the fourth-line treatment, and sixteen as further-line. One patient (4%) achieved partial response (PR), 9 (36%) with stable disease (SD), and 15 (60%) had progressive disease (PD). The disease control rate (DCR) was 40% and the median progression-free survival (PFS) was 1.5 months (95% CI: 0.8-2.4 months). Patients with an initial PFS >6 months had a median PFS after retreatment of 2.2 months, while patients with an initial pemetrexed PFS ≤6 months had a median PFS after retreatment of 1.1 months (P=0.036). The toxicities associated with the 2nd pemetrexed were generally acceptable.

Conclusions: Retreatment of pemetrexed seems to be a potential therapeutic option for treatment of selected advanced NSCLC patients after failure of initial pemetrexed therapy, especially for the patients with a PFS more than 6 months in the initial pemetrexed treatment.

Keywords: Non-small cell lung cancer (NSCLC); pemetrexed; retreatment; efficacy

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Introduction

Pemetrexed is a pyrrolopyrimidine antifolate which inhibits thymidylate synthase, glycinamide ribonucleotide formyltransferase, and ihydrofolate reductase. Pemetrexed was approved by the FDA in 2004 for the treatment of patients with malignant pleural mesothelioma (1) and approved as second-line (2), first-line (3) and maintenance treatment (4,5) in advanced non-small cell lung cancer (NSCLC) now.

Pemetrexed has shown to be less toxic than other chemotherapy regimens, with excellent efficacy and safety in non-squamous NSCLC. There is no guideline for chemotherapy in NSCLC patients who failed second-line/third-line treatment. However, many patients have actually received further-line chemotherapy in the real-world setting. Some studies have conducted trials to evaluate the efficacy of pemetrexed retreatment in patients with malignant pleural mesothelioma (6,7), however, no study investigated the efficacy of pemetrexed retreatment in NSCLC.

In the present study, we investigated the efficacy of

retreatment of pemetrexed after failure of prior pemetrexed-based treatment in advanced NSCLC, and to explore which patients may benefit from retreatment.

Patients and methods

Patient eligibility

Twenty-five consecutive, unselected NSCLC patients, who were admitted to Zhejiang Cancer Hospital from Dec 2009 to Dec 2012, were included in our study. NSCLC staging was performed for all the patients according to the 7th TNM classification. Inclusion criteria were as follows: (I) Pathologically proven non-squamous NSCLC; (II) the disease recurrence was confirmed using chest computed tomography (CT), brain MRI and bone scan as well as ultrasound examination and/or CT of the abdomen; (III) without any local treatment like radiotherapy or interventional therapy during the period of pemetrexed therapy; (IV) at least one measurable lesion and an Eastern Cooperative Oncology Group performance status (PS) of 0 to 2.

Treatment

All patients were given pemetrexed 500 mg/m^2 as a 10-min intravenous infusion on day 1 every 21 days. Dexamethasone (4.5 mg) was taken twice daily on the day before, the day of, and the day after each dose of pemetrexed. Folic acid supplementation and vitamin B12 were taken beginning one week prior to the first dose of pemetrexed and continued until one month after treatment discontinuation.

Response evaluation

All patients were followed up every 6 weeks with imaging examination during treatment with pemetrexed. Objective tumor responses were evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST 1.1). Objective tumor responses included complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD). Disease control rate (DCR) was defined as the addition of objective response and stabilization.

Toxicity evaluation

The toxicity profile of pemetrexed was assessed by reviewing medical records including 25 patients. Severity of adverse reactions was determined based on the requirements of dosage reduction or discontinuation of pemetrexed. All such toxicities were evaluated according to the National Cancer Institute Common Toxicity Criteria version 3.0 (CTC3.0).

Follow-up

All the patients were to be evaluated for tumor response and PFS. Follow-up rate was 100%. The last follow-up date was July 31, 2013.

Statistical analysis

PFS encompassed the time from the first cycle of therapy to documented progression or death from any cause, or until the date of the last follow-up visit for patients who were still alive and who had not progressed. Survival analysis was conducted with a Kaplan-Meier analysis and log-rank test. All statistical tests were analyzed using the computer software SPSS version 17.0 (SPSS Inc, Chicago, IL, USA).

Results

Patient characteristics

A total of 25 patients were included in the study. There were 16 males and 9 females. All of the 25 patients were with advanced stage. Performance score 0-1 was present in 10 patients (40%) and PS 2 accounted for 60%. The median age of the patients was 59.5 years (range, 38-74 years). Twenty-four patients were with adenocarcinoma and one with large cell lung cancer. All of the 25 cases underwent cytotoxic chemotherapy or targeted treatment between the initial and retreatment pemetrexed therapy. Twenty-two patients received EGFR-TKI therapy before pemetrexd retreatment in our study. Among the 22 patients, 11 patients received EGFR-TKI treatment in second-line, 9 in third-line and 2 in fourth-line. Among the 13 patients with initial pemetrexed PFS >6 months, 10 patients treated with EGFR-TKI before pemetrexd retreatment. Patients' characteristics are shown in *Table 1*.

Response data and survival analysis

Twelve patients achieved a PR and thirteen with SD in the initial pemetrexed treatment, accounting for a DCR of 100%. There were one patient with a PR to the retreatment, while 9 patients had SD and 15 patients had PD. Median PFS during initial pemetrexed-based treatment was 6.6 months (95% CI: 5.0-15.5 months), but 1.5 months

Table 1 Baseline characteristics of the study population (n=25)

Variables	Number	Percent
Gender		
Male	16	64
Female	9	36
PS		
0-1	10	40
2	15	60
Age		
Median	59.5	
Mean	58.1±8.5	
Smoking characteristics		
Yes	8	32
No	17	68
Tumor characteristics		
Histology		
Adenocarcinoma	24	96
Large cell	1	4
Retreatment in which line		
Fourth line	9	36
Further line	16	64
Chemotherapy before re-treatment		
Yes	25	100
No	0	0
TKI before re-treatment		
Yes	22	88
No	3	12

PS, performance status.

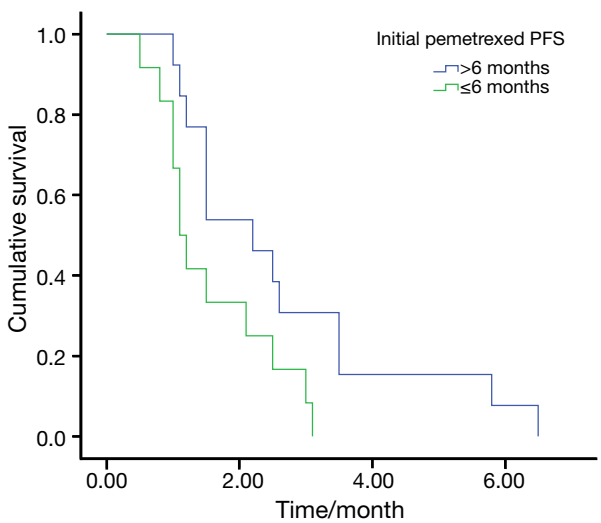

Figure 1 The retreatment PFS in patients with initial pemetrexed PFS >6 months and ≤6 months (P=0.036). PFS, progression-free survival.

during pemetrexed retreatment (95% CI: 0.8-2.4). Thirteen patients had PFS more than six months (>6 months) in the initial pemetrexed treatment and 12 with PFS less than six months (≤6 months). The median survival time for all patients was 21.5 months. The median OS from the beginning of the 2nd pemetrexed was 7.1 months (95% CI: 5.5-10.7 months). Among the 22 patients treatment with EGFR-TKI, 6 tested the EGFR mutation, 2 with EGFR mutation and 4 with wild-type. The PFS of pemetrexed retreatment for the 2 patients with EGFR mutation was 1.2 and 1.0 months for the patients with EGFR wild-type (P=0.14).

The relationship between initial treatment and retreatment efficacy

The overall DCR in the retreatment group was 40%. The retreatment DCR was 46.2% (6/13) in patients who had PFS duration more than 6 months (>6 months) in the prior pemetrexed and 33.3% (4/12) in the patients who had PFS duration less than 6 months (≤6 months), and the PFS was 2.2 and 1.1 months in the two group, respectively, (P=0.036) (*Figure 1*).

Factors affecting PFS by univariate and multivariate analysis

Results of univariate analysis for PFS of retreatment are shown in *Table 2*. The PS score (P=0.031) and initial pemetrexed PFS (P=0.036) were the factors influence the PFS of retreatment pemetrexed (*Table 2*).

A multivariate Cox regression model was constructed with the incorporation of age, sex, PS, smoking history and initial pemetrexed PFS. PS remained as independent prognostic factor for PFS of retreatment pemetrexed (P=0.045).

Toxicities of treatment

Toxicity was evaluated in all the patients. The most common adverse event was hematological toxicities in 16 patients (64.0%), including 5 patients with grade 3-4. Two patients demonstrated fatigue after being retreated with pemetrexed therapy (grade 3). Three dosage reduction were occurred.

Discussion

To the best of our knowledge, our represents the first data

Table 2 Univariate analysis of PFS of retreatment with pemetrexed

	PFS	95% CI	P
Sex			0.07
Male	1.2	0.67-1.7	
Female	2.1	0.6-3.9	
Age			0.6
≥65	1.5	0.1-2.9	
<65	1.5	1.1-1.9	
PS			0.031
0-1	2.4	1.2-3.5	
2	1.1	0.8-1.5	
Retreatment in which line			0.77
Fourth-line	1.5	1.1-1.9	
Further-line	1.5	0.9-2.1	
Smoking history			0.11
Yes	1.2	0.4-2.2	
No	2.1	1.1-3.5	
Initial pemetrexed PFS			0.036
>6 months	2.2	1.9-4.5	
≤6 months	1.1	0.6-2.1	

PFS, progression-free survival.

to assess whether pemetrexed retreatment confers any clinical benefit in patients with advanced NSCLC. In our series, we obtained a DCR of 40% with a median duration of this control of 1.5 months.

It was investigated that second-line therapy was given to 40-60% of patients and 20-30% of patients could receive third-line therapy or further therapy recently (8,9). The availability of new chemotherapy regimens like pemetrexed with low toxicity agents increases the chance for the advanced NSCLC patients to receive further treatment. However, with the decreased of PS and less tolerance of toxicity, few treatment choice was explored for the patients who received further-line treatment.

Two large randomized phase III studies—the JMDB (3) and JMEI (2) trials showed a statistically significant efficacy in patients with non-squamous histology with pemetrexed-based treatment in NSCLC. Another two randomized phase III studies—the JMEN (4) and PARAMOUNT (5) trials also showed that pemetrexed had a well efficacy and less toxicity as maintenance treatment in NSCLC patients.

Retreatment with pemetrexed was reported as effective as second-line therapy in malignant pleural mesothelioma patients who achieved a durable (>12 months) disease control with first-line pemetrexed by Ceresoli *et al.* and Bearz *et al.* studies (6,7). In our study, the DCR and ORR were 40% and 4%, which showed a similar efficacy as second-line treatment.

A limitation of this study may be the retrospective design and small number of patients. However, with no cases in previous clinical studies, our retrospective study can also be considered to be meaningful.

In conclusion, our results indicated that retreatment of pemetrexed could be consider as one of treatment option for the patients with PFS more than six months in the initial pemetrexed-based chemotherapy in NSCLC. Further prospective evaluation of this therapeutic option is warranted.

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References

1. Hazarika M, White RM, Johnson JR, et al. FDA drug approval summaries: pemetrexed (Alimta). Oncologist 2004;9:482-8.
2. Hanna N, Shepherd FA, Fossella FV, et al. Randomized phase III trial of pemetrexed versus docetaxel in patients with non-small-cell lung cancer previously treated with chemotherapy. J Clin Oncol 2004;22:1589-97.
3. Scagliotti GV, Parikh P, von Pawel J, et al. Phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naive patients with advanced-stage non-small-cell lung cancer. J Clin Oncol 2008;26:3543-51.
4. Ciuleanu T, Brodowicz T, Zielinski C, et al. Maintenance pemetrexed plus best supportive care versus placebo plus best supportive care for non-small-cell lung cancer: a randomised, double-blind, phase 3 study. Lancet 2009;374:1432-40.
5. Paz-Ares L, de Marinis F, Dediu M, et al. Maintenance therapy with pemetrexed plus best supportive care versus placebo plus best supportive care after induction therapy with pemetrexed plus cisplatin for advanced non-squamous non-small-cell lung cancer (PARAMOUNT): a double-blind, phase 3, randomised controlled trial. Lancet Oncol 2012;13:247-55.
6. Ceresoli GL, Zucali PA, De Vincenzo F, et al. Retreatment with pemetrexed-based chemotherapy in patients with malignant pleural mesothelioma. Lung

Cancer 2011;72:73-7.
7. Bearz A, Talamini R, Rossoni G, et al. Re-challenge with pemetrexed in advanced mesothelioma: a multi-institutional experience. BMC Res Notes 2012;5:482.
8. Song Z, Yu Y, Chen Z, et al. Third-line therapy for advanced non-small-cell lung cancer patients: feasible drugs for feasible patients. Med Oncol 2011;28 Suppl 1:S605-12.
9. Girard N, Jacoulet P, Gainet M, et al. Third-line chemotherapy in advanced non-small cell lung cancer: identifying the candidates for routine practice. J Thorac Oncol 2009;4:1544-9.

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Surgical Technique

Bronchovascular right upper lobe reconstruction by uniportal video-assisted thoracoscopic surgery

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Abstract: Lung cancer requiring double bronchial and vascular reconstruction of the pulmonary artery is a challenging procedure usually performed by thoracotomy. However, recent development of video-assisted thoracoscopic techniques allows experienced and skilled surgeons to perform these cases through a minimally invasive approach. Most of these complex thoracoscopic resections are performed by using 3 to 4 incisions. We present the first report of a right side combined vascular reconstruction and bronchoplasty performed through a single-incision video-assisted thoracoscopic surgery (VATS) technique.

Keywords: Thoracoscopy/video-assisted thoracoscopic surgery (VATS); minimally invasive surgery; pulmonary artery reconstruction; vasculoplasty; vascular reconstruction; bronchoplasty; surgery/incisions/technique

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Introduction

In the past two decades with increasing frequency video-assisted thoracoscopic surgery (VATS) has been performed for lung cancer treatment. However, complex cases are only performed in a few centers. Most surgeons use a 3-4 port VATS approach for difficult resections such as bronchial or vascular sleeves. This report describes the technique for a right upper lobectomy with bronchoplasty and vascular reconstruction by using a uniportal VATS technique.

Clinical summary

A 73-year-old male, smoker, with severe comorbidities (COPD, obesity, hypertension and cardiomiopathy) was diagnosed with a right upper lobe (RUL) tumor. The CT scan showed the tumor located on the posterior segment of the RUL with bronchial and vascular invasion by lymph nodes (*Figure 1*). The pulmonary function test was normal (FEV1 70%). The patient was proposed for single incision VATS approach.

Surgical technique

Under general anesthesia, a VATS approach using a 4 cm single-incision was made in the 5th intercostal space with no rib spreading (no soft tissue retractor and no direct vision).

A tumor was detected in the RUL and lymph nodes were involving the bronchus and apical-anterior branch of the pulmonary artery (PA) and interlobar artery. The incomplete fissure was divided from anterior to posterior from the hilum to expose and control the artery. The RUL bronchus was not possible to be dissected so it was transected by using a long knife. The main pulmonary artery and the basal trunk were dissected to have adequate proximal and distal vascular control. Before clamping the PA, 5,000 units of heparin were injected intravenously to prevent clotting. The interlobar artery was occluded by using a double vascular sling vessel loop and the main PA was closed using a thoracoscopic clamp (Scanlan International, Inc, Saint Paul, MN, USA). The clamp was placed towards the anterior portion of the incision and the vessel loop was placed in the posterior part. The camera

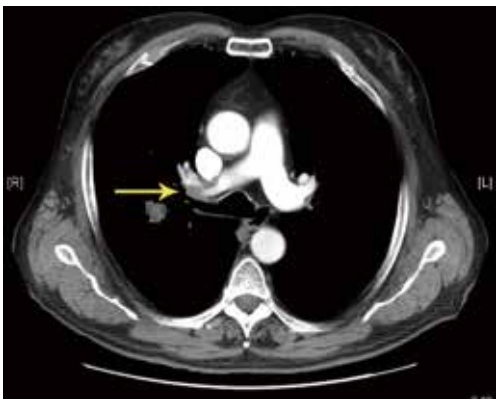

Figure 1 Computed tomography scan showing right upper lobe (RUL) bronchial and vascular lymph node invasion.

Figure 2 Bronchovascular resection and reconstruction.

Figure 3 Surgical image of instrumentation during bronchoplasty.

was located in the posterior part of the incision, and the instrumentation was placed and used below the camera.

The PA was transected laterally and the specimen was temporarily placed in the costo-diaphragmatic space. The defects in the interlobar artery and base of apico-anterior branch were repaired by using interrupted double lateral 4-0 monofilament non-absorbable sutures. The sutures

Figure 4 Postoperative result with chest tube placed in the posterior part of the incision.

were tied and the air was released through one of the posterior branches which was cut and then closed by using a vascular clip (Click aV, Grena^{R}) (*Figure 2*). The specimen was then removed in a protective bag (1).

The bronchus was reconstructed and closed by using interrupted double sutures of 3-0 PDS (*Figure 3*). The lung was inflated and no air leak was observed. Systematic lymph node dissection was then performed to complete the procedure and a single chest tube was placed (*Figure 4*). Total surgery time was 310 min and estimated blood loss was 180 cc. The chest tube was removed on the sixth postoperative day and the patient was discharged on the same day with no complications and excellent recovery.

Discussion

Bronchovascular reconstructive surgery is a technically feasible alternative to pneumonectomy and has the advantage of sparing the functioning of the lung parenchyma. These procedures are very complex and usually performed by open surgery (2). However, with the recent developments in VATS technology and acquired experience, this surgery can be performed thoracoscopically by experienced and skilled VATS surgeons. There are very few articles published in the literature describing a combined bronchial and vascular reconstruction by VATS, and these resections are reported on the left side by using conventional multiport thoracoscopic technique (3,4). We have recently published the first case of double sleeve VATS resection by a single incision approach (5).

As our experience has grown with the single-port VATS approach (6) we have increased the rate of thoracoscopic sleeve procedures and decreased the incidence of

pneumonectomy and today our list of contraindications is very limited (7). Advanced uniportal thoracoscopic procedures as bronchial sleeve lobectomy (8), lobectomy with chest wall resection, vascular reconstruction (9) and double sleeve (5) have already been performed with good postoperative outcomes. To April 2014 we have performed 11 uniportal VATS lobectomies with reconstruction: wedge bronchoplasty (3 cases), bronchial sleeve (5 cases), pulmonary arterioplasty 3 (1 vascular reconstruction, 1 vascular reconstruction combined with bronchoplasty and one double bronchovascular sleeve). The postoperative course of these patients was uneventful. Mean operative time was 215±54 minutes (range, 120-310 minutes) and median hospital stay was 6 days (range, 2-21 days).

The success in performing complex lobectomies by uniportal approach is a result of skills and experience accumulated over time by performing many uniportal VATS surgeries (6). The advantage of uniportal VATS surgery is the vision is direct to the target tissue providing a similar angle of view as for open surgery. Conventional multi-port VATS triangulation creates a new optical plane with genesis of torsional angle that is not favorable with standard two-dimension monitors. Another advantage of the uniportal VATS technique is that instruments inserted parallel to the camera mimic inside the chest maneuvers performed during open surgery. This geometric uniportal VATS concept facilitates the bronchovascular reconstruction in complex resections such as the one described in this article (10).

Most of the published cases requiring sleeve or vascular reconstruction are located on the left side. Infiltration of the PA on the right side requiring arterial reconstruction is less frequent. Reconstruction of the PA enables complete cancer resection while preserving functioning pulmonary tissue, and has a definitive role in the surgical management of lung cancer. In this particular case there was a partial infiltration of the surface of the artery (base of apical-anterior and posterior branch) by lymph nodes needing tangential resection with direct repair, and a total sleeve was not necessary. In this case partial resection of the PA was performed in conjunction with bronchoplasty of the RUL. The use of thoracoscopic instruments with proximal and distal articulation (Scanlan International, Inc, Saint Paul, MN, USA) facilitate the instrumentation through a single-incision, especially for clamping the main pulmonary artery with no interference to the broncho-vascular reconstruction. For clamping the basal trunk we usually prefer to use a double vessel loop or a bulldog-clamp (placed inside the cavity) to facilitate the instrumentation.

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References

1. Gonzalez-Rivas D, Fieira E, de la Torre M, et al. Bronchovascular right upper lobe reconstruction by uniportal video-assisted thoracoscopic surgery. Asvide 2014;1:10.3978/asvide.243.
2. Rendina EA, De Giacomo T, Venuta F, et al. Lung conservation techniques: bronchial sleeve resection and reconstruction of the pulmonary artery. Semin Surg Oncol 2000;18:165-72.
3. Han Y, Zhou S, Yu D, et al. Video-assisted thoracic surgery (VATS) left upper sleeve lobectomy with partial pulmonary artery resection. J Thorac Dis 2013;5:S301-3.
4. Liu L, Mei J, Pu Q, et al. Thoracoscopic bronchovascular double sleeve lobectomy for non-small-cell lung cancer. Eur J Cardiothorac Surg 2014. [Epub ahead of print].
5. Gonzalez-Rivas D, Delgado M, Fieira E, et al. Double sleeve uniportal video-assisted thoracoscopic lobectomy for non-small cell lung cancer. Ann Cardiothorac Surg 2014;3:E2.
6. Gonzalez-Rivas D, Paradela M, Fernandez R, Delgado M, et al. Uniportal video-assisted thoracoscopic lobectomy: two years of experience. Ann Thorac Surg 2013;95:426-32.
7. Gonzalez-Rivas D, Fieira E, Delgado M, et al. Uniportal video-assisted thoracoscopic lobectomy. J Thorac Dis 2013;5:S234-45.
8. Gonzalez-Rivas D, Fernandez R, Fieira E, et al. Uniportal video-assisted thoracoscopic bronchial sleeve lobectomy: first report. J Thorac Cardiovasc Surg 2013;145:1676-7.
9. Gonzalez-Rivas D, Delgado M, Fieira E, et al. Single-port video-assisted thoracoscopic lobectomy with pulmonary artery reconstruction. Interact Cardiovasc Thorac Surg 2013;17:889-91.
10. Bertolaccini L, Rocco G, Viti A, et al. Geometrical characteristics of uniportal VATS. J Thorac Dis 2013;5:S214-6.

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Guideline

Chinese expert consensus on bronchial asthma control

Asthma Workgroup, Chinese Thoracic Society, Chinese Medical Association (CMA)

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Chinese and international guidelines for prevention and treatment of bronchial asthma (hereinafter referred to as asthma) indicate: the goal of asthma management is to achieve and maintain symptom control; treatment protocols need to be adjusted according to asthma control levels during asthma management; asthma management is a long-term process which incorporates three essential aspects—evaluation, treatment, and monitoring (1,2). These guidelines highlight that asthma control as the goal of treatment can be achieved by medication in the majority of patients, particularly with effective asthma management (1,2). The 2009 updates on the Global Initiative for Asthma (GINA) proposed a new conception of "overall asthma control", which expanded the previous understanding of asthma control to emphasize the equal importance of achieving current asthma control and reducing future risk (3). However, a number of surveys in China and other countries have revealed that the rate of asthma control in the real world is far below as proposed by the guidelines (4-9). Poorly controlled asthma was shown to seriously affect the daily life, work and school of patients, and was associated with recurrent asthma exacerbations, unscheduled hospital visits and stay, and impaired lung functions, which eventually lead to increased treatment cost, lost productivity, as well as heavy social and economic burden (4-9). Therefore, with the updated understanding, efforts to improve the current pattern of asthma management and to reinforce the treatment strategies will have a long way to go for better outcomes of overall asthma control. In this regard, the Asthma Workgroup of Chinese Thoracic Society convened a panel meeting of experts in the related fields, which formed the following consensus. In order to guide clinical practicing, the present consensus was developed with reference to related guidelines and consensus documents published elsewhere, in particular, the important papers on asthma control that appeared in the recent years.

Definition of overall asthma control

Overall asthma control is proposed to include two aspects: (I) achieving current control, as characterized by no or few symptoms (≤2 times in a week), no or little need (≤2 times in a week) for reliever medications (such as inhaled short-acting β_2-agonists), normal or nearly normal lung functions, and no limitations in daily activities; and (II) reduced future risks, as reflected by absence of unstable or worsening asthma symptoms, no asthma exacerbations, no persistent decline in lung function, and no adverse reactions related to long-term medications (10).

Overall asthma control is assessed by both the level of asthma control and future risks. The levels of asthma control, based on the patient's symptoms and pulmonary function in the previous 4 weeks, are rated as "controlled", "partially controlled", or "uncontrolled" (*Table 1*). The future risks are evaluated in terms of asthma exacerbations, unstable disease, decline in lung function, and drug-related adverse reactions. Thereby, overall asthma control emphasizes on the relationship between future risks and current control (10).

Relationship between current asthma control and future risks

Evidence has accumulated in successfully preventing and reducing the future risks of several chronic diseases such as hypertension and diabetes mellitus (11-13). Asthma as a chronic disease should be managed with focus not only on achieving current control, but also on reducing the risks of unstable asthma and acute exacerbations in the future. In the

Table 1 Levels of asthma control

Characteristics	Controlled*	Partially controlled†	Uncontrolled
Daytime symptoms	None or ≤2 episodes/week	>2 episodes/week	so rated when at least 3 of the items below "Partially controlled" are met in any week.
Limitations of activities	None	Yes	
Night-time symptoms or awakening	None	Yes	
Need for reliever medications	None or ≤2 times/week	>2 times/week	
Pulmonary function (PEF or FEV_1)	Normal	<80% predicted or personal best (if known)	

*So rated when all items below are met; †so rated when 1 or 2 of the items below are met in any week; PEF, peak expiratory flow; FEV_1, forced expiratory volume in 1 second.

GOAL (Gaining Optimal Asthma controL) study, patients who had achieved 'total control' and 'well control' of asthma in the study phase I continued to achieve their asthma control status in >90% and ~80%, respectively, of the duration in study phase II (14). A global multi-center clinical trial, which incorporated six major strategies of Single inhaler Maintenance And Reliever Therapy (SMART) and used Asthma Control Questionnaire (ACQ)-5 to evaluate asthma control, revealed that ~75% of the patients who had achieved control or partial control would maintain their asthma control levels years later, and that only 6% of the controlled asthma would be likely to revert back to uncontrolled status in the future (10).

The relationship between current control level and future risk is complicated because of multiple influencing factors. A lot more well-designed clinical trials are needed to elucidate on how to achieve an all-round reduction of future asthma risk, so as to provide further evidence for clinical practice. So far, factors known to be related to future risk of asthma include:

(I) Asthma exacerbations. As shown in a five-year follow-up study, decline in lung function was not apparent among asthma patients with no exacerbations who were either on inhaled glucocorticosteroids (hereinafter referred to as steroids) or on placebo; in contrast, among those who had experienced one or more exacerbations and were on placebo, there was a mean reduction by 7% in forced expiratory volume in 1 second (FEV_1), which was significantly greater as compared with asthma patients with no exacerbations. These findings suggested that occurrence of asthma exacerbations may accelerate lung function decline (15).

(II) Level of FEV_1. A baseline FEV_1 level can predict the frequency of future asthma exacerbations. Asthma patients with a lower baseline FEV_1 level tend to have a higher frequency of asthma exacerbations in the future (16,17).

(III) Current asthma control. The clinical characteristics (daytime symptoms, limitation of activities, night-time symptoms, and "as-needed" use of reliever medications) adopted in GINA classification of asthma control level are the most valuable predictors of future risk. A good correlation between the scores of ACQ-5 or Asthma Control Test (ACT) and the GINA asthma control levels (controlled, partially controlled and uncontrolled) has been shown (18,19).

(IV) Exposure to tobacco smoke. Currently smoking asthma patients are less likely to achieve a successful control. Tobacco smoke exposure is the most important modifiable factor that can be controlled to reduce future risk of asthma (2).

(V) Infections. Wheezing in children is typically associated with respiratory viral infections. Respiratory infection with syncytial virus may lead to refractory asthma. Infections with *Aspergillus*, *Mycoplasma pneumoniae*, and *Chlamydia* infection may have a role in adult asthma (2). A recent large-scale retrospective study revealed that influenza virus remains the first leading trigger of asthma exacerbations (20).

(VI) High-dose asthma medications. The need for high-dose inhaled steroids and/or LABAs in asthma patients are frequently predictive of poor control or future risk of asthma (2).

Assessment and monitoring

Proper assessment of asthma control and monitoring of the disease severity are clinically critical for prevention and treatment of asthma (2).

(I) Asthma symptom assessment. Clinically, certain questionnaires such as ACT (*Appendix 1*) and

ACQ (*Appendix 2*) are commonly used to assess the control of asthma symptoms. Owing to satisfactory operability and clinical practicality, these questionnaires are suitable for use at primary healthcare institutions or in clinical trials.

(II) Pulmonary function tests. Pulmonary function tests can be helpful to determine the severity, reversibility and variation of airflow limitation, thereby providing evidence for the diagnosis of asthma and assessment of asthma control. Typically, a post-bronchodilator (e.g., 200-400 μg salbutamol) improvement in FEV_1 ≥12% and ≥200 mL is diagnostic of asthma. However, most asthma patients will not exhibit such reversibility of airflow limitation at each assessment, particularly those who are on treatment. Peak flow meter are inexpensive, portable devices ideal to use in home settings for day-to-day monitoring of airflow limitation. A diurnal variation of ≥20% in peak expiratory flow (PEF) may help determine the diagnosis of asthma and worsening of the disease. Nevertheless, measurement of PEF by no means can be a perfect substitute for spirometry (e.g., FEV_1), as it either overestimates or underestimates the severity of airflow limitation, especially when the condition is worsening.

(III) Airway inflammatory markers: Airway inflammation correlates closely with acute exacerbation and relapse of asthma. While the existing assessment systems of asthma control rely intensively on clinical indicators, it would be important to consider including airway inflammatory markers in these systems. The airway inflammation in asthmatics can be evaluated in many ways, such as measurement of airway hyperresponsiveness, induced sputum cytology, exhaled nitric oxide and breath condensate. However, owing to the methodological diversity, operational complexity and unsatisfactory reproducibility among these tests, technical improvements are further required before their widespread use in clinical practice. Recent literature has shown significant inconsistency between clinical indicators and airway inflammatory markers in patients with asthma, as reflected by earlier improvement in clinical indicators than in inflammatory markers among nearly one-third of the patients. Nevertheless, many studies so far have confirmed that detection of airway inflammatory markers can benefit asthma patients not only in assessing and monitoring current asthma control, but also in predicting future risk.

Achieving overall asthma control

The goal of asthma treatment and management is to achieve "overall asthma control". Towards this goal, the approach of assessment, treatment and monitoring of asthma as proposed by GINA and Chinese Guideline for Prevention and Management of Bronchial Asthma should be followed (1,2).

(I) Proper assessment of asthma control: the levels of asthma control are rated as "controlled," "partially controlled," and "uncontrolled" based on the classification criteria for asthma control. Treatment steps should then be determined according to the specific asthma control level in the patients to achieve the goal of overall asthma control.

(II) Selection of treatment regimens: initial treatment regimen for asthma should be selected (Steps 1 through 5) based on assessment of disease severity (intermittent, mild persistent, moderate persistent and severe persistent asthma). During treatment, patients should be assessed repeatedly for level of asthma control with which the current treatment step is escalated or de-escalated accordingly. Except for those with intermittent asthma, all patients should be given long-term inhaled steroids; for severe cases, the inhaled steroids should be given in combination with other drugs (*Table 2*).

For patients whose symptoms are obviously uncontrolled, treatment should be started at Step 3; for those with uncontrolled asthma and poor lung function (post-bronchodilator FEV_1 <80% predicted), at Step 4. Current guidelines recommend a combination of inhaled corticosteroids (ICS) plus long-acting β2-agonist (LABA) as the first-line choice of initial treatment for patients with moderate to severe asthma (1,2). Budesonide/formoterol and fluticasone/salmeterol are presently the common ICS/LABA combinations used for initial maintenance therapy of moderate to severe asthma, given in recommended daily doses of 640/18 and 500/100 μg, respectively.

According to GINA, combination inhalers containing formoterol and budesonide can be used for both maintenance and rescue. Such a combination therapy has been demonstrated to contribute to enhanced protection from asthma

Table 2 Control-based approach to the management of asthma

← Reduce		Treatment steps	Increase →	
Step 1	Step 2	Step 3	Step 4	Step 5
Asthma education and environmental control				
As needed short-acting β_2-agonist	Select one	Select one	Medium- or high-dose ICS plus one or more	Add either or both
	Low-dose ICS	Low-dose ICS plus LABA	Medium- or high-dose ICS plus LABA	Oral glucocorticosteroid (lowest dose)
	Leukotriene modifier	Medium- or high-dose ICS	Leukotriene modifier	Anti-immunoglobulin E therapy
		Low-dose ICS plus leukotriene modifier	Sustained-release theophylline	
		Low-dose ICS plus sustained-release theophylline		

ICS, inhaled corticosteroid; LABA, long-acting β_2-agonist.

exacerbations and provide improvements in asthma control at relatively low doses of steroids in adult and adolescent patients with asthma (Evidence A) (2). Furthermore, it was also found that the use of a combination inhaler containing a rapid and long-lasting β_2 agonist (formoterol) plus an inhaled steroid (budesonide) as maintenance and rescue is effective in maintaining a high level of asthma control and reduces exacerbations requiring systemic glucocorticosteroids and and hospitalizations (Evidence A) (2). Given these, the guidelines propose a new treatment strategy, the Single inhaler Maintenance and Reliever Therapy (SMART), which involves the ICS/LABA combination for both maintenance and relief. A number of clinical studies have shown that, compared with other treatments, the use of a combination inhaler containing formoterol and budesonide as a SMART strategy may contribute to asthma control and a significant reduction in future risk of asthma among majority of patients, and ultimately help to achieve the overall asthma control (21-25).

(III) Monitoring and maintaining asthma control. Down-stepping of the treatment may be considered only if asthma control has been achieved and maintained for at least 3 months. The severity of asthma should be closely monitored when stepping down the treatment; step up the treatment in response to worsening control (partially controlled or uncontrolled) until asthma control is achieved once again. De-escalation in the therapy is to establish the lowest step and dose of treatment to minimize drug-related adverse effects and medical cost (1,2). In patients who have achieved and maintained asthma control for 3 months on common ICS/LABA combination inhalers containing budesonide/formoterol or salmeterol/fluticasone and starting with an initial maintenance dose of 640/18 μg (budesonide/formoterol 160/4.5 μg, 2 puffs per time, twice daily) or 500/100 μg, respectively, the recommended daily dose for the immediately down-stepped maintenance therapy is 320/9 or 200/100 μg, respectively. Thereafter, for patients who continue to remain their asthma control status for at least 3 more months, consider single use of ICS alone in current dose. If the patient's asthma remains controlled on ICS monotherapy for at least 3 more months, consider titrating ICS to the minimum dose required to maintain control. In individual patients, ICS may be stopped but their condition should be closely monitored so that the treatment is resumed when necessary.

Importance of patient management in improving asthma control

Asthma is a chronic disease that constitutes significant impact on patients, their family and the society. Good patient management may help achieve complete control of asthma. Owing to the disparities in culture and healthcare

systems, the implementation of asthma management can vary from country to country, but typically involves five aspects as follows (1,2):

(I) Patient education. Medical education on asthma can help patients recognize early symptoms of asthma and minimize misunderstandings about the disease and treatment, so that they can seek timely medical attention. Patient education also helps establish a close partnership between healthcare workers and patients (and their family members) which will enable "guided self-management" of asthma. Obviously, asthma education is a long-term ongoing project that requires repetition, reinforcement, regular updates and perseverance.

(II) Monitoring asthma by integrating evaluation of symptoms and measurement of lung function. The shortened 5-questioned ACQ (ACQ-5) is commonly used in clinical research settings. Pulmonary function test can provide objective monitoring of asthma severity and patient response to therapy, thereby avoiding inadequate treatment due to underestimation of asthma symptoms by individual patients or healthcare providers. PEF monitoring is an easy-to-use procedure most commonly used for simple evaluation of the pulmonary function. Patients are advised to take home-monitoring of their PEF twice daily early during the treatment of asthma, and may reduce the frequency of PEF measurement when asthma control is achieved. Long-term PEF monitoring on a regular basis should be recommended particularly for patients who have ever been hospitalized for asthma or with poor perception of airflow limitation, in order to help them recognize early symptoms and avert the risk of fatal asthma attacks.

(III) Identifying and avoiding exposure to risk factors. A variety of environmental factors, including allergens, pollutants, food and drugs, may be involved in the onset of asthma or triggering an exacerbation. Patients and their family should be educated on how to identify these risk factors, and how these factors intersect with the development of asthma; they should be helped with effective measures to avoid exposure to risk factors.

(IV) Planning for long-term management based on regular follow-up consultations. Prompt adjustment of asthma medication is needed according to the control level; the patient's inhaler device techniques also need corrections and re-checking by physicians. All these rely on patient-physician interactions during routine follow-up consultations. At these visits, the patient's questions are discussed, and any problems with asthma and its initial treatment are reviewed.

(V) Planning the prevention of acute exacerbations. Prevention against acute asthma attacks requires collaborative efforts from patients and healthcare workers. Firstly, patients should be encouraged to have long-term monitoring of asthma and stay on adequate medications. Secondly, patients should be educated on how to identify a worsening of their condition as early as possible. In this regard, the importance of pulmonary function testing should be particularly underlined, since a decline in lung function (typically as reflected by PEF) may precede any uncomfortable symptoms, and therefore prompt the patient to see a doctor earlier. Patients with acute exacerbations should be encouraged to seek medical attention quickly at healthcare institutions they usually visit. Those at high risk of asthma-related death require urgent care and closer attention. Those who have recovered from an asthma exacerbation should be advised to stay on regular and adequate maintenance treatment and avoid risk factors to prevent relapse.

Directions for future research

Although many clinical trials have shown that ICS/LABA-based treatment regimens can result in favorable clinical control and reduced future risks, the rates of asthma control in the real world are much lower than expected. Many factors may account for this, including poor patient compliance, inadequate treatment, heterogeneity in manifestations of asthma, and the yet incompletely understood pathogenesis of asthma. There is thus a dire need for perseverant insight with more basic research and clinical studies towards improving asthma control. For instance, a series of studies on airway remodeling and relevant therapeutic targets are on-going; in-depth studies on neutrophilic asthma, individualized treatment strategies and bronchial thermoplasty are expected to offer new promises for asthma control.

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References

1. Asthma Workgroup, Chinese Thoracic Society, Chinese Medical Association. Guidelines on the prevention and treatment of bronchial asthma: definition, diagnosis, treatment, and management. Chin J Tuber Respir Dis 2008;31:177-85.
2. Global Initiative for Asthma. Global strategy for asthma management and prevention. Available online: http://www.ginasthma.org/local/uploads/files/CINA-Report2011-May4.pdf
3. National Institutes of Health, National Heart, Lung, and Blood Institute. Pocket guide for asthma management and prevention: a pocket guide for physicians and nurses. Philadelphia: Diane Publishing Company, 1995.
4. Lai CK, Ko FW, Bhome A, et al. Relationship between asthma control status, the Asthma Control TestTM and urgent health-care utilization in Asia. Respirology 2011;16:688-97.
5. Holgate ST, Price D, Valovirta E. Asthma out of control? A structured review of recent patient surveys. BMC Pulm Med 2006;6 Suppl 1:S2.
6. Partridge MR, van der Molen T, Myrseth SE, et al. Attitudes and actions of asthma patients on regular maintenance therapy: the INSPIRE study. BMC Pulm Med 2006;6:13.
7. FitzGerald JM, Boulet LP, McIvor RA, et al. Asthma control in Canada remains suboptimal: the Reality of Asthma Control (TRAC) study. Can Respir J 2006;13:253-9.
8. Benkheder A, Bouacha H, Nafti S, et al. Control of asthma in the Maghreb: results of the AIRMAG study. Respir Med

2009;103 Suppl 2:S12-20.
9. Peters SP, Jones CA, Haselkorn T, et al. Real-world Evaluation of Asthma Control and Treatment (REACT): findings from a national Web-based survey. J Allergy Clin Immunol 2007;119:1454-61.
10. Bateman ED, Reddel HK, Eriksson G, et al. Overall asthma control: the relationship between current control and future risk. J Allergy Clin Immunol 2010;125 600-8, 608.el-608.e6.
11. Mancia G, Laurent S, Agabiti-Rosei E, et al. Reappraisal of European guidelines on hypertension management: a European Society of Hypertension Task Force document. J Hypertens 2009;27;2121-58.
12. Liu LS, Writing Group of 2010 Chinese Guidelines for the Management of Hypertension. 2010 Chinese guidelines for the management of hypertension. Zhonghua Xin Xue Guan Bing Za Zhi 2011;39:579-615.
13. Nathan DM, Buse JB, Davidson MB, et al. Management of hyperglycemia in type 2 diabetes: A consensus algorithm for the initiation and adjustment of therapy: a consensus statement from the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care 2006;29:1963-72.
14. Bateman ED, Bousquet J, Busse WW, et al. Stability of asthma control with regular treatment: an analysis of the Gaining Optimal Asthma controL (GOAL) study. Allergy 2008;63:932-8.
15. Bai TR, Vonk JM, Postma DS, et al. Severe exacerbations predict excess lung function decline in asthma. Eur Respir J 2007;30:452-6.
16. Kitch BT, Paltiel AD, Kuntz KM, et al. A single measure of FEVl is associated with risk of asthma attacks in long-term follow-up. Chest 2004;126:1875-82.
17. Fuhlbrigge AL, Kitch BT, Pahiel AD, et al. FEV(1) is associated with risk of asthma attacks in a pediatric population. J Allergy Clin Immunol 2001;107:61-7.
18. O'Byrne PM, Reddel HK, Eriksson G, et al. Measuring asthma control: a comparison of three classification systems, Eur Respir J 2010;36:269-76.
19. Chen H, Gould MK, Blanc PD, et al. Asthma control, severity, and quality of life: quantifying the effect of uncontrolled disease. J Allergy Clin lmmunol 2007;120:396-402.
20. Reddel HK, Jenkins C, Quirce S, et al. Effect of different asthma treatments on risk of cold-related exacerbations. Eur Respir J 2011;38:584-93.
21. O'Byrne PM, Bisgaard H, Godard PP, et al. Budesonide/formoterol combination therapy as both maintenance and reliever medication in asthma. Am J Respir Crit Care Med 2005;171:129-36.
22. Scicchitano R, Aalbers R, Ukeua D, et al. Efficacy and safety of budesonide/formoterol single inhaler therapy versus a higher dose of budesonide in moderate to severe asthma. Curr Med Res Opin 2004;20:1403-18.
23. Kuna P, Peters MJ, Manjra AI, et al. Effect of budesonide/formoterol maintenance and reliever therapy on asthma exacerbations. Int J Clin Pract 2007; 61:725-36.
24. Bousquet J, Boulet LP, Peters MJ, et al. Budesonide/formoterol for maintenance and relief in uncontrolled asthma vs. high-dose salmeterol/fluticasone. Respir Med 2007;101:2437-46.
25. Rabe KF, Atienza T, Magyar P, et al. Effect of budesonide in combination with formoterol for reliever therapy in asthma exacerbations: a randomised controlled, double-blind study. Lancet 2006;368:744-53.

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Appendix 1

Asthma Control Test™ (ACT)

(I) In the past 4 weeks, how much of the time did your asthma keep you from getting as much done at work, school, or home?

(i) All of the time
(ii) Most of the time
(iii) Some of the time
(iv) A little of the time
(v) None of the time

(II) During the past 4 weeks, how often have you had shortness of breath?

(i) More than once a day
(ii) Once a day
(iii) Three to 6 times a week
(iv) Once or twice a week
(v) Not at all

(III) During the past 4 weeks, how often did your asthma symptoms (wheezing, coughing, shortness of breath, chest tightness, or pain) wake you up at night or earlier than usual in the morning?

(i) Four or more nights a week
(ii) Two to 3 nights a week
(iii) Once a week
(iv) Once or twice
(v) Not at all

(IV) During the past 4 weeks, how often have you used your rescue medication (such as Albuterol)?

(i) Three or more times per day
(ii) One or 2 times per day
(iii) Two or 3 times per week
(iv) Once a week or less
(v) Not at all

(V) How would you rate your asthma control during the past 4 weeks?

(i) Not controlled at all
(ii) Poorly controlled
(iii) Somewhat controlled
(iv) Well controlled
(v) Completely controlled

A total point value of 25 means completely controlled; 20-24, well controlled; and <20, not well controlled.

Appendix 2

Asthma Control Questionnaire 5-item version (ACQ-5)

(I) On average, during the past week, how often were you woken up by your asthma during the night?

(i) Never
(ii) Hardly ever

(iii) A few times
(iv) Several times
(v) Many times
(vi) A great many times
(vii) Unable to sleep because of asthma

(II) On average, during the past week, how bad were your asthma symptoms when you woke up in the morning?
(i) No symptoms
(ii) Very mild symptoms
(iii) Mild symptoms
(iv) Moderate symptoms
(v) Quite severe symptoms
(vi) Severe symptoms
(vii) Very severe symptoms

(III) In general, during the past week, how limited were you in your activities because of your asthma?
(i) Not limited at all
(ii) Very slightly limited
(iii) Slightly limited
(iv) Moderately limited
(v) Very limited
(vi) Extremely limited
(vii) Totally limited

(IV) In general, during the past week, how much shortness of breath did you experience because of your asthma?
(i) None
(ii) Very little
(iii) Little
(iv) Moderate amount
(v) Quite a lot
(vi) A great deal
(vii) Very great deal

(V) In general, during the past week, how much of the time did you wheeze?
(i) Not at all
(ii) Hardly any of the time
(iii) A little of the time
(iv) Some of the time
(v) Lot of the time
(vi) Most of the time
(vii) All the time

An average score of <0.75 means completely controlled; 0.75-1.5, well controlled; and >1.5, uncontrolled.

Case Report

Bronchial aneurysm secondary to tuberculosis presenting with fatal hemoptysis: a case report and review of the literature

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Abstract: Fatal hemoptysis is a serious complication of pulmonary tuberculosis, which can rarely be due to a bronchial aneurysm. Here, we present a case of fatal hemoptysis due to bronchial aneurysm occurred in a 59-year-old patient of Han ethnicity suffering from active pulmonary tuberculosis. Meanwhile, we perform a literature review of reported cases to stress detailed radiological examination is needed prior to bronchoscopy to exclude the possibility of aneurysm existent in proximity to the lesions caused by mycobacterium tuberculosis. Urgent and effective treatment is needed to control such life-threatening bleeding, such as rigid bronchoscopy, endovascular therapy and surgery, and endovascular stent-graft repair.

Keywords: Hemoptysis; tuberculosis; aneurysm

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Case presentation

A 59-year-old female with repeatedly dry cough for half a year and low-grade fever for four days was referred to our hospital. There was no associated history of hemoptysis. She went to a local hospital where antibiotics (specific type unknown) were prescribed, but her symptoms were not relieved. A chest CT scan was done which revealed multiple focal nodules in the right lung field, enlarged mediastinal lymph nodes and atelectasis (*Figure 1A-C*). She also had a past medical history of cirrhosis and gastroesophageal varices secondary to chronic viral hepatitis C for which endoscopic band ligation was performed twice to prevent massive bleeding due to gastroesophageal varices.

On admission, physical examination was remarkable only for fever of 37.9 ℃. The laboratory results were as follows: white blood cell (WBC) count was 4,600 cells/μL with 81.8% neutrophils, C reactive protein (CRP) level was 81.3 mg/L, and erythrocyte sedimentation rate (ESR) level was 77 mm in the first hour. Serum tumor markers were negative. Liver and renal function tests were within normal limits. Coagulation profile was normal. This patient then underwent a bronchoscopy that revealed an obvious pulsatile aneurysm (4 mm × 4 mm) in her right intermediate bronchus (*Figure 1D*) and its tunica mucosa was irregular. Microbiological tests of sputum specimens were positive for acid fast bacilli. During the bronchoscopy procedure, an unexpected massive episode of hemoptysis developed due to rupture of the aneurysm. Emergency intubation was performed to keep the respiratory tract patent but copious amount of blood emerged from the cannula. Unfortunately, this patient died after 4 hours despite of giving the best supportive care.

Discussion

Fatal hemoptysis is a serious complication of pulmonary tuberculosis (1). We present a rare case of fatal hemoptysis due to bronchial aneurysm in a patient with active pulmonary tuberculosis. Pulmonary tuberculosis is complicated with vascular lesions including pulmonary or bronchial arteritis and thrombosis, bronchial artery dilatation, and Rasmussen aneurysm (2). Meanwhile, tuberculosis can also rarely cause aneurysm formation in other organs, such as brain and aorta (3). The pathogenesis of vascular lesions may be associated with chronic inflammation leading to neovascularization and

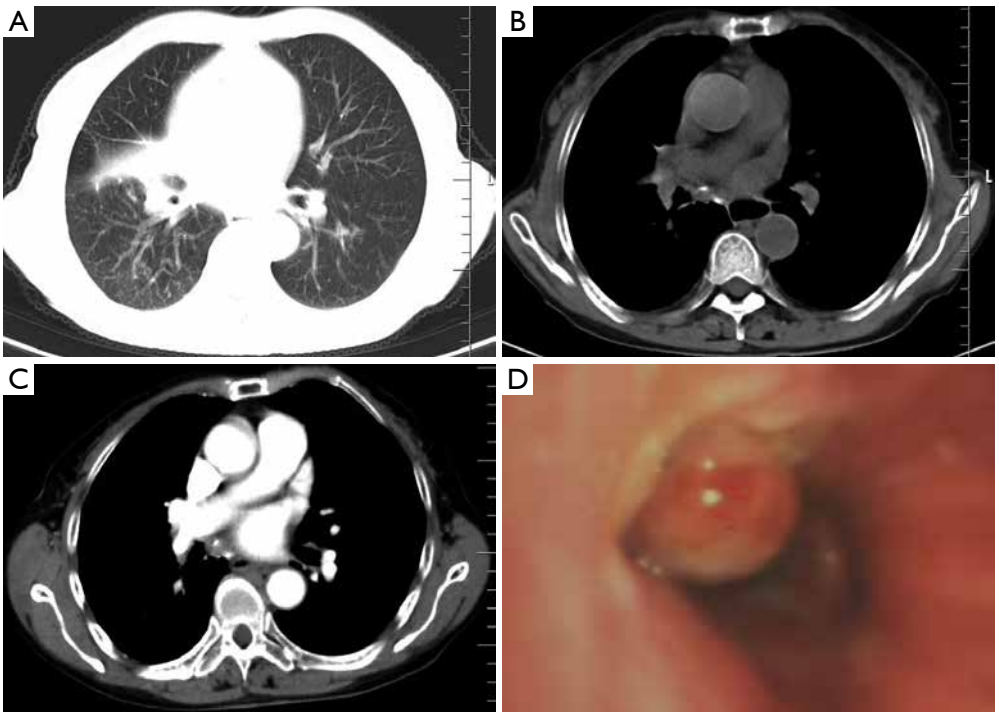

Figure 1 The radiological findings in contrast CT scan and bronchoscopy. (A) Chest CT showed multiple focal nodules in right lung field, and atelectasis of middle lobe of right lung as well as luminal stenosis; (B,C) contrast enhanced CT scan revealed no signs of pulmonary artery aneurysm (PAA) and arterial pulmonary hypertension; (D) bronchoscopy showed an obvious pulsatile aneurysm along with mucosal lesions and luminal stenosis.

increased collateral supply from nearby systemic arteries. These newly formed collateral vessels have weak arterial wall and are prone to rupture (4). A rare case of bronchial artery aneurysm presenting with massive hemoptysis due to pulmonary tuberculosis was previously reported, and the definitive diagnosis was made using multidetector computed tomographic (MDCT) angiography (4).

Autopsy and MDCT angiography strategies are more reliable than bronchoscopy for diagnosis of bronchial artery aneurysm. In our case, we attribute the cause of fatal hemoptysis to the rupture of bronchial artery aneurysm which was associated with vascular lesions due to mycobacterium tuberculosis infection. The clinical findings in favor of this are primarily the presence of an obvious pulsatile aneurysm which was detected by bronchoscopy, lesions in the mucosa, luminal stenosis, and microbiological tests of sputum specimens indicative of acid fast bacilli. Additionally, rupture of bronchial aneurysm occurred during bronchoscopy. Secondly, although bronchial and lower tracheal varices, pulmonary hypertension and elevation of pulmonary venous pressure can occur in end-stage alcoholic liver disease with portal hypertension, which has led to massive hemoptysis in previous case reports (5), there were no signs of such bronchial and lower tracheal varices detected by bronchoscopy in our case. Besides, our patient had undergone endoscopic band ligation twice in the past to prevent massive bleeding due to gastroesophageal varices. Thirdly, arterial pulmonary hypertension formation may be due to the presence of portocaval shunts that can cause massive hemoptysis (6), but its detection by radiological imaging is limited. Finally, endobronchial mycotic pulmonary artery aneurysm (PAA) is another cause of massive hemoptysis (7), but no fungus was detected from appropriate sputum sample.

Conclusions

Bronchial artery aneurysm is a rare complication of mycobacterium tuberculosis infection, which may lead to fatal hemoptysis and should always be considered in the differential diagnosis. Detailed radiological examination to rule out the possibility of aneurysm existing in the nearby

lesions caused by infectious disease is of prime importance prior to bronchoscopy. Effective and emergent treatment should be performed to control such life-threatening bleeding, including rigid bronchoscopy, endovascular therapy and surgery (8), and endovascular stent-graft repair (9).

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Disclosure: The authors declare no conflict of interest.

References

1. Lee BR, Yu JY, Ban HJ, et al. Analysis of patients with hemoptysis in a tertiary referral hospital. Tuberc Respir Dis (Seoul) 2012;73:107-14.
2. Kim HY, Song KS, Goo JM, et al. Thoracic Sequelae and Complications of Tuberculosis. Received 2001;21:839-58; discussion 859-60.
3. Saraf R, Limaye U. Ruptured intracranial tubercular infectious aneurysm secondary to a tuberculoma and its endovascular management. Br J Neurosurg 2013;27:243-5.
4. Karmakar S, Nath A, Neyaz Z, et al. Bronchial artery aneurysm due to pulmonary tuberculosis: detection with multidetector computed tomographic angiography. J Clin Imaging Sci 2011;1:26.
5. Youssef AI, Escalante-Glorsky S, Bonnet RB, et al. Hemoptysis secondary to bronchial varices associated with alcoholic liver cirrhosis and portal hypertension. Am J Gastroenterol 1994;89:1562-3.
6. Radulescu D, Duncea C, Donca V. Hepatic cirrhosis associated with arterial pulmonary hypertension. Rom J Gastroenterol 2004;13:341-3.
7. Dransfield MT, Johnson JE. A mycotic pulmonary artery aneurysm presenting as an endobronchial mass. Chest 2003;124:1610-2.
8. Sakr L, Dutau H. Massive hemoptysis: an update on the role of bronchoscopy in diagnosis and management. Respiration 2010;80:38-58.
9. Kasashima F, Endo M, Kosugi I, et al. Mediastinal bronchial artery aneurysm treated with a stent-graft. J Endovasc Ther 2003;10:381-5.

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Case Report

Lophomonas blattarum infection presented as acute exacerbation of chronic obstructive pulmonary disease

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Abstract: The present case described a 70-year-old male who was initially diagnosed and treated as acute exacerbation of chronic obstructive pulmonary disease (AECOPD). Ultimately Lophomonas blattarum (L. blattarum), a rare protozoan causing opportunistic infection, was found in suction sputum smear. Bronchoscopy showed a lot of purulent sputum in airways, diffusely swelling and friable mucus on bronchus. After single tinidazole treatment, symptoms and image showed marked improvement. It indicates though in the untraditional immunocompromised case, the suspect of opportunistic diseases is necessary, especially in the cases failed to improvement under empirical treatment. It also supports the tinidazole treatment is efficacy in L. blattarum infection.

Keywords: Lophomonas blattarum infection; chronic obstructive pulmonary disease (COPD)

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Introduction

Lophomonas blattarum (L. blattarum) is a rare protozoan, which parasitizes in intestinal tracts of some special arthropods, such as termites and cockroaches (1). The presenting data supports that L. blattarum is a kind of opportunistic pathogens, causing bronchopulmonary infection in immunocompromised cases, especially in the organ transplantation patients with immunosuppression treatment (2-4).

Chronic obstructive pulmonary disease (COPD) is defined as progressive and persistent airflow limitation with chronic cough, sputum, dyspnea and some other diversity symptoms (5). Acute exacerbation of chronic obstructive pulmonary disease (AECOPD) is characterized as worsen of symptoms beyond daily variation, which might result from bacterial and viral infection. On the other hand, due to the long term use of corticosteroids and bacterial colonization, bronchopulmonary infection is frequently seen in COPD patients, which might mimic or aggravate exacerbation of COPD. Here we presented a L. blattarum infection case, which was misdiagnosed as AECOPD initially.

Case (*Figure 1*)

A 70-year-old male farmworker with COPD (group D) had been hospitalized 3 times in the previous 12 months for exacerbations. His latest pulmonary function showed forced expiratory volume in the first second was 0.92 L, 32% predicted; and forced vital capacity was 2.6 L, after bronchodilating. He was on inhaled tiotropium 18 mcg once daily and fluticasone-salmeterol 500/50 mcg twice daily. For the serious carbon dioxide retention (arterial CO_2 pressure 55 mmHg in stable stage), this patient was ventilated with noninvasive Bipap (S/T-D Ventilatory Support System, inspiratory positive airway pressure: 12 cm H_2O, and expiratory positive airway pressure: 4 cm H_2O) 6 hours daily. Fortunately, except COPD history, this man donot have any other disease histories.

At an outside hospital, the patient complained of

worsening cough, sputum and dyspnea for 5 days. Physical examination revealed a temperature of 36.2 ℃, heart rate of 102 beats per minute, respiratory rate of 20 beats per minute, blood pressure of 120/80 mmHg and an oxygen saturation of 92% on 2 L/min nasal oxygen supplement. Lung examination only found coarse, but decreased breath sounds. And the other system examinations were all unremarkable. Blood routine revealed white blood cell count of 7.5×10^9/L and an increased neutriphil percentage of 80.3%. Chest radiography did not find any infiltration, masses and lymphadenopathy (*Figure 1A*). This patient was presumed as AECOPD with a possible infection of bronchus, and treated in the institute with intravenous ceftazidime and azithromycin, and nebulized ipratropium and budesonide. Due to the increased pCO_2 of 60 mmHg, the Bipap ventilation was prolonged to 12 hours per day with the same index.

After 4 days of treatment, the patient was transferred to our ward emergently with a marked exacerbation of dyspnea and cough, and a newly unconsciousness. Physical examination revealed cyanosis and an oxygen saturation of 80% on 4 L/min nasal oxygen supplement. Lung examination showed decreased breath sounds, and small amount of bilateral rhonchi and rales, and the other signs were almost the same as previous. Blood routine showed: white blood cell count of 9.1×10^9/L and neutriphil percentage of 87.94%. The arterial gas analysis showed highly increased CO_2 pressure, 145 mmHg, with a crucial PH 7.18. This critical case was diagnosed as hospital acquired pneumonia, COPD with pulmonary encephalopathy, and type 2 respiratory failure. He was nasal intubation immediately, and ventilated in synchronized intermittent mandatory ventilation mode. Bronchoscopy showed a lot of purulent sputum, diffusely swelling and congestion mucus on bronchus, especially in bilateral superior lobar trachea and right midtrachea (*Figure 1D,E*). Meropenem was used, plus bronchodilators, mucolytic and nutritional supports. Despite the strong treatment, the chest X-ray presented bilateral effusion, infiltrated and linear opacities (*Figure 1B*) with unimproved symptoms. The laboratory studies including erythrocyte sedimentation rate, glucose, liver test, renal function, IgE, human immunodeficiency virus, tuberculosis, virus and fungus were negative. Fortunately, the sputum smear directly from bronchoscopy revealed motile organism with wave flagellates, which was identified as L. blattarum (*Figure 1F,G*). Then intravenous tinidazole 0.4 g once daily had been administrated. One week later, the symptoms and images were improved (*Figure 1C*), and intubation ventilation was turned to noninvasive Bipap (S/T-D Ventilatory Support System, inspiratory positive airway pressure: from 16 to 12 cm H_2O, and expiratory positive airway pressure: from 6 to 4 cm H_2O). After discharge, tinidazole 0.5 g had been orally administrated twice daily for 1 month. The follow up found the variation of symptoms and image opacities were resolved (*Figure 1H,I*).

Discussion

L. blattarum is a rare and host-specific protozoan in the muggy environment (1), and it could be spread by waste and dust during the crawling of host. The infection of L. blattarum in only found in respiratory system so far (2-4), it might indicate the most possible transmitting pattern is air borne.Though it needs further study, it seems that L. blattarum infection is an organ specific disease, indicting bronchopulmonary epithelium and microenviroment might be beneficial for L. blattarum.

L. blattarum infection presents untypical symptoms, such as cough, sputum and dyspnea (2-4). All of these symptoms are commonly attributed to common bronchopulmonary infection or AECOPD, which might obscure the diagnosis and treatment. Reflecting in this case, it happened that the patient had worse respiratory symptoms and underlied with COPD. Though it was initially suspected as AECOPD, the empirical treatment for AECOPD did not show any improvement in the case. Even the ordinary sputum smear did not find any clues to protozoan and fungus infection. Diagnosis was delayed until the direct suction smear under bronchoscopy revealed the appearance of motive L. blattarum. This present case suggests the importantce of L. blattarum infection in the differential diagnosis of AECOPD and other common bronchopulmonary infection cases, especially who fails to empiric antibiotic treatments. As other cases, this patient recovered after the single tinidazole treatment, despite the failure of the initial antibiotic treatments. It seems tinidazole is enough for L. blattarum infection, and some other antibiotic or anti-fungus drugs might be unnecessary or even useless.

L. blattarum infection is so rare that only reported less than 70 cases in the whole world (2-4). It often based on immunocompromise, especially renal transplantation with receiving immunosuppression. Though high-dose inhaled corticosteroids have been demonstrated to increase the risk of bacteria, virus and fungus infection, this case is not a classical immunocompromised host. And we did not find any evidences of virus and fungus infection, which frequently occur in immunodeficiency cases. Since there are not

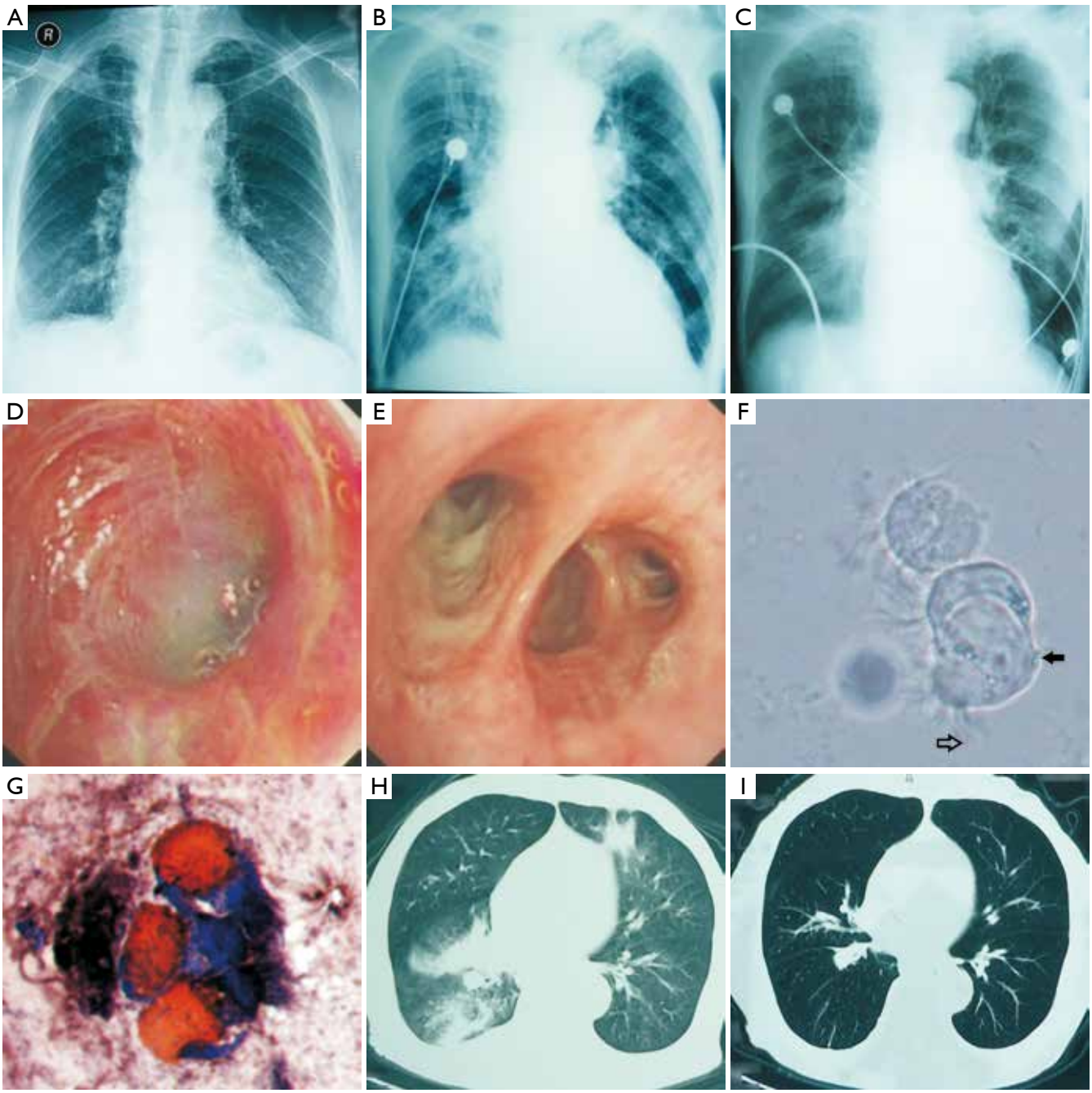

Figure 1 Image, bronchoscopy and smear finding in this case. (A) chest X-ray showed no signs of any infiltration, masses and lymphadenopathy, at the fifth day from onset; (B) chest X-ray showed bilateral effusion, infiltrated and linear opacities, at the nineth day from onset; (C) chest X-ray showed improvement after tinidazole injection, at the fifteenth day from onset; (D) bronchoscopy showed a lot of purulent sputum, and diffusely swelling and friable mucus on the left lingual bronchus; (E) bronchoscopy showed a lot of purulent sputum, and diffusely swelling and congestion mucus on the right middle bronchus; (F) (×400); (G) (Leifson, ×400): Lophomonas blattarum clustered in direct suction specimen. ➡, body of Lophomonas blattarum; ⇨ , wave flagellates; (H) pulmonary CT scan presented bilateral effusion, infiltrated, at the twenty fifth day from onset; (I) pulmonary CT scan presented improvement and resolved of lesion, 3 months later after discharge.

any reliable L. blattarum infection models, the immunity pathogenesis is still unclear. There is an unbalance of CD8+ and CD4+ T cell in COPD pathogenesis (5). And the unbalance also exists in the protozoan infection. It is presumptively that COPD is an additional predisposing factor in L. blattarum infection beyond corticosteroids.

Sometimes, it is difficult to differentiate L. blattarum and bronchial ciliated cells. In Leifson's stain, the bronchial ciliated cell is also presented with columnar shape, red nuclei, bluish cytoplasms, and inserted cilia, which is like waving flagella. However, the ciliated cells are often degeneration or necrosis in the bronchial secretion. In this case, these cells were still alive after 72 hours culture, indicating these cells were different from the bronchial ciliated cell. On the other hand, rather than meropenem and some other broad-spectrum antibiotics, the tinidazole brought exciting improvement in the case, suggesting there was L. blattarum infection. Because of the augment of chronic patients impairing the immunity by diseases or medications, the incidence of opportunistic diseases is mounting. Due to the specific host location, cases of L. blattarum infection are all found in warm and muggy districts (4). It suggests that chronic patients should keep clean and dry of residence and ventilation duct.

Conclusions

This case illustrated the misdiagnosis of L. blattarum, a rare opportunistic pathogen, until the bronchoscopy provided the positive direct sputum smear. It indicates though in the untraditional immunocompromised case, the suspect of opportunistic diseases is necessary, especially in the cases failed to improvement under empirical treatment.

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Consent: This work was approved by the Ethical Committee of the Second Xiangya Hospital. Written informed consent was obtained from the patient for publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Authors' contributions: HZ carried out the following up and drafting. XK carried out the picture and radiograph collecting. XC participated the identification of Lophomonas blattarum HL and PC supervised the diagnosis and treatments in this case. YC diagnosed and treated this case, and coordinated and helped to draft the manuscript.

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References

1. Ohkuma M, Noda S, Hongoh Y, et al. Inheritance and diversification of symbiotic trichonymphid flagellates from a common ancestor of termites and the cockroach Cryptocercus. Proc Biol Sci 2009;276:239-45.
2. Wang Y, Tang Z, Ji S, et al. Pulmonary Lophomonas blattarum infection in patients with kidney allograft transplantation. Transpl Int 2006;19:1006-13.
3. He Q, Chen X, Lin B, et al. Late onset pulmonary Lophomonas blattarum infection in renal transplantation: a report of two cases. Intern Med 2011;50:1039-43.
4. Martinez-Girón R, van Woerden HC. Lophomonas blattarum and bronchopulmonary disease. J Med Microbiol 2013;62:1641-8.
5. Vestbo J, Hurd SS, Agustí AG, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. Am J Respir Crit Care Med 2013;187:347-65.

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Case Report

Pulmonary alveolar proteinosis

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Abstract: We report a 68-year-old female who presented with chronic cough and progressive dyspnoea. Computed tomography of the thorax and subsequent bronchoscopy confirmed the diagnosis of pulmonary alveolar proteinosis (PAP), which was treated with whole lung lavage. This case is reported in view of the low incidence of PAP.

Keywords: Pulmonary alveolar proteinosis (PAP); rare lung diseases; surfactant

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Introduction

Pulmonary alveolar proteinosis (PAP) is an uncommon pulmonary disease characterised by abnormal excessive alveolar accumulation of surfactant. We present a case of PAP in an elderly female that was successfully treated with whole lung lavage.

Case report

A 68-year-old female presented to the Respiratory Clinic for chronic cough and dyspnoea of six months duration. There was no fever or weight loss. Her symptoms did not improve despite previous courses of antibiotics and oral steroids. She had a medical history of ischemic heart disease for which she was on clopidogrel, telmisartan, metoprolol and rosuvastatin. There was otherwise no significant family or contact history. She denied exposure to cigarette smoke, occupational hazards or toxic fumes. Respiratory examination revealed scattered lung crepitations. Cyanosis and clubbing were not noted. Pulse oximetry on room air was 93%. Her leucocyte count at presentation was 9.59×10^9/L (normal range 4×10^9-10×10^9/L) and serum LDH was elevated at 1,282 U/L (normal range 180-380 U/L). A chest radiograph was performed which showed bilateral alveolar opacities (*Figure 1*). A CT thorax demonstrated bilateral ground-glass opacities and septal reticulations forming a "crazy paving pattern" (*Figure 1C*).

Bronchoalveolar lavage revealed grainy eosinophilic debris (*Figure 2*) whilst transbronchial lung biopsy of the left lower lobe demonstrated preserved alveolar spaces filled with amorphous grainy material (*Figure 2B,C*). Lavage cultures were negative. A diagnosis of PAP was made.

The bronchoscopy was complicated by hemoptysis which resolved spontaneously and a left pneumothorax which necessitated placement of a left chest tube. She subsequently underwent two sequential whole lung lavages under general anesthesia, with the aim of treating the left lung first followed by the right lung during the second lavage 48 hours later. It was anticipated that the second lavage would pose a ventilator challenge as single lung ventilation would be performed on the lung affected by the previous hemorrhage and pneumothorax. Apart from frequent bronchoscopy to aspirate any spillover lavage fluid, we opted to reduce the volume of each aliquot as guided by oxygen saturations. The lowest oxygen saturation recorded was 82% (on 100% inspired oxygen fraction) which was transient, and this improved to 100% at the end of the lavage. In total, 16.1 L of saline was used for the first procedure, and 13 L was used for the second lavage.

The patient was monitored in the intensive care unit for 24 hours after each lavage. Post procedural chest X-rays did not demonstrate pneumothoraces, allowing removal of the chest tube. Her symptoms improved and at time of discharge 48 hours after the second lavage, her pulse oximetry was 96% on room air.

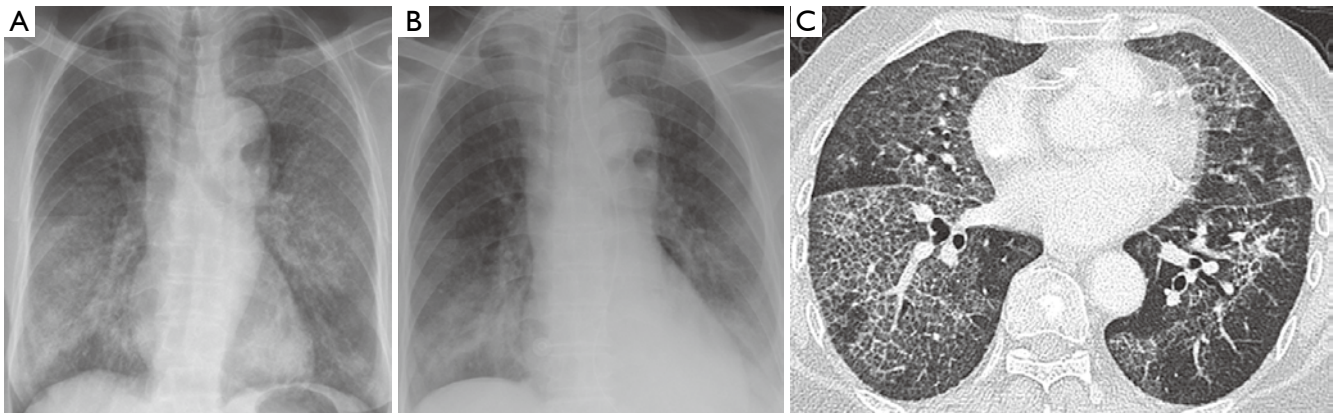

Figure 1 (A) Chest radiograph demonstrated diffuse alveolar opacities with a peri-hilar distribution; (B) a repeat chest radiograph performed following whole lung lavages demonstrated an improvement of the alveolar opacities; (C) a CT scan of the chest demonstrating bilateral ground-glass opacities and septal reticulations.

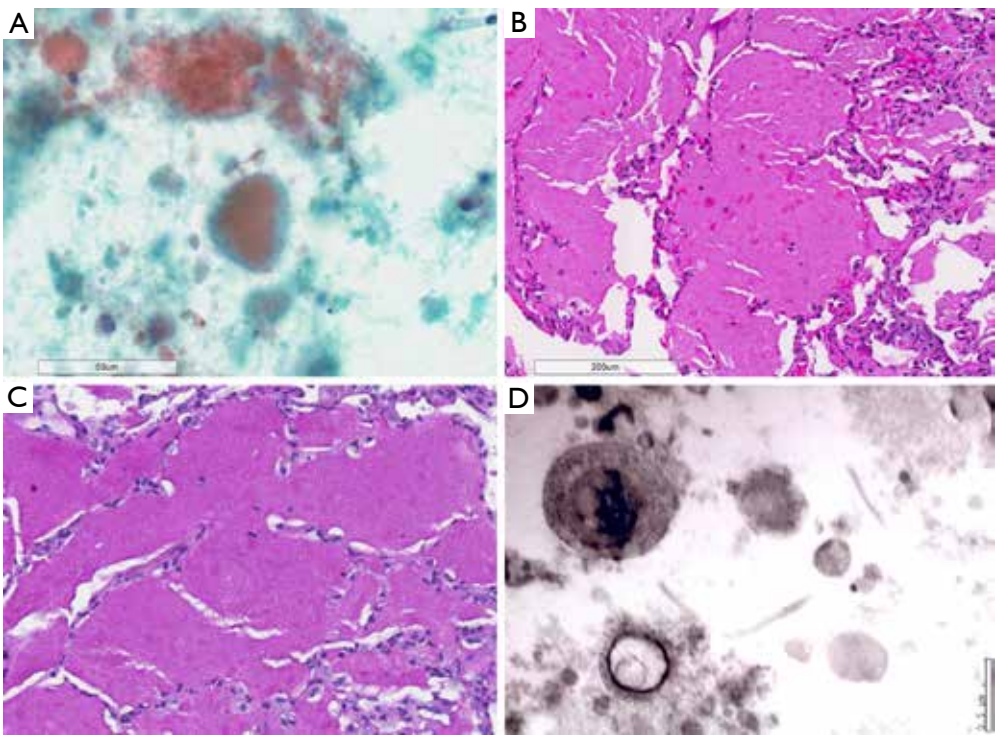

Figure 2 Bronchoalveolar lavage demonstrated eosinophilic amorphous granules with sharp edges as well as cellular debris and rare macrophages (A, 400× magnification). Transbronchial lung biopsy showed preservation of the alveolar architecture, relative lack of inflammation and filling of alveolar spaces with amorphous eosinophilic granular material (B, H & E stain, 200× magnification) which was intensely PAS positive (C, PAS stain, 200× magnification). Electron microscopic (EM) study of the bronchoalveolar lavage material showed lamellar bodies corresponding to degraded surfactant (D, 28,000× magnification).

Figure 3 Fluid derived from whole lung lavage which gave an initial "milky" appearance with sediments (Bottle 1) and gradually turned less opaque (Bottle 7). The initial bloody appearance was attributed to the bleeding that occurred during the prior transbronchial lung biopsy.

Discussion

PAP is an uncommon condition that was first described in a 27-patient case-series by Rosen *et al*. in 1958 (1). This condition arises from defective clearance and accumulation of surfactant in pulmonary alveoli, for which granulocyte macrophage-colony stimulating factor (GM-CSF) plays a critical role in enabling surfactant catabolism by alveolar macrophages. Understanding the pathogenesis of this disease was made possible in 1994 when it was reported that knockout mice that had GM-CSF deficiency developed similar disease (2). This knowledge was enhanced by the finding of a neutralizing IgG antibody against GM-CSF (3,4), and this autoantibody was subsequently noted to also impair neutrophil function, predisposing patients to increased risk of infections (5). Based on the etiology, three forms are currently recognized (6): genetic (arising from mutations in surfactant proteins or GM-CSF receptor genes), autoimmune, and secondary to toxic inhalation or haematological disorders. Autoimmune PAP is the most common form (representing about 90% of all cases) (7).

Common clinical manifestations are cough and dyspnea occurring in the third to sixth decades, although fever, chest pain and hemoptysis may also occur. The diagnosis is suspected by a CT thorax demonstrating the characteristic "crazy paving" pattern of reticulations superimposed on ground glass opacities, the latter arising from the accumulation of lipoproteinaceous material in the alveolar spaces (8). Bronchoalveolar lavage confirms the diagnosis by demonstrating foamy macrophages and eosinophilic granules that are positive for periodic acid-Schiff (PAS) staining (9).

Five-year survival without treatment has been reported to be 85% (10). Whole lung lavage is the first-line therapy, with the purpose of washing out the lipoproteinacous material from the alveoli (11) (*Figure 3*). It improves symptoms 5-year survival to 94% (8). Symptoms, radiologic abnormalities and physiologic parameters are also improved. The duration of response is currently unclear, with the median duration of benefit reported to be 15-36 months (10,12). Up to 66% of patients may require a repeat lavage (10). Given the rarity of PAP, proper prospective studies evaluating the optimal technique, safety and duration of benefit have not been performed. Complications of whole lung lavage include low oxygen saturation, hemodynamic instability, convulsions, pneumothorax, pleural effusion and fever have been reported. Whilst generally 15-20 L of saline (based on aliquots of 1 L) is required for each lavage to produce clinical improvement, this must be weighed against the potential risk of peri-procedural desaturations. We suggest adjusting the volumes of aliquots to avoid precipitous drops in oxygen saturations.

Corticosteroids are not recommended for treating PAP (10). Other promising therapies for autoimmune PAP include GM-CSF supplemental therapy (13) or aiming to reduce levels of anti-GM-CSF antibodies by using plasmapheresis (14) or rituximab (15), although these therapeutic options require further studies.

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References

1. Rosen SH, Castleman B, Liebow AA. Pulmonary alveolar proteinosis. N Engl J Med 1958;258:1123-42.
2. Dranoff G, Crawford AD, Sadelain M, et al. Involvement of granulocyte-macrophage colony-stimulating factor in pulmonary homeostasis. Science 1994;264:713-6.
3. Tanaka N, Watanabe J, Kitamura T, et al. Lungs of patients with idiopathic pulmonary alveolar proteinosis express a factor which neutralizes granulocyte-macrophage colony stimulating factor. FEBS Lett 1999;442:246-50.

4. Kitamura T, Tanaka N, Watanabe J, et al. Idiopathic pulmonary alveolar proteinosis as an autoimmune disease with neutralizing antibody against granulocyte/macrophage colony-stimulating factor. J Exp Med 1999;190:875-80.
5. Uchida K, Beck DC, Yamamoto T, et al. GM-CSF autoantibodies and neutrophil dysfunction in pulmonary alveolar proteinosis. N Engl J Med 2007;356:567-79.
6. Trapnell BC, Whitsett JA, Nakata K. Pulmonary alveolar proteinosis. N Engl J Med 2003;349:2527-39.
7. Inoue Y, Trapnell BC, Tazawa R, et al. Characteristics of a large cohort of patients with autoimmune pulmonary alveolar proteinosis in Japan. Am J Respir Crit Care Med 2008;177:752-62.
8. Holbert JM, Costello P, Li W, et al. CT features of pulmonary alveolar proteinosis. AJR Am J Roentgenol 2001;176:1287-94.
9. Wells AU. The clinical utility of bronchoalveolar lavage in diffuse parenchymal lung disease. Eur Respir Rev 2010;19:237-41.
10. Seymour JF, Presneill JJ. Pulmonary alveolar proteinosis: progress in the first 44 years. Am J Respir Crit Care Med 2002;166:215-35.
11. Michaud G, Reddy C, Ernst A. Whole-lung lavage for pulmonary alveolar proteinosis. Chest 2009;136:1678-81.
12. Brach BB, Harrell JH, Moser KM. Alveolar proteinosis. Lobar lavage by fiberoptic bronchoscopic technique. Chest 1976;69:224-7.
13. Kavuru MS, Sullivan EJ, Piccin R, et al. Exogenous granulocyte-macrophage colony-stimulating factor administration for pulmonary alveolar proteinosis. Am J Respir Crit Care Med 2000;161:1143-8.
14. Luisetti M, Rodi G, Perotti C, et al. Plasmapheresis for treatment of pulmonary alveolar proteinosis. Eur Respir J 2009;33:1220-2.
15. Borie R, Debray MP, Laine C, et al. Rituximab therapy in autoimmune pulmonary alveolar proteinosis. Eur Respir J 2009;33:1503-6.

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Case Report

Image-guided bronchoscopy for histopathologic diagnosis of pure ground glass opacity: a case report

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Abstract: Guided bronchoscopy has been found to be useful for the diagnosis of solid peripheral pulmonary lesions (PPLs) but more evidence on ground glass opacities (GGOs), especially those without a solid component, are lacking. A 69-year-old male, asymptomatic, heavy smoker was referred to our department for non-surgical diagnosis of a focal pure GGO in the right upper lobe that was found incidentally on computed tomography (CT). Transbronchial biopsy (TBB) with the aide of endobronchial ultrasound with a guide sheath (EBUS-GS), virtual bronchoscopic navigation (VBN), and fluoroscopy was performed for sampling. There were no complications after the procedure. The diagnosis of adenocarcinoma with lepidic growth pattern was established from the fourth and fifth TBB specimens and was confirmed on subsequent surgical resection. Image-guided bronchoscopy with TBB was successful for the diagnosis of a pure GGO. Use of a larger biopsy device may be helpful for the histopathologic diagnosis of lung adenocarcinoma with lepidic growth.

Keywords: Endobronchial ultrasound with a guide sheath (EBUS-GS); radial EBUS; ground glass opacity (GGO); peripheral pulmonary lesion (PPL); transbronchial biopsy (TBB)

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Introduction

Particular interest in peripheral pulmonary lesions (PPLs) and ground glass opacities (GGOs) of the lung has grown in recent years. If malignancy is suspected, the strategies for evaluation are positron emission tomography scan, non-surgical biopsy, or surgical resection (1). For non-surgical procedures, guidelines recommend extensive tissue sampling for accurate histopathologic and molecular diagnosis (2,3). Previous studies on transthoracic needle aspiration (TTNA) have reported that a higher percentage of GGO component contributed to a lower diagnostic yield for these types of PPLs (4,5).

Bronchoscopy is another non-surgical option for diagnosis of peripheral lung cancer (1). Endobronchial ultrasound with a guide sheath (EBUS-GS) had been reported to increase the diagnostic yield of transbronchial biopsy (TBB) (6,7) and the addition of virtual bronchoscopic navigation (VBN) can improve this yield further (8). Majority of studies on TBB for PPLs included solid lesions but reports on how to improve the procedure for GGOs are lacking. In this report, we present the case of a patient with pure GGO of the lung that was successfully diagnosed by TBB.

Case presentation

A 69-year-old male was referred to our department because of an incidental chest computed tomography (CT) scan finding of a pure GGO PPL, measuring 38 mm in the largest diameter, and located in the right segment 2 (*Figure 1*). He was asymptomatic, a 50-pack year tobacco smoker, and without history of malignancy.

This study was approved by the Institutional Review

Board of the hospital and informed consent was obtained from the patient.

Bronchoscopy was performed under local anesthesia with conscious sedation using BF 1T260 (outer diameter of 5.9 mm, working channel diameter of 2.8 mm; Olympus, Tokyo, Japan). VBN (LungPoint; Broncus Technologies, Inc., Mountain View, CA, USA) was used to plan the route to the target lesion (*Figure 2A*). Radial EBUS probe (UM-S20-20R, Olympus, Tokyo, Japan) with a guide sheath (2.6 mm compatible channel diameter, 2.55 mm maximum outer diameter; GS Kit K-203, Olympus, Tokyo, Japan) was inserted into the affected bronchus under fluoroscopy guidance (*Figure 2B*). The EBUS image showed blizzard sign (9) (*Figure 2C*). Cytology specimen was collected by brush (length 10 mm, diameter 2.0 mm); standard biopsy forceps with a 1.9 mm maximum outer diameter was used to collect a total of five histology samples (*Figure 2D*). There were no complications after bronchoscopy.

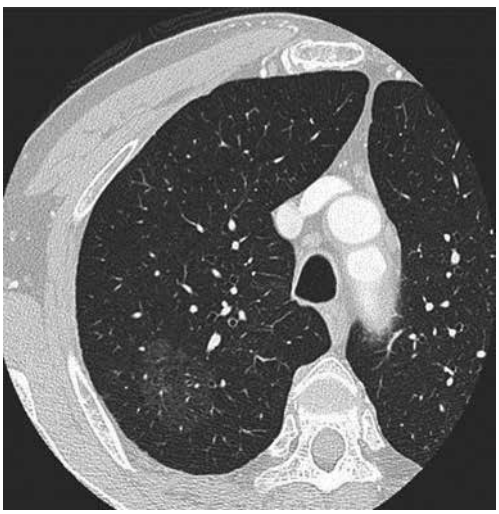

Figure 1 Thin-slice computed tomography (CT) scan in the axial plane shows a focal pure ground glass opacity measuring 38 mm (major axis) in the right segment 2a of the lung. There was no mediastinal lymph node enlargement.

Cytology examination was negative for malignant cells. The consecutive TBB specimens were examined histopathologically in the order that they were collected. The first and second specimens were negative for malignant cells, the third showed a few atypical cells, and the fourth and fifth specimens showed adenocarcinoma with lepidic growth pattern (*Figure 3*). After right upper lobectomy, the definitive diagnosis was confirmed to be minimally invasive adenocarcinoma (*Figure 4*) pstage IB (T2aN0M0). No further adjuvant therapy was administered.

Discussion

The introduction of chest CT scan screening has led to an increased interest in PPLs, particularly GGOs, which can be pre-invasive lesions in the lung adenocarcinoma growth spectrum. Non-surgical options for pathology diagnosis are by bronchoscopic or transthoracic approach. Studies on TTNA of GGOs demonstrated that obtaining core tissue

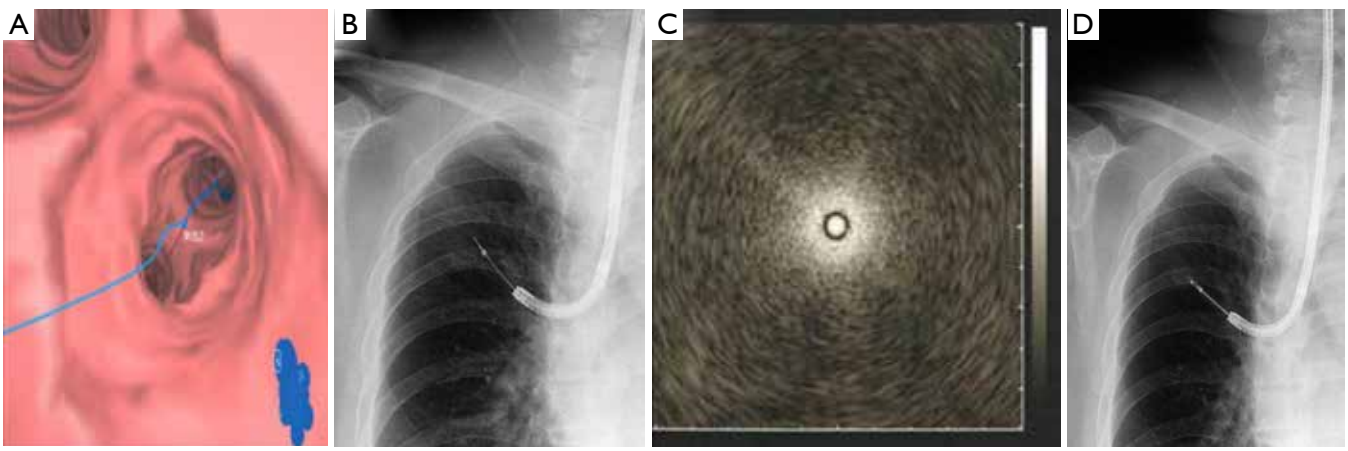

Figure 2 The bronchoscopy procedure. (A) Virtual bronchoscopy navigation (LungPoint) indicated that the bronchus leading to the target site was right B2a; (B) fluoroscopy image during radial probe EBUS scanning of the target site; (C) EBUS image of the blizzard sign, a hyperechoic shadow that was subtly more intense than the typical snowstorm appearance ascribed to normal alveolar tissue during scanning; (D) fluoroscopy image during transbronchial biopsy (TBB) using standard biopsy forceps (1.9 mm maximum outer diameter) with a guide sheath.

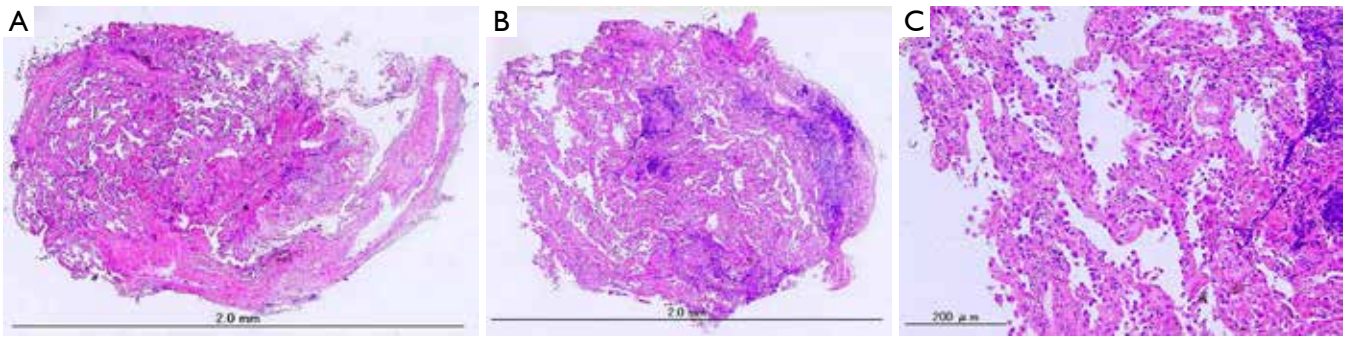

Figure 3 Photomicrographs of the diagnostic transbronchial biopsy (TBB) specimens on hematoxylin and eosin staining. The initial three TBB specimens were non-diagnostic, but the fourth (A, 40×) and fifth (B, 40×) specimens revealed adenocarcinoma with lepidic growth pattern; (C) higher magnification (100×) of the fifth specimen.

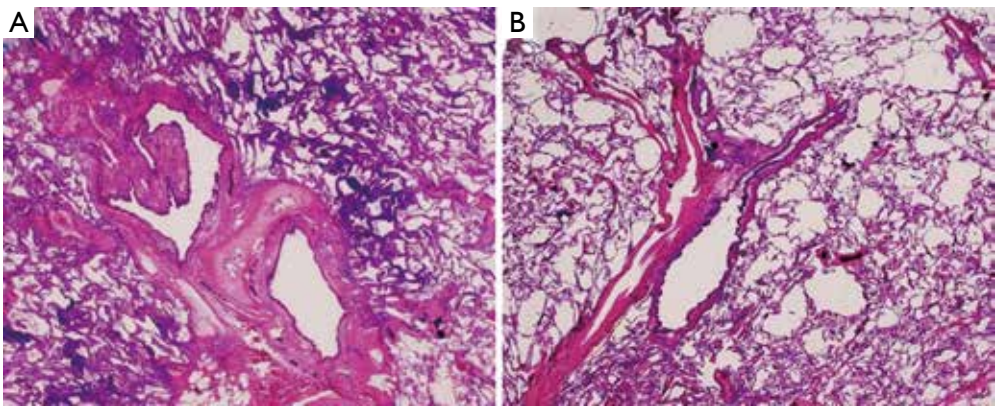

Figure 4 Photomicrographs of the surgical specimen (hematoxylin and eosin staining, 1.25×) show that in a tissue section from the tumor lesion of resected specimen (A), the smooth muscle layer of the terminal bronchial wall was relatively thick compared to that of a tissue section from the adjoining non-pathologic alveolar tissue (B).

resulted to high diagnostic yields and that pure GGOs were more difficult to diagnose (4,5,10). On the other hand, more studies on bronchoscopy as a diagnostic tool for GGOs are lacking. One study reported that EBUS-GS TBB of GGO-dominant lesions had a diagnostic yield of less than 60 percent (11). Further refinements that focus on tissue procurement from GGO-predominant or pure GGO PPLs are needed.

For EBUS-GS transbronchial sampling, it has been reported that an EBUS probe within a lesion and VBN were useful for tumor localization prior to biopsy, leading to improved diagnostic yields (6,8). We previously reported blizzard sign as a specific EBUS image for GGO (9). For this particular case, this sign helped us locate the biopsy site after it was detected by EBUS within the bronchial subsegment that was specified by VBN. At present, guide sheath kits (forceps, brush, and guide sheath) come in two sizes, large and small. Generally, bronchoscopists choose one of them according to PPL characteristics and physician preferences. For this patient, we chose to use the large size for the purpose of obtaining extensive amounts of tissue.

Histologically, GGOs are described to have replacing growth patterns along thickened but preserved alveolar walls; this lepidic growth usually corresponds to the ground glass component (3). It was recommended that high quality tissue samples were more effective than cytology samples for diagnosis of GGOs (2,3). To our knowledge, there have been no reports yet describing the optimal number of TBB specimens required to diagnose GGOs. In this case, cytology samples were negative and only the TBB samples were diagnostic. Specifically, the structural integrity of adenocarcinoma with lepidic growth pattern was demonstrated in the latter (fourth and fifth) TBB specimens. Correlating this with the histopathologic findings of thickened bronchial wall in the resected specimen (*Figure 4*), we considered that obtaining around five histology samples

by a larger device would be required to penetrate the thick smooth muscle layer.

In conclusion, image-guided bronchoscopy and use of a larger sampling device during TBB may be helpful for histopathologic diagnosis of pure GGO.

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References

1. Detterbeck FC, Lewis SZ, Diekemper R, et al. Executive Summary: Diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines. Chest 2013;143:7S-37S.
2. Travis WD, Brambilla E, Noguchi M, et al. Diagnosis of lung cancer in small biopsies and cytology: implications of the 2011 International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society classification. Arch Pathol Lab Med 2013;137:668-84.
3. Travis WD, Brambilla E, Noguchi M, et al. International association for the study of lung cancer/american thoracic society/european respiratory society international multidisciplinary classification of lung adenocarcinoma. J Thorac Oncol 2011;6:244-85.
4. Kim TJ, Lee JH, Lee CT, et al. Diagnostic accuracy of CT-guided core biopsy of ground-glass opacity pulmonary lesions. AJR Am J Roentgenol 2008;190:234-9.
5. Shimizu K, Ikeda N, Tsuboi M, et al. Percutaneous CT-guided fine needle aspiration for lung cancer smaller than 2 cm and revealed by ground-glass opacity at CT. Lung Cancer 2006;51:173-9.
6. Kurimoto N, Miyazawa T, Okimasa S, et al. Endobronchial ultrasonography using a guide sheath increases the ability to diagnose peripheral pulmonary lesions endoscopically. Chest 2004;126:959-65.
7. Wang Memoli JS, Nietert PJ, Silvestri GA. Meta-analysis of guided bronchoscopy for the evaluation of the pulmonary nodule. Chest 2012;142:385-93.
8. Tamiya M, Okamoto N, Sasada S, et al. Diagnostic yield of combined bronchoscopy and endobronchial ultrasonography, under LungPoint guidance for small peripheral pulmonary lesions. Respirology 2013;18:834-9.
9. Sasada S, Izumo T, Chavez C, et al. Blizzard sign as a specific endobronchial ultrasound image for ground glass opacity: a case report. Respiratory Medicine Case Reports 2014;12:19-21.
10. Lu CH, Hsiao CH, Chang YC, et al. Percutaneous computed tomography-guided coaxial core biopsy for small pulmonary lesions with ground-glass attenuation. J Thorac Oncol 2012;7:143-50.
11. Izumo T, Sasada S, Chavez C, et al. The diagnostic utility of endobronchial ultrasonography with a guide sheath and tomosynthesis images for ground glass opacity pulmonary lesions. J Thorac Dis 2013;5:745-50.

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Case Report

Conservative management of post-intubation tracheal tears—report of three cases

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Abstract: Iatrogenic tracheal rupture is a rare complication after intubation. We present three patients with tracheal tears. In all of these patients, a common finding was a lesion of the posterior tracheal wall with postoperative subcutaneous and emphysema as the first clinical sign of the rupture. Diagnosis and follow-up were based on clinical and endoscopic findings and chest computed tomography (CT) scans. In our cases with progressive subcutaneous and mediastinal emphysema or dyspnea, we performed a tracheotomy and bypassed the lesion with a tracheostomy tube to avoid an increase in air leakage into the mediastinum. Under broad-spectrum antibiotic therapy, no mediastinitis occurred and all patients survived without sequelae. Closure of tracheostomy was scheduled for 1-2 months after tracheal injury. Analysis of surgical and anesthesiological procedures revealed no abnormalities and the accumulation of tracheal injuries was considered as accidental. We found that in clinically stable patients with spontaneous breathing and with no mediastinitis, a conservative management of tracheal tears is a safe procedure.

Keywords: Intratracheal intubation; laceration; subcutaneous emphysema; mediastinal emphysema; mediastinitis; tracheostomy

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Introduction

These patients, as presented below, underwent routine otolaryngological surgery in a tertiary referral center. We tried to elucidate these cases in critical incident audits. A multidisciplinary team, consisting of otolaryngologists (the operating surgeons involved), anesthetists and an external thoracic surgeon consultant, discussed the course of events. We carried out literature research in PubMed, Web of Science and Cochrane databases with the search strings "subcutaneous emphysema, otolaryngology", "subcutaneous emphysema, laryngoscopy", "iatrogenic tracheal rupture", or "tracheal rupture, intubation".

Case reports

Clinical history of our three cases is listed in *Table 1*. Two operations were carried out by an attending surgeon and one was performed by a resident registrar under the supervision of an attending surgeon. Operating teams and anesthetists were different in each case. All operations were done in the same operating theater. Instruments for general anesthesia were checked regularly and after all events. During this period, we used the products from the same endotracheal tube manufacturer and supplier. Difficult but atraumatic intubation was noted in case 3. Operations under anesthetic were uneventful except for case 1.

Table 1 Clinical history of our three patients having tracheal tear

	Case 1	Case 2	Case 3
Age (years), Gender	75, Male	55, Female	70, Male
Initial surgery	Voice prosthesis re-implantation after previous laryngectomy	Subtotal parotidectomy and neck dissection because of epithelial-myopeithelial carcinoma	Panendoscopy because of a floor of the mouth carcinoma (no biopsy taken)
Symptoms	Intra-operative cathecolamine therapy, post-operative subcutaneous emphysema	Left sided supraclavicular emphysema, good general condition without any pulmonary symptoms	Progressive subcutaneous emphysema of the neck, face and chest, later on angina pectoris and dyspnea
Clinical findings	Gaping lesion (3 cm) of tracheal mucosa, 2 cm above carina, pneumothorax, mediastinal emphysema	Tracheal tear in the middle portion on the right side (posterior wall), no pneumothorax	Pneumothorax on both sides (chest CT), two parallel mucosal tracheal tears of the right posterior wall
Therapy	ICU monitoring, thorax drainage, meropenem i.v., cuffed endotracheal tube	ICU monitoring, meropenem i.v.; tracheotomy because of rapid increase of subcutaneous and mediastinal emphysema; surgical closure of the tracheastoma after two months	ICU monitoring, tracheotomy, meropenem i.v.; surgical closure of tracheastoma after 45 days

CT, computed tomography.

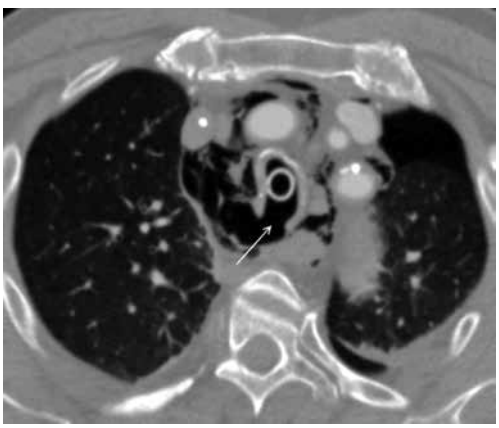

Figure 1 Case 1. Transverse computed tomography (CT) scan reveals a full perforation of the pars membranacea (arrow) and the resulting mediastinal emphysema and left pneumothorax.

In case 1, the tracheal lesion was diagnosed intra-operatively and the patient was transferred to the ICU immediately after surgery (*Figure 1*). In this previously laryngectomized patient, we positioned a tracheal tube with cuff distally to the tear to prevent further air leakage through the injury site (1-3). A prerequisite for this method was an intact portion of trachea above the carina (3). Bridging the rupture with the tube and drainage of concomitant pneumothorax allowed a rapid stabilization of vital signs. As this individual was considered a high-risk patient for open-chest surgery (repair of the tear), conservative treatment was initialized. This multimorbid patient had a favorable outcome without closure of the tracheal rupture. A video clip about shows the healed trachea three weeks after injury (*Figure 2*). In the other two cases, tracheal laceration was diagnosed post-operatively. Subcutaneous emphysema was the first symptom of mucosal injury in these two cases, always appearing post-operatively with a delay of 3-15 h. Once the diagnosis of tracheal injury was made, all patients were continuously monitored for vital signs. Empiric broad-spectrum antibiotic therapy was administered to all patients to prevent mediastinitis. Since these patients presented progressive subcutaneous and mediastinal emphysema or dyspnea or both, we performed tracheostomy to maintain safe airways and to decrease intra-tracheal pressure and air leakage. In these two cases, the need for a secure airway emerged, when the symptoms progressed post-operatively at various intervals. In case 2 for example, this delayed progress appeared one week after the injury. Our experience supports literature data reporting a rapid settling of subcutaneous emphysema and pneumomediastinum after tracheotomy (3-5).

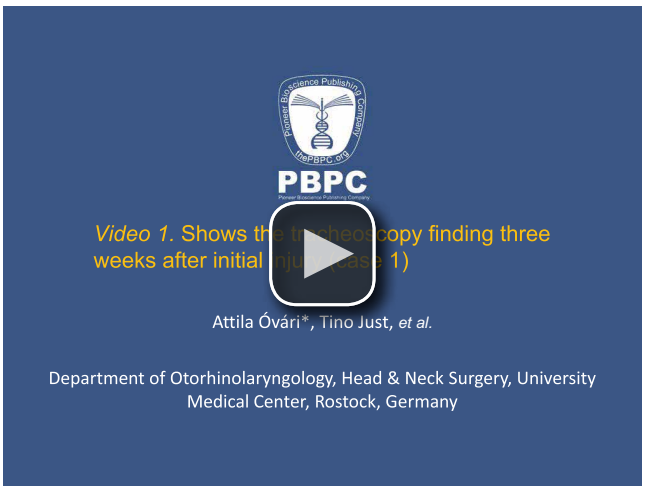

Figure 2 Shows the tracheoscopy finding three weeks after initial injury (case 1). Note the scar of the laceration on the posterior tracheal wall (3-14 s). Voice prosthesis *in situ* (laryngectomized patient).

Surgical and anesthesiological procedures were evaluated by an external consultant specialist, who found no abnormalities. The accumulation of events was considered as accidental. After analyzing the possible promoting factors in our case series, we believe that repeated ex- and intubations led to tracheal injury in case 1. In case 2, chronic tracheo-bronchitis and inhalative corticoid treatment might have contributed to increased vulnerability of the tracheal mucosa. In case 3, we did not find any risk factor for the injury.

Discussion

Diagnostic process in post-operative subcutaneous emphysema

If patients develop subcutaneous emphysema after surgery, mucosal injury must be identified, and potential concomitant pneumomediastinum or pneumothorax should be excluded or diagnosed. Emphysema is not specific to tracheal laceration. Diffuse air entrapment in soft tissues of the neck, head and chest may impede the localization of the lesion, and the extent of emphysema does not necessarily reflect the severity of injury (6).

In the literature, combination of chest computed tomography (CT) scans and tracheoscopy are recommended to diagnose a suspected tracheal injury. CT gives evidence of air leakage into the mediastinum and detects a pneumothorax, however, the tracheal injury site seen on CT scans does not always correspond with tracheoscopy findings. CT scans have only 85% sensitivity for detecting tracheal injury (7). Therefore, endoscopic evaluation of the upper aero-digestive tract is the mainstay of diagnosis of tracheal lacerations.

Causes of post-intubation tracheal laceration

Surgical trauma of the mucosa is the main cause of emphysema. However, elevated intrathoracic pressure (physical strain, forced blowing, ventilation with positive pressure, coughing when the expiration vent is closed), lung diseases such as bronchial asthma, or perforation of a lung bulla may provoke subcutaneous emphysema. Iatrogenic tracheal rupture after intubation is very rare. Incidence ranges between 1:20,000 and 1:75,000 for intubation with a 'single lumen' endotracheal tube. 'Double lumen' endotracheal tubes have a larger diameter and intubation with these tubes causes tracheal rupture more frequently (0.05-0.19%) (8,9). Typically, these lesions are longitudinal lacerations of the posterior tracheal wall (paries membranaceus) (8,10). Due to the anatomic situation with the esophagus supporting the membranous trachea on the left side, tracheal tears are localized more frequently on the right side. Seldom, tracheal lacerations spread out into the bronchi (10).

A meta-analysis of 182 cases (50 reports) demonstrated that emergency intubation is a risk factor of tracheal tears (1). Tracheal rupture occurs mainly in women, the reason is that airways in females have a narrower diameter and tracheas are shorter and less resistant (8,10-14). The precise cause of post-intubation tracheal tears is unclear. It may occur during passage of the endotracheal tube, when the tube's tip wounds the mucosa, even without difficult intubation (10,15). The use of a stylet as a guide for the tube may also cause tracheal injury by the same mechanism (15). Overinflation of the endotracheal tube cuff is the most common cause of tracheal tears (15-17). Since "high-volume-low-pressure" cuffs have been introduced, this complication is found less frequently, even though not completely eliminated. Accidental cuff overinflation with higher pressure is an obvious explanation; relative overinflation is possible if the cuff is filled just above the carina where the trachea has its largest diameter and the tube is pulled back to its correct position (12). Generally, tube repositioning without cuff deflation should be avoided to prevent mucosal injury. Intra-operative repositioning of the patient's head or body can also displace an endotracheal tube and promote tracheal injury (1,18). Diffusion of anesthetic N_2O gas into the cuff can also increase pressure

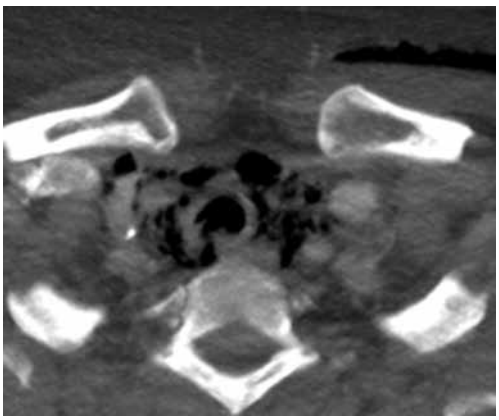

Figure 3 Stages of the spontaneous healing of a tracheal tear (case 2). Computed tomography (CT) scan shows the lesions of the right posterior tracheal wall, as well as soft tissue beginning to overlap the defect (first CT-scan).

by overinflating the cuff (19,20), however, N_2O was not given in our cases.

Therapeutic approaches for tracheal tears

If a tracheal lesion is confirmed in a patient, anesthetists and thoracic surgeons should be consulted to set up an individualized therapy regime. Delayed clinical presentation of subcutaneous emphysema prolongs diagnosis and therapeutic intervention. Any delay in diagnosis favors deterioration of the clinical situation, i.e., mediastinitis and pneumothorax. Miñambres *et al*., however, found that diagnosis time does not have an influence on mortality (1). Presumably, the cause that required intubation is a greater factor in clinical outcome than the tracheal injury itself (1).

Traditionally, tracheal tears have usually been treated surgically (1,9,12,17,21). For example, lesions discovered during an open thoracic surgery procedure are repaired at the same time (1,10,21). Nevertheless, delayed surgical repair of the tear doubles the risk of death in patients whose tracheal rupture is detected after surgery, compared to a patient group with simultaneous repair (1). In particular, in severely ill patients, surgical closure via open chest surgery has a mortality up to 71% (13). Increasing evidence is being presented in case series consisting of small numbers of patients, suggesting conservative management of lacerations as a viable option (1). There is a trend toward non-surgical therapy although consensus has not been reached and clear guidelines are lacking (1,14).

The main factors discussed in the literature determining the treatment of choice are, first, the clinical situation of the patient and, second, the properties of the tracheal lesion (localization, length and depth). Recent studies suggest conservative management of tracheal lacerations when patients have minimal, non-progressive symptoms (subcutaneous emphysema or pneumomediastinum), no air leakage, no respiration difficulty, no esophageal injury, and patients who are breathing spontaneously or whose extubation is expected within 24 h (1,10,17).

Some authors emphasized the significance of the length of the rupture and suggested conservative treatment in selected, clinically stable patients having tracheal ruptures less than 2 cm (1,22). Lesions over 2 cm in length are thought to be better treated surgically (1,17,19); others, however, perform conservative therapy in cases with tears up to 4 cm in length (23). Small tracheal lesions localized in the upper third of the trachea can be managed conservatively in patients without respiratory distress or mediastinitis (19). Lacerations of the upper two-thirds of the trachea, and not involving all tracheal layers, are also suitable for non-surgical therapy (2). Cardillo *et al*. underlined the significance of the depth of the lesion for determining further treatment (14). They suggest immediate surgical repair for cases with esophageal injury and/or mediastinitis. All other injuries of the tracheal wall, including fully penetrating lacerations are managed conservatively with fibrin glue (14).

Tracheal stenting of post-intubation lacerations is a relatively new therapeutic approach (24-26). Expandable tracheal stents represent an alternative to surgical therapy, especially in patients with an unacceptably high risk of surgery-related mortality (26). Problems of airway stents such as halitosis or granulation tissue formation can be treated with inhalation and intraluminal laser vaporization (26). Newly developed removable tracheal stents can prevent these late complications (25).

Tracheotomy is a simple, less invasive alternative to surgical repair as it reduces intratracheal pressure and air leakage through the tear, thereby allowing spontaneous healing of the rupture (3,4,6). Additional CT-scans and endoscopic findings are presented demonstrating the stages of tear healing (*Figures 3*-7). Direct intraluminal suture of proximal tracheal lacerations can be performed through the tracheal stoma in a minimally invasive manner (18). Moreover, a tracheal stoma makes tracheoscopic control easier.

Failure management after the reported events

In our department we changed anesthetic management

Figure 4 Stages of the spontaneous healing of a tracheal tear (case 2). Virtual computed tomography (CT) tracheoscopy shows the longitudinal lesion of the tracheal wall (first CT-scan).

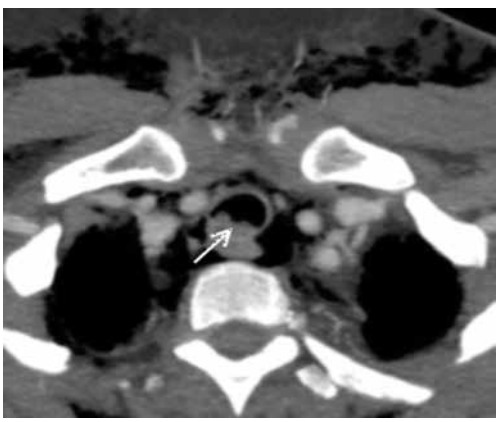

Figure 5 Stages of the spontaneous healing of a tracheal tear (case 2). Seventh day after injury: full coverage of the defect due to protrusion of the soft tissue of esophagus (arrow).

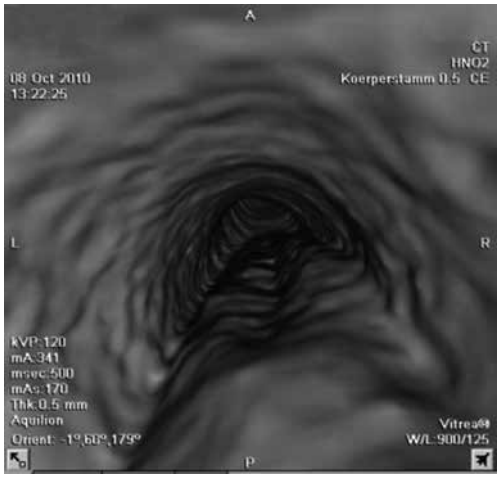

Figure 6 Stages of the spontaneous healing of a tracheal tear (case 2). Seventh day after injury: virtual computed tomography (CT) tracheoscopy showing intraluminal soft tissue covering the defect. A, anterior; P, posterior; R, right; L, left.

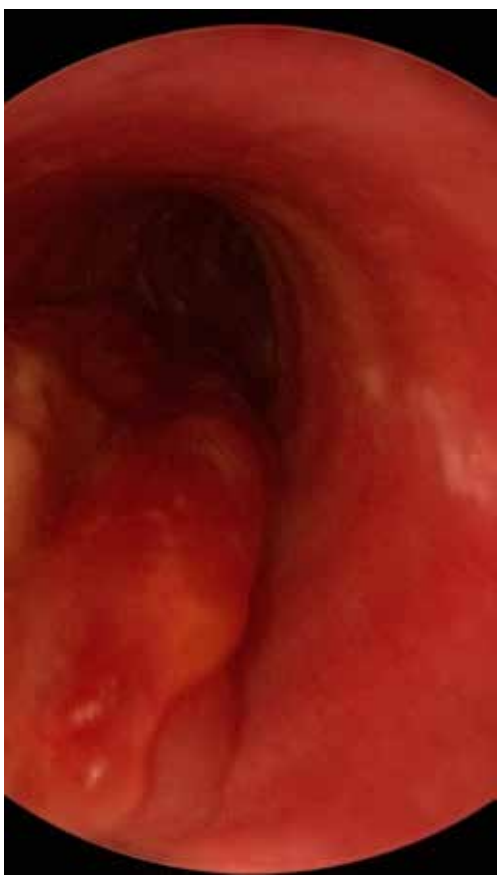

Figure 7 Stages of the spontaneous healing of a tracheal tear (case 2). Seventh day after injury: tracheoscopic view of the tear with protrusion of the soft tissue of the esophagus.

to prevent further tracheal injuries. In patient's informed consent, the possibility of unforeseeable and very rare airway injuries is now explicit mentioned. Continuous cuff pressure monitoring was introduced and all anesthesia devices were supervised. To be able to examine potential material faults, used endotracheal tubes are retained until the discharge of each patient. Ultimately, the manufacturer of endotracheal tubes was changed. After these measures were taken, we have not diagnosed a new case with tracheal injury.

Conclusions

In our three cases, a conservative management (tracheotomy + empiric broad-spectrum antibiotic therapy) of post-intubation tracheal laceration proved to be a safe and effective procedure. All tracheal ruptures healed without sequelae. No mediastinitis or injury-related deaths occurred. Tracheal stoma was surgically closed in all but one case (laryngectomized patient) with good cosmetic results. Nevertheless, we believe that the risk for intubation-related tracheal injuries can only be minimized but not completely eliminated, being inherent complications of the procedure.

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References

1. Miñambres E, Burón J, Ballesteros MA, et al. Tracheal rupture after endotracheal intubation: a literature systematic review. Eur J Cardiothorac Surg 2009;35:1056-62.
2. Gabor S, Renner H, Pinter H, et al. Indications for surgery in tracheobronchial ruptures. Eur J Cardiothorac Surg 2001;20:399-404.
3. Mullan GP, Georgalas C, Arora A, et al. Conservative management of a major post-intubation tracheal injury and review of current management. Eur Arch Otorhinolaryngol 2007;264:685-8.
4. Peña MT, Aujla PK, Choi SS, et al. Acute airway distress from endotracheal intubation injury in the pediatric aerodigestive tract. Otolaryngol Head Neck Surg 2004;130:575-8.
5. Óvári A, Just T, Dommerich S, et al. Conservative management of post-intubation tracheal tears—report of three cases. Asvide 2014;1:10.3978/asvide.242
6. Seidl RO, Todt I, Nielitz T, et al. Tracheal ruptures in endotracheal intubation. Diagnosis and therapy. HNO 2002;50:134-8.
7. Chen JD, Shanmuganathan K, Mirvis SE, et al. Using CT to diagnose tracheal rupture. AJR Am J Roentgenol 2001;176:1273-80.
8. Lampl L. Tracheobronchial injuries. Conservative treatment. Interact Cardiovasc Thorac Surg 2004;3:401-5.
9. Schneider T, Volz K, Dienemann H, et al. Incidence and treatment modalities of tracheobronchial injuries in Germany. Interact Cardiovasc Thorac Surg 2009;8:571-6.
10. Conti M, Pougeoise M, Wurtz A, et al. Management of postintubation tracheobronchial ruptures. Chest 2006;130:412-8.
11. Hofmann HS, Rettig G, Radke J, et al. Iatrogenic ruptures of the tracheobronchial tree. Eur J Cardiothorac Surg 2002;21:649-52.
12. Massard G, Rougé C, Dabbagh A, et al. Tracheobronchial lacerations after intubation and tracheostomy. Ann Thorac Surg 1996;61:1483-7.
13. Meyer M. Latrogenic tracheobronchial lesions--a report on 13 cases. Thorac Cardiovasc Surg 2001;49:115-9.
14. Cardillo G, Carbone L, Carleo F, et al. Tracheal lacerations after endotracheal intubation: a proposed morphological classification to guide non-surgical treatment. Eur J Cardiothorac Surg 2010;37:581-7.
15. Jo YY, Park WY, Choi E, et al. Delayed detection of subcutaneous emphysema following routine endotracheal intubation -A case report-. Korean J Anesthesiol 2010;59:220-3.
16. Striebel HW, Pinkwart LU, Karavias T. Tracheal rupture caused by overinflation of endotracheal tube cuff. Anaesthesist 1995;44:186-8.
17. Marty-Ané CH, Picard E, Jonquet O, et al. Membranous tracheal rupture after endotracheal intubation. Ann Thorac Surg 1995;60:1367-71.
18. Walles T, Friedel G, Stöltzing H, et al. Case studies of iatrogenic tracheal injury during intraoperative ventral positioning. Symptoms, diagnostics, and differential therapy. Chirurg 2007;78:374-8.
19. Sippel M, Putensen C, Hirner A, et al. Tracheal rupture after endotracheal intubation: experience with management in 13 cases. Thorac Cardiovasc Surg 2006;54:51-6.
20. Tu HN, Saidi N, Leiutaud T, et al. Nitrous oxide increases endotracheal cuff pressure and the incidence of tracheal lesions in anesthetized patients. Anesth Analg 1999;89:187-90.
21. Borasio P, Ardissone F, Chiampo G. Post-intubation tracheal rupture. A report on ten cases. Eur J Cardiothorac Surg 1997;12:98-100.

22. Carbognani P, Bobbio A, Cattelani L, et al. Management of postintubation membranous tracheal rupture. Ann Thorac Surg 2004;77:406-9.
23. Jougon J, Ballester M, Choukroun E, et al. Conservative treatment for postintubation tracheobronchial rupture. Ann Thorac Surg 2000;69:216-20.
24. Yopp AC, Eckstein JG, Savel RH, et al. Tracheal stenting of iatrogenic tracheal injury: a novel management approach. Ann Thorac Surg 2007;83:1897-9.
25. Creagh-Brown B, Sheth A, Crerar-Gilbert A, et al. A novel approach to the management of acute tracheal tear. J Laryngol Otol 2008;122:1392-3.
26. Madden BP, Sheth A, Ho TB, et al. Novel approach to management of a posterior tracheal tear complicating percutaneous tracheostomy. Br J Anaesth 2004;92:437-9.

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Case Report

Pulmonary benign metastasizing leiomyoma: a case report and literature review

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Abstract: Benign metastasizing leiomyoma (BML) is a rare condition that occurs in all age groups and that is particularly prevalent among women of late childbearing age. All patients have a history of uterine leiomyoma and/or myomectomy, often associated with distant metastases from the uterus, which commonly occurs in the lung. We report the case of a 32-year-old young woman suffering from chest stuffiness, labored respiration and weakness after a myomectomy performed one month earlier. The chest CT showed a diffuse miliary shadow in both sides of her lungs, but serum tumor markers such as CA125, CA199, carcinoembryonic antigen (CEA), neuron specific enolase (NSE), and CYFRA21-1 were normal. The patient underwent a lung biopsy by thoracoscopic surgery after four weeks of anti-TB treatment; there were no significant changes in the chest CT. H&E staining showed that the tumor cells had characteristics of smooth muscle cell differentiation. Immunohistochemical staining showed a low tumor cell proliferation index, which indicated that the likelihood of a malignancy was not high. There was no expression of CD10, indicating a diagnosis of pulmonary benign metastasizing leiomyoma (PBML). Smooth muscle actin (SMA) and desmin as specific markers of smooth muscle and the estrogen receptor (ER) and progesterone receptor (PR) were all strongly positive, which is characteristic of PBML. The patient was given the anti-estrogen tamoxifen for 3 months. With no radiological evidence of disease development and further distant metastasis, the patient will continue to be followed.

Keywords: Benign metastasizing leiomyoma (BML); myomectomy; pulmonary metastases; lung biopsy; immunohistochemistry

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Introduction

Benign metastasizing leiomyoma (BML) is a rare condition that occurs in all age groups, mostly between age 30 and 74, and is particularly prevalent among women of late childbearing age. Its clinical course is closely associated with sex hormone levels. All BML patients have a history of uterine leiomyoma and/or myomectomy. Symptoms usually develop and a diagnosis is made within 15 years (1), and BML is often associated with distant metastases from the uterus, such as benign leiomyoma in the lungs, paraaortic lymph nodes, abdominal lymph nodes, heart, breasts, liver, esophagus, trachea, limb striated muscles, skeletal muscle, skin, scars and central nervous system (2-7). The lungs are the most common site of metastasis, with characteristic scattered nodules (8-12). The pathological results of a pulmonary nodule examination usually indicate a specific smooth muscle phenotype with a low proliferation index and slow growth. The nodules are also positive for the estrogen receptor (ER) and the progesterone receptor (PR), revealing its uterine origin (13). This type of disease is usually called pulmonary benign metastasizing leiomyoma (PBML).

Since the first case of metastasizing leiomyoma was

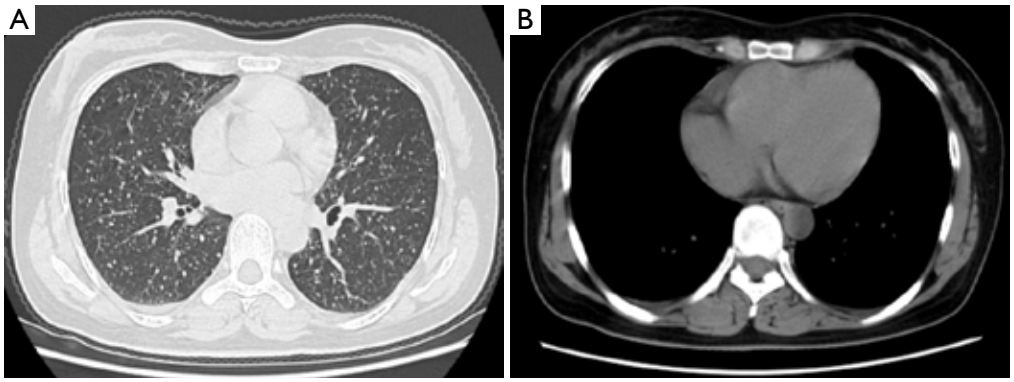

Figure 1 (A) A diffuse miliary shadow in both lungs; (B) the mediastinal window scan was normal.

discovered and reported by Steiner (14) in 1939, more than 100 cases of PBML have been reported. Most cases have been discovered by chest X-ray or CT scan during routine examinations. Some patients have symptoms such as cough, dyspnea or chest pain; PBML is quite difficult to diagnose by simple medical imaging or physical examination and is often misdiagnosed as pneumonia, bronchitis, phthisic or metastasizing lung cancer. Open lung biopsy (OLB) is the standard diagnostic procedure for PBML.

We reported a case of PBML. The pulmonary metastasis appeared only 1 month after the myomectomy, which was performed earlier than in any other cases. The clinical pathology, imaging features, bronchoscopy results, immunohistochemical staining features, diagnosis, treatment and prognosis are analyzed and discussed in this article, and the literature is reviewed.

Case report

A 32-year-old non-smoking woman (BMI 26) with a history of blood transfusion was hospitalized with chest tightness and labored breathing for one month. The patient suffered from chest tightness, labored breathing and weakness after a myomectomy that had been performed one month earlier, but there was no patent cough or expectoration. The patient gave birth to a child at 30 years of age. The first menstruation of the patient was at 12 years of age and was regular thereafter, occurring every 27-28 days and lasting 4-5 days. The chest CT revealed diffuse pulmonary lesions. The laboratory examinations were positive for PPD, negative for serum anti-tuberculosis antibodies, and negative for T-spot; the blood sedimentation rate was 20 mm/h, and C-reactive protein was within the normal range. The results of a routine blood test, routine stool test, routine urine test, ESR, blood coagulation function, and myocardial enzyme determination were all within normal limits. Serum concentrations of cancer markers such as CA125, CA199, CA153 and CA242 were all within normal ranges. Carcinoembryonic antigen (CEA), neuron specific enolase (NSE) and cytokeratin 19 fragments (CYFRA21-1) were also all within normal limits. An abdominal MRI showed a hemangioma over the posterior segment of the liver and a cyst over the anterior segment of the liver. The results of a bronchoscopy were normal, and a TBLB was negative. The results of an ECT bone scan were normal. The lung function tests were within the normal limits except for a slightly low residual volume. The diffusion lung capacity was normal. Deep venous ultrasonography of the lower limbs showed no thrombosis. The patient felt no improvement taking isoniazid, rifampicin and ethambutol associated with levofloxacin antituberculosis treatment for 4 weeks. Another chest CT (*Figure 1*) showed a diffuse miliary shadow in both lungs, small multiple lymph nodes in the mediastinum and bilateral axillaries. Subsequently, a lung biopsy was taken by thoracoscopic surgery, which showed obvious nodules in the right lung. Multiple tissues were taken for biopsy using the thoracoscope.

H&E staining (*Figure 2*) showed spindle-shaped smooth muscle tumor cells of different differentiations. The tumors appeared multi-nodular and isolated. The realm was clear, the nodular surface was coated with alveolar epithelium, some nodules had a hardened area, and the cell morphology was consistent without atypia and with rare mitoses. Immunohistochemical staining is shown in *Figure 3*; CD10 (*Figure 3A*) is the key to the differential diagnosis; we were able to make a differential diagnosis of

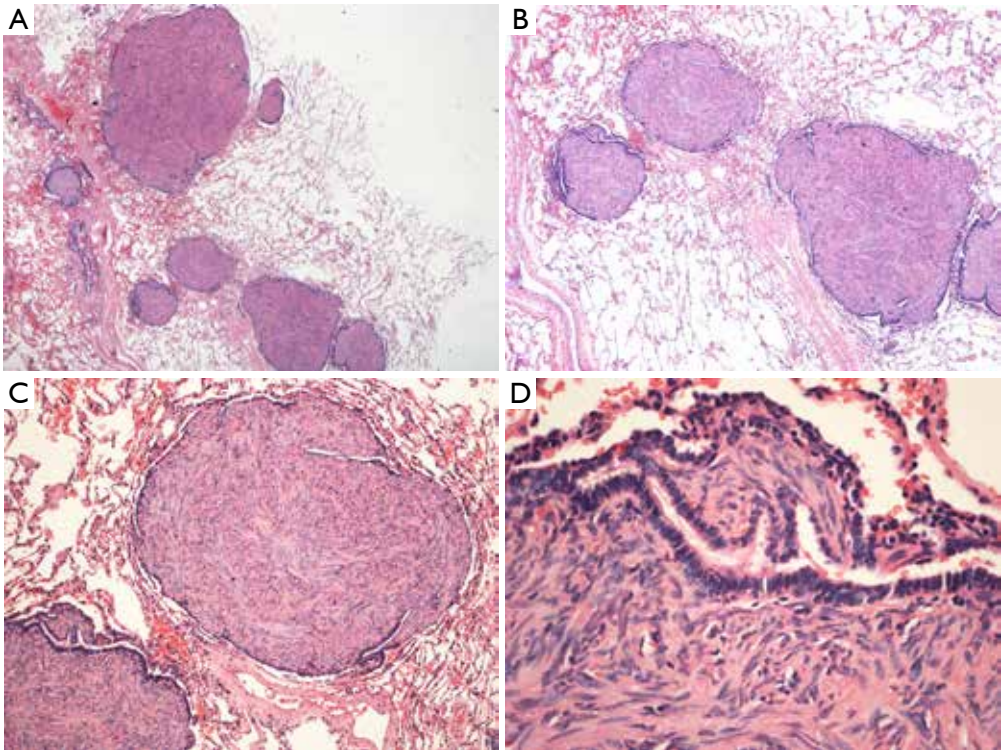

Figure 2 Pathological findings in the right lung through a thoracoscopic lung biopsy by H&E staining showed spindle smooth muscle cells of different differentiations. The tumors had multiple nodules and were isolated. The realm was clear, and the cell morphology was consistent, without atypia and with rare mitoses (A, ×100; B, ×100; C, ×200; D, ×400).

PBML. Solitary fibrous tumors and endometrial stromal sarcoma, which are both spindle cell tumors, were evident in the lung. CD10 expression was positive in the latter two, and the CD10 expression in the PBML was negative. The negative expression of CD10 confirmed the diagnosis of PBML. Positivity for SP-B (*Figure 3B*) indicates a primary lung tumor, and negativity for SP-B (as in *Figure 3B*) is related to lung metastases. The low proliferative activity of Ki-67 [*Figure 3C*; Ki-67(+) <5%] showed a low tumor cell proliferation index. CK (*Figure 3D*) is an epithelial marker, so the PBML was inevitably negative. Positivity for α-smooth muscle actin (SMA) (*Figure 3I*) and desmin (*Figure 3E,F*) suggested that the tumor was derived from smooth muscle. Strong positivity for the ER and the PR indicated that the tumor cells originated in the uterus and were regulated by the ER and PR (*Figure 3G,H*). Based on all of these results, the patient was diagnosed with PBML.

The patient was given the anti-estrogen tamoxifen for 3 months. No further development of the disease or distant metastasis was found. The patient has been followed for 4 months and will continue to be followed.

Discussion

Hysteromyoma is a common gynecologic tumor, 50% of which occurs in women over 30 years of age (15). Most hysteromyoma is benign, and malignant cases account for 0.13-6% (15). PBML usually refers to benign hysteromyoma metastasizing to the lung (1,7-10,16,17), similar to distant endometriosis (13).

There are several main hypotheses for the pathological origin of PBML (18-20) to be confirmed: (I) it is derived from low-grade leiomyomatosis; (II) it is derived from benign metastasizing hysteromyoma; or (III) it is derived from a multicenter-growing leiomyomatosis. Additionally, some researchers hypothesize that BML is related to angioleiomyoma (13). The main pathological PBML lesions show no significant differences from leiomyomatosis in other parts of the body. The characteristic findings are well-defined nodules varying from miliary size to 10 cm, interwoven with a pale solid ductile cut surface with no hemorrhage or necrosis. Immunohistochemical staining of tumor issues in the lung showed that the tumor cells

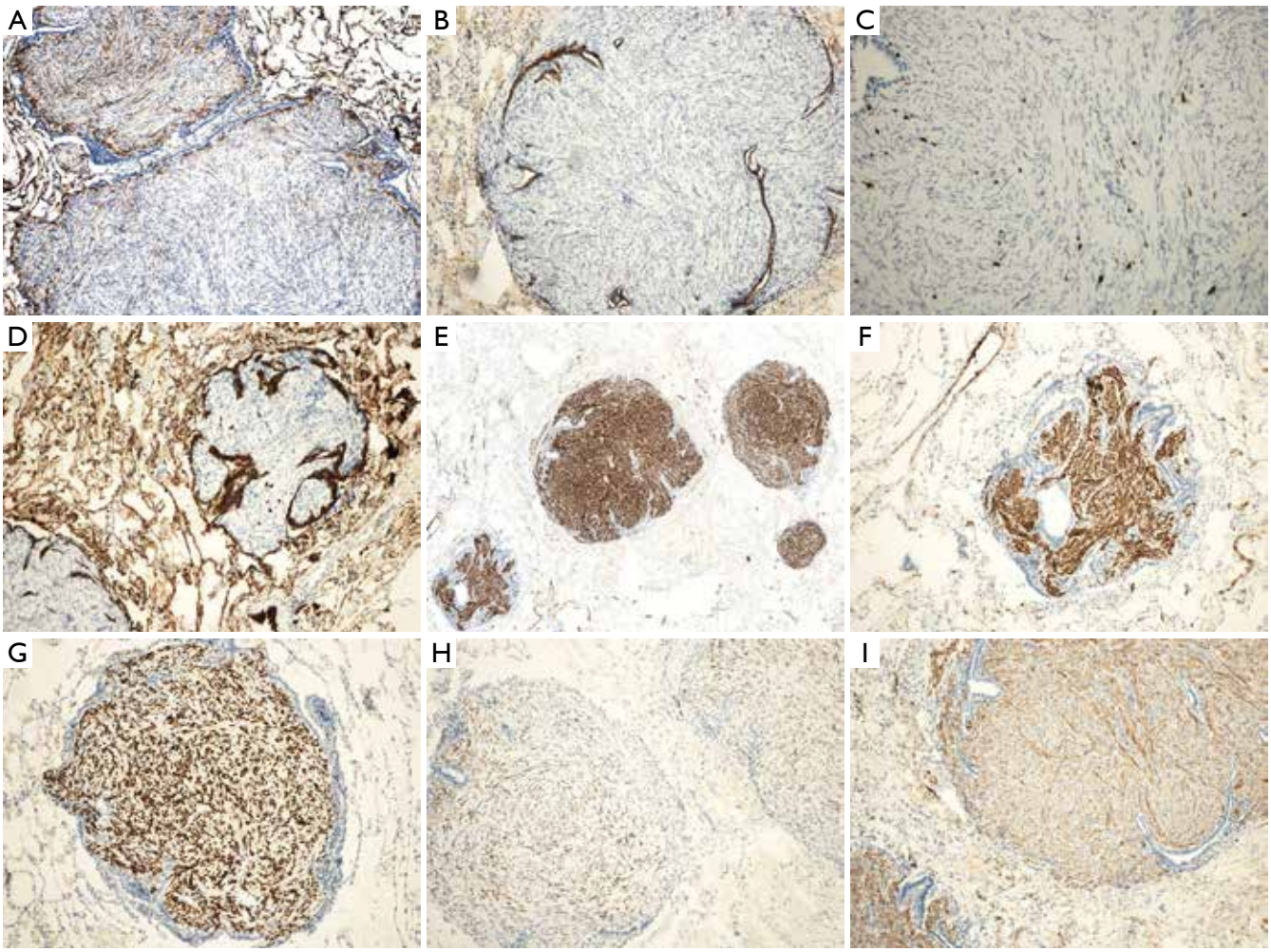

Figure 3 Groups of immunohistochemical staining results. (A) Negativity for CD10 throughout the tumor suggested that this was not a solitary fibrous tumor or an endometrial stromal sarcoma but rather PBML, ×400; (B) negativity for SP-B throughout the tumor suggested lung metastases, ×400; (C) weak positivity for Ki-67 (<5%) suggested that the low proliferation index of tumor cells, ×400; (D) negativity for CK throughout the tumor suggested that the tumor was not of epithelial origin, ×400; (E) strong positivity for desmin throughout the tumor suggested that the tumor was derived from smooth muscle, ×400; (F) strong positivity for desmin-2 throughout the tumor suggested that the tumor was derived from smooth muscle, ×400; (G) strong positivity for the progesterone receptor (PR) throughout the tumor suggested that the tumor cells originated in the uterus and were regulated by the PR, ×400; (H) strong positivity for the estrogen receptor (ER) throughout the tumor suggested that the tumor cells originated in the uterus and were regulated by the ER, ×400; (I) strong positivity for SMA showed that the tumor was derived from smooth muscle, ×400.

are characteristic of smooth muscle cell differentiation (α-SMA+, Desmin+), and the low expression of Ki-67 indicates a low tumor cell proliferation index, which indicates that the likelihood of malignancy was not high.

PBML is generally considered a monoclonal benign tumor that is potentially metastatic (21). It is hormone dependent, and its growth mainly depends on estrogen and progesterone. Estrogen causes the tumor to progress, whereas progesterone causes the tumor to subside. The tumor subsides when the patient is pregnant or menopausal. However, its pathogenesis remains unknown (21). According to a popular theory, the tumor is believed to hematogenously spread. When uterine surgery is undertaken, including uterine curettage, hysteromyomectomy and hysterectomy, the possibility of hematogenous spread by surgical induction increases and the tumor may disseminate to the lung through the venous circulation (7). However, this theory cannot explain how the lung nodules exist before a hysterectomy in some cases (22).

There were miliary changes in both of the patient's lungs only one month after the hysteromyomectomy, which suggested hematogenous metastasis to the lung. However, there was no obvious relation between the severity of the lesions and the influences of the disease on the lung. The lung function of the patient was not significantly affected even though there were miliary changes in the lung.

Because PBML progresses slowly, it is often discovered accidently during physical examination. It has a small influence on lung function. Some patients present with fever, chest stuffiness, pant, dry cough and hemoptysis, whereas most patients have no symptoms. Only when the tumor grows larger or multiplies will some patients have symptoms such as hemoptysis and chest pain. As the disease progresses, the patient may die of respiratory failure.

The plain chest film, chest CT and/or CT-PET scan of the PBML patients show several multiple or solitary shadows of different sizes that are noncalcified and with smooth edges or round lobular or round soft tissues, either well-circumscribed or cavitary, and with small quantities of pleural effusion or mild thickening of the pleura (23). Some patients have lungs densely covered by miliary nodules, with narrow or blocked tracheas and bronchi. However, there are normally no influences on the endobronchus and pleura and no mediastinal lymphadenectasis (24). Horstmann *et al.* (19) conducted a retrospective study of the imaging findings of 23 PBML cases. The most common features (16 cases, 70%) were multiple nodules in both lungs with smooth edges that were either lobulated or cavitary. There were also one-sided multiple lumps (4 cases, 17%) and solitary lumps (3 cases, 13%). The rare features were diffuse miliary lesions. The chest CT scan of the patient showed small miliary nodules with slight mediastinal lymphadenectasis.

It is indispensable to identify PBML from multiple pulmonary nodules or lump diseases such as benign or malignant tumor, pulmonary lymphangioleiomyomatosis, tuberculoma, sarcoidosis, pneumoconiosis, lung collagen vascular disease, inflammatory pseudotumor, metastatic carcinoma of the lung and so on. Because the imaging features of PBML are nonspecific, it is quite difficult to diagnose and differentiate. Only when the malignancy is excluded can the dissemination and metastasis of a benign tumor be confirmed (25,26).

The OLB/thoracoscopic lung biopsy is indispensable and the standard diagnosis for PBML. The OLB or thoracoscopic lung biopsy cannot be replaced with the percutaneous lung puncture biopsy. Women of childbearing age with a history of uterine myoma, especially uterine tumor surgery, should be considered for the possible diagnosis of PBML when pulmonary nodules and diffuse lesions are present.

There are several treatment options for PBML. The first choice is excision by surgery to remove the foci (27). The patients should be followed after surgery to observe closely if a new focus develops. The second option includes hysterectomy, bilateral adnexectomy and long-term hormone therapy, which blocks the hormone release to stabilize the pulmonary lesions (28). The third option is inferior vena cava filter implantation, which has a potent effect on preventing primary lesion metastasis to the lungs (26). The patients should be followed to observe closely the sizes and numbers of tumors with a thoracoscopic tumor resection or partial pulmonary lobectomy.

Because PBML normally expresses the ERs and PRs, it is crucial to control estrogen levels. Although bilateral adnexectomy has an obvious effect on tumor growth control, drug treatment has the advantages of minimal trauma and reversibility, with oral drugs relieving symptoms of patients who cannot have surgery. It is reported (6,7,13,19,29,30) that hormone therapy, such as tamoxifen, raloxifene and so on, is effective. It is largely reported that controlling estrogen levels is effective in controlling tumor growth and providing a good prognosis. The overall survival period is 6-101 months, and the median is 94 months (23). There were both cases of spontaneous recovery and cases of death (7).

To summarize, although PBML is a rarely reported disease, women of childbearing age with a history of uterine myoma, especially uterine tumor operation, should be considered for the possible diagnosis of PBML when pulmonary nodules and diffuse lesions are present. Surgery is the first choice for patients who can tolerate the operation; a biopsy should be taken for multiple lesions to identify the pathological types and sources as soon as possible. If surgery is not a good choice, anti-hormone therapy may be the only effective treatment option, and we should monitor the sex hormone levels and the relapse or distant metastasis of PBML. In our case, the patient's imaging findings were not typical: the HRCT showed numerous miliary nodules in diffuse and random distributions in both lungs, which complicated our diagnosis. In the case reports, the times at which metastasizing leiomyoma appeared in the lung after a hysteromyomectomy were between several months and 15 years (1); however, the time to appearance of the metastasizing leiomyoma in the lung after the hysteromyomectomy was only 1 month. This result may

indicate that PBML can also develop in a short period of time.

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References

1. Kayser K, Zink S, Schneider T, et al. Benign metastasizing leiomyoma of the uterus: documentation of clinical, immunohistochemical and lectin-histochemical data of ten cases. Virchows Arch 2000;437:284-92.
2. Kwon YI, Kim TH, Sohn JW, et al. Benign pulmonary metastasizing leiomvomatosis: case report and a review of the literature. Korean J Intern Med 2006;21:173-7.
3. Jo JH, Lee JH, Kim DC, et al. A case of benign metastasizing leiomyoma with multiple metastasis to the soft tissue, skeletal muscle, lung and breast. Korean J Intern Med 2006;21:199-201.
4. Yoon G, Kim TJ, Sung CO, et al. Benign metastasizing leiomyoma with multiple lymph node metastasis: a case report. Cancer Res Treat 2011;43:131-3.
5. Kang SA, Choi SI, Kim YA, et al. A case of benign metastasizing pulmonary leiomyoma. Tuberc Respir Dis 2005;58:614-8.
6. Egberts JH, Schafmayer C, Bauerschlag DO, et al. Benign abdominal and pulmonary metastasizing leiomyoma of the uterus. Arch Gynecol Obstet 2006;274:319-22.
7. Abramson S, Gilkeson RC, Goldstein JD, et al. Benign metastasizing leiomyoma: clinical, imaging, and pathologic correlation. AJR Am J Roentgenol 2001;176:1409-13.
8. Patton KT, Cheng L, Papavero V, et al. Benign metastasizing leiomyoma: clonality, telomere length and clinicopathologic analysis. Mod Pathol 2006;19:130-40.
9. Esteban JM, Allen WM, Schaerf RH. Benign metastasizing leiomyoma of the uterus: histologic and immunohistochemical characterization of primary and metastatic lesions. Arch Pathol Lab Med 1999;123:960-2.
10. Jautzke G, Müller-Ruchholtz E, Thalmann U. Immunohistological detection of estrogen and progesterone receptors in multiple and well differentiated leiomyomatous lung tumors in women with uterine leiomyomas (so-called benign metastasizing leiomyomas). A report on 5 cases. Pathol Res Pract 1996;192:215-23.
11. Nucci MR, Drapkin R, Dal Cin P, et al. Distinctive cytogenetic profile in benign metastasizing leiomyoma: pathogenetic implications. Am J Surg Pathol 2007;31:737-43.
12. Tietze L, Günther K, Hörbe A, et al. Benign metastasizing leiomyoma: a cytogenetically balanced but clonal disease. Hum Pathol 2000;31:126-8.
13. Nuovo GJ, Schmittgen TD. Benign metastasizing leiomyoma of the lung: clinicopathologic, immunohistochemical, and micro-RNA analyses. Diagn Mol Pathol 2008;17:145-50.
14. Steiner PE. Metastasizing fibroleiomyoma of the uterus: Report of a case and review of the literature. Am J Pathol 1939;15:89-110.7.
15. Robboy SJ, Bentley RC, Butnor K, et al. Pathology and pathophysiology of uterine smooth-muscle tumors. Environ Health Perspect 2000;108 Suppl 5:779-84.
16. Canzonieri V, D'Amore ES, Bartoloni G, et al. Leiomyomatosis with vascular invasion. A unified pathogenesis regarding leiomyoma with vascular microinvasion, benign metastasizing leiomyoma and intravenous leiomyomatosis. Virchows Arch 1994;425:541-5.
17. Koh DM, Burn PR, King DM. Benign metastasizing leiomyoma with intracaval leiomyomatosis. Br J Radiol 2000;73:435-7.
18. Martin E. Leiomyomatous lung lesions: a proposed classification. AJR Am J Roentgenol 1983;141:269-72.
19. Horstmann JP, Pietra GG, Harman JA, et al. Spontaneous regression of pulmonary leiomyomas during pregnancy. Cancer 1977;39:314-21.
20. Fu Y, Li H, Tian B, et al. Pulmonary benign metastasizing leiomyoma: a case report and review of the literature. World J Surg Oncol 2012;10:268.
21. Radzikowska E, Szczepulska-Wójcik E, Langfort R, et al. Benign pulmonary metastasizing leiomyoma uteri. Case report and review of literature. Pneumonol Alergol Pol 2012;80:560-4.
22. Rivera JA, Christopoulos S, Small D, et al. Hormonal manipulation of benign metastasizing leiomyomas: report of two cases and review of the literature. J Clin Endocrinol Metab 2004;89:3183-8.
23. Ahmad SZ, Anupama R, Vijaykumar DK. Benign metastasizing leiomyoma - case report and review of literature. Eur J Obstet Gynecol Reprod Biol 2011;159:240-1.
24. Ni Y, Shi G, Wan H, et al. Pulmonary benign metastasizing leiomyoma: case report and review of the literature. Clin Exp Obstet Gynecol 2012;39:249-51.
25. Goyle KK, Moore DF Jr, Garrett C, et al. Benign metastasizing leiomyomatosis: case report and review. Am J Clin Oncol 2003;26:473-6.

26. Yamazaki K. CD10- and CD34-positive periglandular stromal cells in pulmonary benign metastasizing leiomyoma with metaplastic adenomyomatous glands: an ultrastructural and immunohistochemical study. Virchows Arch 2005;446:270-7.
27. Yonezawa K, Yokoo N, Yamaguchi T. Effectiveness of an inferior vena caval filter as a preventive measure against pulmonary thromboembolism after abdominal surgery. Surg Today 1999;29:821-4.
28. Abu-Rustum NR, Curtin JP, Burt M, et al. Regression of uterine low-grade smooth-muscle tumors metastatic to the lung after oophorectomy. Obstet Gynecol 1997;89:850-2.
29. Mogi A, Hirato J, Kosaka T, et al. Benign metastasizing leiomyoma of the lung: report of a case. Gen Thorac Cardiovasc Surg 2013;61:719-22.
30. Lewis EI, Chason RJ, DeCherney AH, et al. Novel hormone treatment of benign metastasizing leiomyoma: an analysis of five cases and literature review. Fertil Steril 2013;99:2017-24.

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Case Report

An anterior mediastinal mass: delayed airway compression and using a double lumen tube for airway patency

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Abstract: Perioperative management of patients with an anterior mediastinal mass is difficult. We present a 35-year-old woman who showed delayed compression of the carina and left main bronchus despite no preoperative respiratory signs, symptoms, or radiologic findings due to an anterior mediastinal mass and uneventful stepwise induction of general anesthesia. Even use of a fiberoptic bronchoscope (FB) after induction of anesthesia was not helpful to predict delayed compression of the airway. Therefore, the anesthesiologist and the cardiothoracic surgeon must prepare for unexpected delayed compression of the airway, even in low risk patients who are asymptomatic or mildly symptomatic without postural symptoms or radiographic evidence of significant compression of structures. We also describe successful management for the compressed carina and left main bronchus with a double lumen tube (DLT) as a stent during surgery. FB guided DLT intubation is a possible solution to maintain airway patency.

Keywords: Anterior mediastinal mass; delayed airway compression; double lumen tube (DLT)

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Introduction

Induction and management of general anesthesia in patients with a mediastinal mass can be challenging and cases related to mediastinal masses have been continuously reported (1-3). For a patient's safety, anesthesiologists and cardiothoracic surgeons need to evaluate the pathophysiologic consequences of mediastinal masses and cooperate closely.

A careful preoperative evaluation of thoracic structures and stepwise induction of anesthesia, including initiating cardiopulmonary bypass (CPB) with continuous monitoring of ventilation and hemodynamics, are essential. This is particularly the case in high risk patients with a mediastinal mass related signs, symptoms, or radiologic findings such as severe postural symptoms, stridor, cyanosis, tracheal compression (>50%) or with associated bronchial compression, pericardial effusion or superior vena cava syndrome (4-6). Intermediate risk patients who have mild to moderate symptoms and/or tracheal compression (<50%) are also assessed and perioperative care plan should be planned for airway security. In contrast, low risk patients who are asymptomatic or mildly symptomatic, without postural symptoms or radiologic findings of significant compression of structures, endure ordinary general anesthesia (6,7).

This report describes delayed compression of the airway after uneventful induction of anesthesia occurred even in a low risk patient who was not expected to show any airway compromises. This report also showed how to resolve it with a rather simple technique: using a double lumen tube (DLT) and fiberoptic bronchoscope (FB) as a possible solution to maintain airway patency.

Case report

A 35-year-old previously healthy female (163 cm/58 kg) presented for excision of an anterior mediastinal mass, found incidentally on a chest radiograph taken as part of a routine checkup. She had no signs or symptoms related to the mass such as cough, chest pain, superior vena cava obstruction,

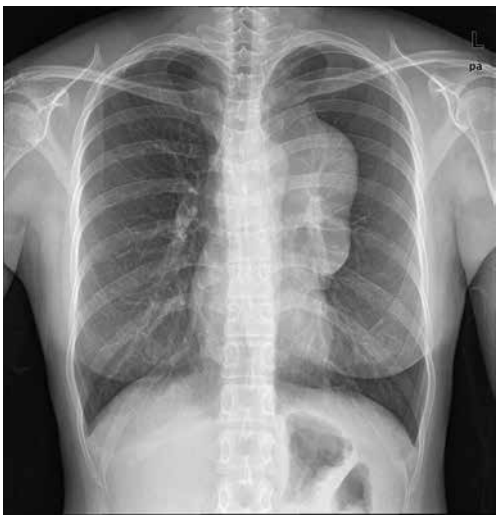

Figure 1 Chest radiograph (postero-anterior view) shows a large mediastinal mass occupying the left side without deviation or compression of the distal trachea, carina, or main bronchi.

hoarseness, syncope, dysphagia, dyspnea; or noisy breathing at rest, on exertion, in the supine position, or during sleep. Her chest X-ray and computed tomography (CT) scan, which were taken one day before surgery, suspected a teratoma (7.5 cm × 9.2 cm × 14 cm) in the left anterior to middle mediastinum, compressing the brachiocephalic vein, but did not show any airway compromise (*Figures 1*,*2*).

A median sternotomy and surgical excision of the mediastinal mass was planned. The cardiothoracic surgeon and the anesthesiologist agreed to perform stepwise induction of anesthesia without initiation of CPB because the patient did not show any preoperative airway compromise related to the anterior mediastinal mass (low risk).

The patient was transferred to the operating room premedicated with 0.2 mg glycopyrrolate. Routine anesthetic monitoring was applied. Induction was achieved with an initial dose of 50 mg propofol followed by two times of 30 mg. The patient's spontaneous mask ventilation was maintained without any respiratory difficulties. Seventy five mg succinylcholine was administered to achieve tracheal intubation. A size 7.0 cuffed endotracheal tube was placed easily via direct laryngoscopy demonstrating a Cormack-Lehane grade I view with concomitant placement of a right radial arterial catheter. FB (PortaView-LF^{TM}, Olympus Medical Systems Corp., Tokyo, Japan) revealed no compression of the trachea or both main bronchi. Bilateral breathing sounds and bilateral chest expansion were also confirmed and 4 mg of vecuronium was then administered.

Sevoflurane and remifentanil were used to maintain anesthesia. A central venous catheter was placed in the right internal jugular vein without difficulty. The elapsed time was about 20 minutes from the beginning of induction to central venous catheterization.

At this time, the peak inspiratory pressure began to increase to over 40 cmH_2O. Breathing sounds from the left lung diminished considerably and chest wall expansion was limited. Arterial saturation began decreasing despite manual ventilation with continuous positive airway pressure and 100% oxygen was added. After placing the patient in the left lateral decubitus and the intermittent oxygen flush via FB which was pushed distal to the compressed part of the left main bronchus, oxygen saturation started to increase.

FB revealed total compression of the left main bronchus and partial compression of the carina. The entire length of the left main bronchus was, however, not compressed (less than 2 cm) and the left upper and lower lobar bronchi were intact (*Figure 3*); thus, we considered to re-intubate with a left-sided DLT (32 Fr. Mallinckrodt^{TM} Endobronchial tube, Covidien, Tullamore, Ireland) expecting the rigid DLT to maintain airway patency.

Preparation for emergent CPB started after the patient was laid in the supine position. At the same time, the endotracheal tube was withdrawn, and the trachea was intubated using a DLT via direct laryngoscopy. We inserted the FB into the bronchial lumen and introduced the DLT and FB from the trachea to the left main bronchus, and further into the distal border of the compressed site with the FB as a guide. We were able to place the DLT in the appropriate position to prevent compression of the carina and left main bronchus. After the FB was withdrawn, the DLT was connected to the ventilator and conventional mechanical ventilation was initiated. FB revealed that the tip of the right lumen did not enter the left main bronchus. Bilateral breathing sounds were clearly auscultated. The elapsed time was 3 minutes during the procedure. The plan for emergent CPB was rediscussed and then canceled.

On median sternotomy, a round mass with a smooth margin was found located in the left anterior to middle mediastinum and was compressing the carina, left main bronchus, and brachiocephalic vein. The mass was totally removed with minimal blood loss. End-tidal carbon dioxide and peak inspiratory pressure remained within the normal range and ventilation was kept stable through surgery (*Table 1*). FB was performed frequently to access airway patency. After relieving the airway, the DLT was replaced with a size 7.0

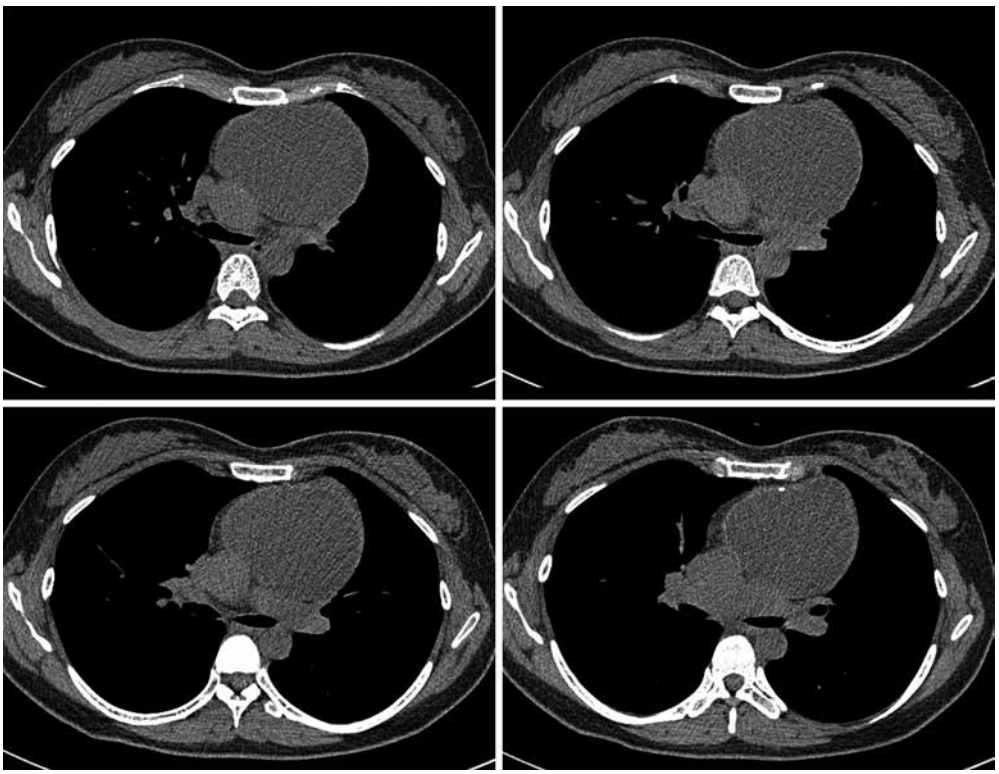

Figure 2 Chest computed tomography scans show an anterior mediastinal mass causing extrinsic compression of the brachiocephalic vein to the posterolateral side (not shown). No airway compromise is shown at the level of the carina and main bronchi.

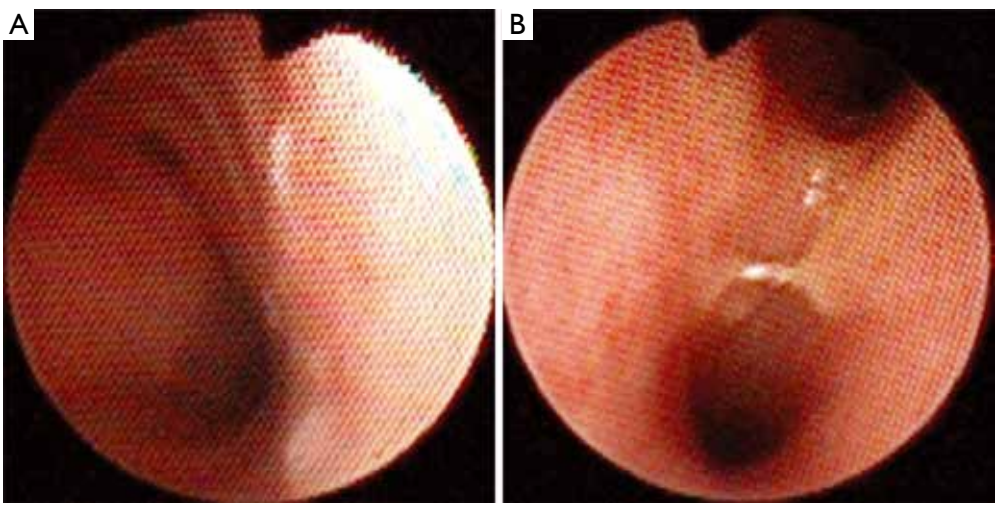

Figure 3 The carina is compressed (A) and the left upper and lower labar bronchi (B) are intact. The pictures were taken in the left lateral decubitus position.

cuffed endotracheal tube.

At the end of the operation, the patient was extubated uneventfully. She did not show any difficulties breathing in the post-anesthetic care unit. No injuries on the trachea and bronchus caused by the mediastinal mass and DLT were present intraoperatively and postoperatively. The histopathological diagnosis confirmed the mass as a mature teratoma.

Table 1 Peak inspiratory pressure, partial pressure of end-tidal CO_2, pulse oxygen saturation and arterial blood gas analysis during the induction and maintenance of general anesthesia for the resection of the anterior mediastinal mass

Time after endotracheal intubation (min)	PIP (cmH_2O)	pH	$EtCO_2$ or $PaCO_2$ (mmHg)	PaO_2 (mmHg)	SpO_2 or SaO_2 (%)	FiO_2
0 (After SLT intubation)	18		32		100	0.5
20	42		23		75	0.5
25 (after LLD position)	25		35		97	1.0
35 (Before supine position)	18		30		100	1.0
40 (after DLT intubation)	18		31		100	1.0
70*	17	7.41	34.6	512	99.9	1.0
130*	15	7.43	30.8	170	99.5	0.5
250*	16	7.41	33.8	182	99.4	0.5

*, arterial blood gas analysis results; DLT, double lumen tube; $EtCO_2$, partial pressure of end-tidal carbon dioxide; FiO_2, fraction of inspired oxygen; LLD, left lateral decubitus; $PaCO_2$, partial pressure of carbon dioxide in arterial blood; PaO_2, partial pressure of oxygen in arterial blood; PIP, peak inspiratory pressure; SaO_2, arterial oxygen saturation; SpO_2, pulse oxygen saturation; SLT, single lumen tube.

Discussion

Anesthetic management of patients with anterior mediastinal masses is very complicated. Clinicians should look for any symptoms such as cough, dyspnea on exertion, chest pain, fatigue, or vocal cord paralysis (4,5). A careful evaluation of the size and the location of the mass are important for predicting the physiological effects it will have on surrounding mediastinal and other thoracic structures (8). Based on this information, an anesthetic plan should be formulated prior to induction of anesthesia. This is, however, particularly the case in intermediate to high risk patients with a mediastinal mass related signs, symptoms, or radiologic findings (4-6).

In contrast, low risk patients such as our patient tolerate ordinary general anesthesia (6,7). Our patient did not have any signs and symptoms related to the mediastinal mass and the CT scan showed that the airway was intact; thus, stepwise induction with propofol and succinylcholine was performed. FB revealed that airway patency was maintained after tracheal intubation; therefore, vecuronium was given. However, this patient showed delayed compression of the airway after induction of anesthesia. It means that clinicians should pay attention to low risk patients as well as intermediate and high risk patients.

This compression seemed to be related to the anesthetic agents and muscle relaxants that trigger a decrease in respiratory muscle tone, producing reduced lung volume and loss of the outward force of the chest. Furthermore, anesthetic agents relax the airway smooth muscle; thus, the airway is compressed more easily (9-12). Hemorrhage inside the mass after needle biopsy or a rapid-growing mass may compress the airway as well. However, our patient's chest X-ray and CT scan were taken one day before surgery and a needle biopsy had not been performed. Internal jugular vein catheterization was also unlikely to cause the airway compression, because there were not any difficulties during catheterization.

When ventilatory difficulties ensue after induction of anesthesia, reported management options include repositioning the patient, placing rigid bronchoscope distal to the obstruction, or initiating CPB (4,8). When our patient's oxygenation level began to be compromised, repositioning her in the left lateral decubitus was life-saving. We decided that a rigid bronchoscope was not necessary because the FB slid smoothly through the compressed part of the left main bronchus, and repositioning the patient was effective for oxygenation.

A DLT is usually intended for one lung ventilation but in this case, with the help of FB, we used it like a rigid stent that opened up the compressed carina and left main bronchus as we pushed the tip distal to the obstruction. The DLT was rigid enough to push away the mass even in the supine position. Even though the airway in this case was maintained with a DLT, DLT intubation was not our first choice because our patient had a low risk of airway compromises and DLT intubation causes more frequent complications such as sore throat, hoarseness, vocal cords injuries, and tracheobronchial injuries (13,14).

To use a DLT, several limitations should be considered. First, use of a DLT is an option for some patients with masses externally compressing the carina or bronchus, it is not an option, however, for patients with intrinsic airway

tumors. Second, the nature of the mass may also influence the successful insertion of a DLT. Solid mass may be difficult to move. In this case, CT scans indicated that the mass was not so hard. Third, a DLT cannot be a possible solution to maintain airway patency if the main bronchus is entirely collapsed. The present patient showed that the length of the collapsed left main bronchus was only 2 cm and the left upper and lower lobar bronchi were intact. Fourth, because the right main bronchus is shorter than the left main bronchus, it may be much more difficult to place a DLT in a patient with compression of the right main bronchus. Lastly, this procedure is not for patients with hemodynamic compromise.

Positioning the patient for DLT intubation was also considered. It was possible to intubate a DLT in the lateral position. However, we positioned the patient in the supine position because intubation in the supine position is easier and if needed, repositioning the patient in the lateral position could relieve airway compression.

In conclusion, even though patients may not have any preoperative signs, symptoms, or radiologic findings related to an anterior mediastinal mass, and the airway is secured after uneventful induction of anesthesia, these do not guarantee that airway patency is maintained during surgery. Therefore, anesthesiologists and cardiothoracic surgeons have to prepare for delayed compression of the airway, even in low risk patients. Using a DLT in patients undergoing or expected to be in ventilatory jeopardy due to anterior mediastinal masses during induction of anesthesia is a possible solution to maintain airway patency.

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References

1. Tauro LF, Shetty P, Kamath A, et al. Double whammy - mediastinal and ovarian teratoma: a rare clinical co-existence. J Thorac Dis 2012;4:434-6.
2. La Mantia E, Franco R, Rocco R, et al. Spindle cell lipoma: a rare tumor of the mediastinum. J Thorac Dis 2013;5:E152-4.
3. Tan PC, Esa N. Anesthesia for massive retrosternal goiter with severe intrathoracic tracheal narrowing: the challenges imposed -A case report-. Korean J Anesthesiol 2012;62:474-8.
4. Slinger P, Karsli C. Management of the patient with a large anterior mediastinal mass: recurring myths. Curr Opin Anaesthesiol 2007;20:1-3.
5. Tempe DK, Arya R, Dubey S, et al. Mediastinal mass resection: Femorofemoral cardiopulmonary bypass before induction of anesthesia in the management of airway obstruction. J Cardiothorac Vasc Anesth 2001;15:233-6.
6. Blank RS, de Souza DG. Anesthetic management of patients with an anterior mediastinal mass: continuing professional development. Can J Anaesth 2011;58:853-9, 860-7.
7. Béchard P, Létourneau L, Lacasse Y, et al. Perioperative cardiorespiratory complications in adults with mediastinal mass: incidence and risk factors. Anesthesiology 2004;100:826-34; discussion 5A.
8. Atlee JL. eds. Complications in Anesthesia (Second Edition). Philadelphia: WB Saunders;2006:670-2.
9. Bergman NA. Reduction in resting end-expiratory position of the respiratory system with induction of anesthesia and neuromuscular paralysis. Anesthesiology 1982;57:14-7.
10. Hedenstierna G, Edmark L. The effects of anesthesia and muscle paralysis on the respiratory system. Intensive Care Med 2005;31:1327-35.
11. Neuman GG, Weingarten AE, Abramowitz RM, et al. The anesthetic management of the patient with an anterior mediastinal mass. Anesthesiology 1984;60:144-7.
12. Dikmen Y, Eminoglu E, Salihoglu Z, et al. Pulmonary mechanics during isoflurane, sevoflurane and desflurane anaesthesia. Anaesthesia 2003;58:745-8.
13. Ceylan KC, Kaya SO, Samancilar O, et al. Intraoperative management of tracheobronchial rupture after double-lumen tube intubation. Surg Today 2013;43:757-62.
14. Gilbert TB, Goodsell CW, Krasna MJ. Bronchial rupture by a double-lumen endobronchial tube during staging thoracoscopy. Anesth Analg 1999;88:1252-3.

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Case Report

Surgery for giant emphysematous bullae: case report and a short literature review

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Abstract: Giant bullous emphysema (GBE), known as 'vanishing lung syndrome', usually occurs in association with chronic obstructive pulmonary disease (COPD). In this report, we describe a patient with giant emphysematous bulla occupying approximately 95% of the right hemithorax, who had history of repeated attacks of acute exacerbation of COPD. About 95% of right pulmonary parenchyma was removed. Delightfully, the 5% of residual lung compressed for 4 years gradually inflated, and occupied the whole hemithorax. Preoperatively he was functionally and clinically severely disabled while improved markedly after bullectomy.

Keywords: Giant pulmonary bulla; video-assisted thoracoscopic surgery; preoperative evaluation; lung function

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Introduction

Giant bullous emphysema (GBE), referred to as vanishing lung syndrome as a clinical syndrome, was first described by Burke in 1937 (1). Fifty years after that, Roberts *et al.* established the radiographic criteria for this syndrome as the presence of giant bullae in one or both upper lobes occupying at least one-third of the hemithorax and compressing the normal surrounding parenchyma (2). Chronic obstructive pulmonary disease (COPD)-related emphysematous bullae are the most common type lead to GBE (3). With progression of COPD, the obstruction increases in severity and eventually becomes irreversible. When the giant bulla occupies the entire hemithorax, and the remaining lung have been collapsed for a long period, it is difficult to predict the postoperative outcome of a bullectomy, and greatly increases the risks of surgery. Surgical bullectomy is a valid treatment option for patients with GBE (4), whereas bullectomy with end-stage COPD have very rarely been reported. We present a case of a patient with severely impaired lung function underwent successful bullectomy and is currently without residual symptoms.

Case report

A 59-year-old Chinese man, a nonsmoker presented in February 2012 with increasing cough and exertional dyspnea for 7 days, has suffered from repeated attacks of COPD over the past 4 years.

On admission, there was severe orthopnea and he was too breathless to leave the house (MRC grade 4) (5). Oxygen saturation was 93 percent while he was breathing ambient air. Arterial blood gas analysis revealed that his PaO_2 was low at 67 mmHg, with $PaCO_2$ 47 mmHg. Chest computed tomography (CT) (*Figure 1*) revealed the giant bulla had occupied approximately 95% of his right hemithorax, with only a small volume of ventilated lung in the lower lung field. We were not able to measure the ventilatory function, because the severity of his dyspnea prohibited him from holding his breath long enough. However, a previous test on March 7, 2011 showed: forced expiratory volume in one second (FEV_1) 0.93 L (31.30% predicted); forced vital capacity (FVC) 1.81 L; FEV_1/FVC 51.27%; and maximum ventilatory volume (MVV) 34.75 L (30.80% predicted). We also used the physical functioning domain of the Medical Outcomes study 36-Item Short-Form Health

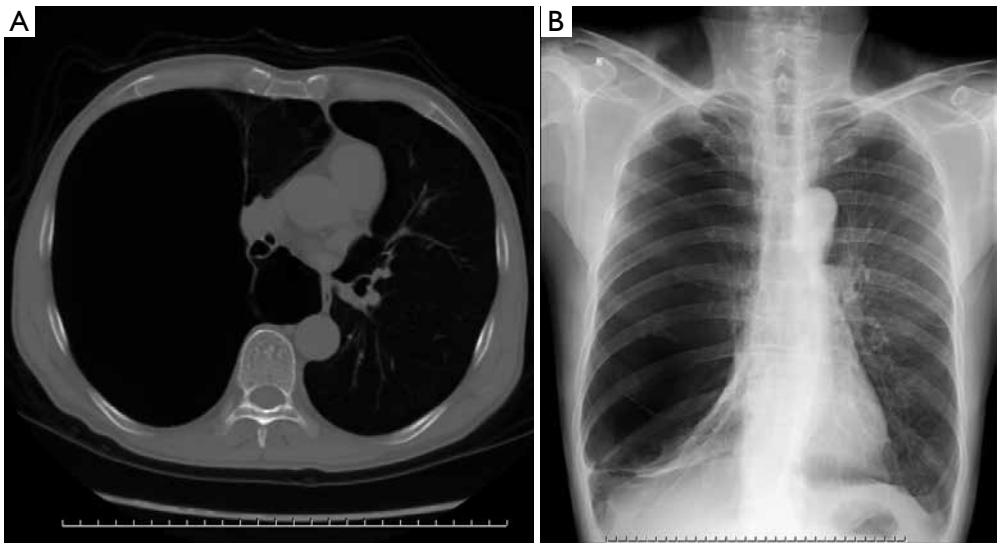

Figure 1 Chest computed tomography (A) and radiograph (B) revealed bilateral giant emphysematous changes. In particular, the giant emphysematous bulla occupied approximately 95% of his right hemithorax, with only a small volume of ventilated lung in the lower lung field.

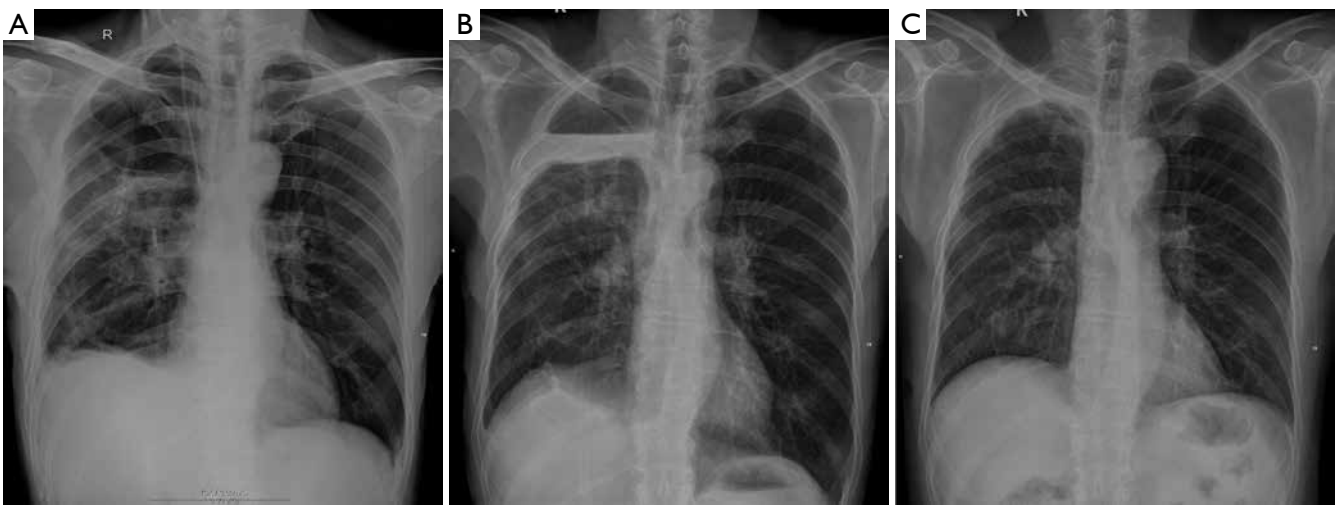

Figure 2 (A) The chest radiograph showed there was a residual cavity in the upper right thoracic cavity and the lung compressed about 30% when discharged; (B) the chest radiograph performed 2 months after surgery showed the right lung inflated well, but an air-fluid level was reserved; (C) chest radiography performed at the last follow-up, ten months after surgery.

Survey (6) to assessed the quality of life, which score was 0. The differential diagnosis was made against lung cancer. According to the CT scanning results, there's no obvious mass growth in the lung, so the diagnosis of cancer can be excluded.

Bullectomy was performed using the video-assisted thoracoscopic surgery approach. Intraoperatively, we saw multiple bullaes in the upper, middle, and lower lobe. About 95% of right pulmonary parenchyma was removed. Soon after the operation, the subjective symptoms improved. The chest radiograph performed showed there was a residual cavity in the upper right thoracic cavity and the lung compressed about 30% (*Figure 2A*). Although an air-fluid level was reserved, the chest radiograph (*Figure 2B*) performed after 2 months showed the lung inflated well suggesting that the patient made a good recovery. Meanwhile, a lung function test showed: FEV_1 1.33 L (45.20% predicted); FVC 3.09 L; FEV_1/FVC 43.04%;

MVV 39.85 L (35.60% predicted). Arterial blood gas analysis revealed that his PaO_2 was at 85 mmHg while $PaCO_2$ was 39 mmHg. The lung expanded completely at the last follow-up (*Figure 2C*), ten months after surgery, when he did general activities without restriction. He got SF-36 physical functioning scale scores of 75. But the lung function had no further improvement.

Discussion

Today's surgeons focus not only on operative mortality but also on the quality of daily life for patience after surgery. In almost all studies performed on patients with bullous lung disease, dyspnea is the most common complaint (7-9), so dyspnea becomes an important indicator of the life quality. Palla *et al*. investigated patients with GBE during a 5-year-follow-up period, and concluded the degree of dyspnea decreased markedly soon after surgery and kept diminishing until the fourth year of follow-up (4). In this case, the man had history of COPD over the previous 4 years. With progression of this disease, the severity of airways obstruction deteriorated and eventually became irreversible. The patient could do normal activities without restriction (MRC grade 4) postoperatively. Six-minute walk distance increased significantly postpulmonary rehabilitation. What's more, health related quality of life as measured by the SF-36 Physical Functioning Scale showed marked improvement from a baseline preoperative score of 0 to a 10-month postoperative score of 75. From our point of view, for patients with giant pulmonary bullae occurring in association with end-edge COPD, the decrease in dyspnea sensation with exercise, are important to operate on.

The selections of patients suffering GBE for surgery have been widely reported (10-12). It is accepted that patients who have nonfunctioning bullae that compresses normal lung and occupies space in the chest cavity will benefit most from a surgical procedure (13), but there is no clear guidelines on the severely impaired lung function. Gunstensen *et al*. have suggested on the basis of their own surgical results that the more severe the preoperative impairment of FEV_1 the less likely the chance of marked improvement after operation (14). Nakahara *et al*. concluded that those patients with an FEV_1% less than 35% did not show as much benefit from bullectomy (15). In this case, pre- and postoperative FEV_1% value was 31.3% and 45.2%, respectively. And FEV_1 improved more than 0.4 of a liter. Obviously, there was significant change after surgery. So FEV_1% less than 35% should not be considered a contraindication to bullectomy. Patients with very low preoperative FEV_1 still have striking improvements.

Though the case of the spontaneous resolution of a giant pulmonary bulla have been reported (16), surgical intervention was the only alternative in our patient, given that he had giant bullous disease-associated end-edge COPD, which responded poorly to conventional treatment. Previous literature stressed the importance of resecting as little nonfunctioning lung as possible-performing bullectomy (13,17-19). There has been controversy on operation range of diffuse bullaes. In the preoperative assessment, MVV% value was far less than 55%, which indicated that the man could not tolerate complete lung excision (20). As stated above, we removed about 95% of right pulmonary parenchyma. Delightfully, the 5% of residual lung compressed for 4 years gradually inflated, and occupied the whole hemithorax ten months after surgery. A few lung tissues (about 5%) is still able to well re-expand to occupy whole hemithorax, which can be considered in the controversy on operation range of diffuse bullaes. Pneumonectomy should be avoided. Although the patient had a prolonged air leak postoperatively, which was the most common complication for bullectomy (21-23), it did not prevent compressed lung tissue from expanding.

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References

1. Burke R. Vanishing lungs: a case report of bullous emphysema. Radiology 1937;28:367-71.
2. Roberts L, Putman CE, Chen JT, et al. Vanishing lung syndrome: upper lobe bullous pneumopathy. Rev Interam Radiol 1987;12:249-55.
3. Ruan SY, Huang CT, Chien JY, et al. Non-surgical management of giant lung bullae during mechanical ventilation. Respir Care 2011;56:1614-6.
4. Palla A, Desideri M, Rossi G, et al. Elective surgery for giant bullous emphysema: a 5-year clinical and functional follow-up. Chest 2005;128:2043-50.
5. Surveillance for respiratory hazards in the occupational setting [American Thoracic Society]. Am Rev Respir Dis 1982;126:952-6.
6. Ware JE Jr, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care 1992;30:473-83.

7. Shah SS, Goldstraw P. Surgical treatment of bullous emphysema: experience with the Brompton technique. Ann Thorac Surg 1994;58:1452-6.
8. Liu HP, Chang CH, Lin PJ, et al. An alternative technique in the management of bullous emphysema. Thoracoscopic endoloop ligation of bullae. Chest 1997;111:489-93.
9. Fatimi SH, Riaz M, Hanif HM, et al. Asymptomatic presentation of giant bulla of the left apical and anterior segment of the left upper lobe of the lung with near complete atelectasis of the remaining left lung. J Pak Med Assoc 2012;62:165-6.
10. Kinnear WJ, Tattersfield AE. Emphysematous bullae. BMJ 1990;300:208-9.
11. Nickoladze GD. Functional results of surgery for bullous emphysema. Chest 1992;101:119-22.
12. Lin KC, Luh SP. Video-assisted thoracoscopic surgery in the treatment of patients with bullous emphysema. Int J Gen Med 2010;3:215-20.
13. Greenberg JA, Singhal S, Kaiser LR. Giant bullous lung disease: evaluation, selection, techniques, and outcomes. Chest Surg Clin N Am 2003;13:631-49.
14. Gunstensen J, McCormack RJ. The surgical management of bullous emphysema. J Thorac Cardiovasc Surg 1973;65:920-5.
15. Nakahara K, Nakaoka K, Ohno K, et al. Functional indications for bullectomy of giant bulla. Ann Thorac Surg 1983;35:480-7.
16. Scarlata S, Cesari M, Caridi I, et al. Spontaneous resolution of a giant pulmonary bulla in an older woman: role of functional assessment. Respiration 2011;81:59-62.
17. Benfield JR, Cree EM, Pellett JR, et al. Current approach to the surgical management of emphysema. Arch Surg 1966;93:59-70.
18. Potgieter PD, Benatar SR, Hewitson RP, et al. Surgical treatment of bullous lung disease. Thorax 1981;36:885-90.
19. Laros CD, Gelissen HJ, Bergstein PG, et al. Bullectomy for giant bullae in emphysema. J Thorac Cardiovasc Surg 1986;91:63-70.
20. Miller JI Jr. Physiologic evaluation of pulmonary function in the candidate for lung resection. J Thorac Cardiovasc Surg 1993;105:347-51; discussion 351-2.
21. Divisi D, Battaglia C, Di Francescantonio W, et al. Giant bullous emphysema resection by VATS. Analysis of laser and stapler techniques. Eur J Cardiothorac Surg 2002;22:990-4.
22. Schipper PH, Meyers BF, Battafarano RJ, et al. Outcomes after resection of giant emphysematous bullae. Ann Thorac Surg 2004;78:976-82; discussion 976-82.
23. Gunnarsson SI, Johannesson KB, Gudjonsdottir M, et al. Incidence and outcomes of surgical resection for giant pulmonary bullae--a population-based study. Scand J Surg 2012;101:166-9.

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Case Report

Nodular fasciitis on chest wall in a teenager: a case report and review of the literature

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Abstract: We report the case of a 16-year-old boy with a rapid growing mass on his left anterior chest wall. The mass was completely resected, and pathological examination confirmed nodular fasciitis. Benign chest tumors rarely occur in childhood. Nodular fasciitis is a benign proliferation of myofibroblast that is often mimicked by a sarcoma of the soft tissue. Physicians should consider the possibility of nodular fasciitis in chest wall tumors in the pediatric population.

Keywords: Benign neoplasm; thoracic wall; myofibroblast

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Introduction

Chest wall tumors rarely occur in childhood, accounting for only 1.8% of the solid tumors in the chest wall (1). The ribs are the most common site of chest wall tumors, but the clavicle, sternum, scapula and soft tissues are also involved. Sarcomas make up for the majority of chest wall tumors, and benign tumors, such as eosinophilic granuloma, aneurysmal bone cyst, hamartoma, osteoma, osteochondroma, and chondroma are rarer than malignant tumors.

Nodular fasciitis is a benign, rapid proliferation of fibroblasts and myofibroblasts in the subcutaneous tissues and resembles malignant soft tissue sarcomas from a clinical and pathologic point of view (2).

Its rapid growth, abundant cellularity, and mitotic activity often lead to a misdiagnosis of sarcoma. Nodular fasciitis is relatively common in the adult population; however, it is rarely reported chest wall tumor among children (3).

We report a rare case of nodular fasciitis in a 15-year-old boy that developed in the wall of his chest.

Case report

A 16-year-old boy presented with a palpable mass on his left anterior chest wall at the level of the fifth intercostal space. The mass had first been noticed 2 months ago and had grown rapidly. The patient denied any history of trauma, infection, or tuberculosis. On the physical examination, a hard, round and smooth-surfaced mass that was affixed to the chest wall was palpated. The skin over the lesion was neither tender nor hot.

The chest X-ray and bone scan showed no abnormal findings. The chest computed tomography scan revealed a well-enhanced ovoid mass approximately 3 cm × 2 cm in size in the anterior portion of the patient's left lower chest wall (*Figure 1*). The central portion of this mass showed poor enhancement, while the peripheral portions showed intense enhancement. To obtain a pathologic confirmation and to manage the mass, surgical resection was decided. The mass was located between the pectoralis muscle and the ribs. Its exterior surface was well demarcated by the pectoralis muscle, but the interior surface was firmly attached to the periosteum of the ribs, intercostal muscles and fascia. The mass was completely resected, including the intercostal muscles and surrounding soft tissues.

The mass had a hypercellular pattern, and upon microscopic examination, short and irregular bundles of spindle cells with microhemorrhage and inflammatory cells were noted.

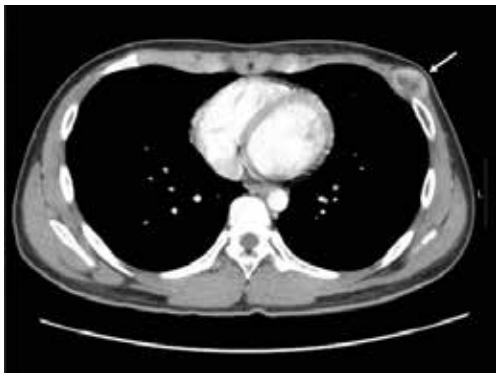

Figure 1 Chest computed tomography scan showing a well-enhanced ovoid mass approximately 3 cm × 2 cm in size in the anterior portion of the left lower chest wall. White arrow: mass.

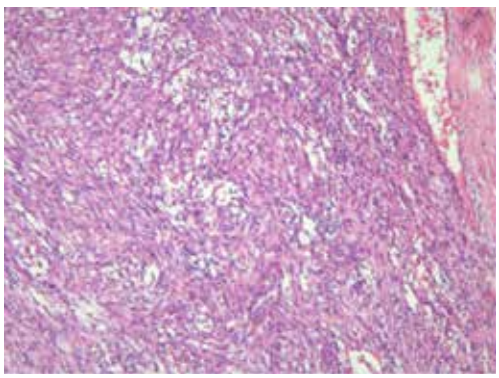

Figure 2 Hypercellular nodular fasciitis. Short and irregular bundles of spindle cells with microhemorrhage and inflammatory cells were discovered (hematoxylin and eosin, ×100).

However, intermittent myxoid and hypocellular areas were also observed and mitotic activity was not found (*Figure 2*).

The patient recovered without any complications and remains free of recurrence 2 years after surgery.

Discussion

Nodular fasciitis is a benign mesenchymal tumor that often presenting as a rapidly growing soft tissue mass. It can be easily mistaken for a malignancy because its clinical and histologic characteristics are similar to malignancies such as sarcoma (4).

Nodular fasciitis was first described by Konwaler *et al.* in 1955 and was initially named pseudosarcomatous fibromatosis. Other terms, such as pseudosarcomatous fasciitis, infiltrative fasciitis, and proliferative fasciitis, have also been used synonymously. Price *et al.* (5) first used the term "nodular fasciitis" because the origin of tumor was from the superficial and deep fascial layers.

Nodular fasciitis is most commonly observed in young adults between 20 and 40 years of age (6,7). Approximately 10% of the lesions occur in children (7). Although men and women equally affected, in childhood, the lesions may occur predominantly in boys.

The lesions are generally small and solitary and commonly appear in the upper extremities in adults. After the upper extremity, the next most common site of involvement is the lower extremity, with only 9% located in the chest wall. In infants and children, the head and neck are commonly involved.

Bemrich-Stolz *et al.* reported 18 cases of nodular fasciitis in children (8), 7 of which occurred in the head and neck, 5 in the upper and lower extremities, and 5 in back. The average age was 9 years, with a range of 5 months to 18 years; the male gender was predominant (72%). All lesions were solitary.

Nodular fasciitis has characteristic pseudosarcomatous features and is generally considered to be a benign and a reactive fibroblastic growth. Nodular fasciitis is a benign myofibroblastic proliferation that usually occurs in the subcutaneous tissues of the upper extremities, and trunk, as well as in the head and neck of young adults. Most patients present with a rapidly growing painless solitary mass. Grossly, the masses are solid, nodular, rubbery, or firm.

Nodular fasciitis is subdivided into three types based on their predominant histological features: myxoid (type 1), cellular (type 2), and fibrous (type 3) (5,9). Type 1 lesions are composed of spindle, plump or stellate fibroblast-like cells embedded in myxomatous stroma rich in hyaluronidase-digestible acid mucopolysaccharide. This type demonstrates an abundance of immature capillaries running in a parallel direction, frequent red cell extravasation and considerable inflammatory changes. Type 2 lesions have a higher cellularity and less plentiful ground substances. The fibroblast-like spindle cells have been revealed to be large and plump with vesicular nuclei. Type 3 lesions are characterized by an increased collagen production, and fibroblast-like cells are more slender and spindle-shaped. Nodular fasciitis may have a variable histologic appearance, depending on the preoperative duration of the lesion, and a correlation between histological patterns and clinical features has been suggested (5,9). The histological appearance of nodular fasciitis may change in time from active myxoid to cellular, before changing lastly to the mature fibrous type. The myxoid type of nodular fasciitis should be differentiated from the myxoid variant of

malignant fibrous histiocytoma, which usually occurs in older patients and forms a large tumor. The cellular type of fasciitis can easily be mistaken for a sarcoma, while the fibrous type may be confused with other benign lesions.

Surgical excision is treatment of choice. These lesions rarely recur and sometimes even slowly regressed (8); furthermore, they do not metastasize and are readily cured by local excision.

Nodular fasciitis may be misdiagnosed as a sarcoma due to its rapid growth, abundant cellularity, mitotic activity and poorly circumscribed nature. A previous study demonstrated that recurrent nodular fasciitis was often a misdiagnosed malignancy (10). While spontaneous resolution after fine needle aspiration has reported, surgical excision should be considered because of its potential diagnostic confusion with sarcoma, a rare incidence in the pediatric population, and its minimal morbidity during surgical excision.

Although nodular fasciitis is rare among chest wall tumor of the pediatric population (11), it should be considered during the evaluation of chest wall tumors in childhood for an accurate diagnosis and proper intervention.

Acknowledgements

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References

1. Kumar AP, Green AL, Smith JW, et al. Combined therapy for malignant tumors of the chest wall in children. J Pediatr Surg 1977;12:991-9.
2. Wagner LM, Gelfand MJ, Laor T, et al. A welcome surprise: nodular fasciitis presenting as soft tissue sarcoma. J Pediatr Hematol Oncol 2011;33:316-9.
3. Tomita S, Thompson K, Carver T, et al. Nodular fasciitis: a sarcomatous impersonator. J Pediatr Surg 2009;44:e17-9.
4. Di Serafino M, Maurea S, Vallone G. Nodular fasciitis of the chest: case report of a rare presentation. Musculoskelet Surg 2011;95:251-3.
5. Price EB Jr, Silliphant WM, Shuman R. Nodular fasciitis: a clinicopathologic analysis of 65 cases. Am J Clin Pathol 1961;35:122-36.
6. Stout AP. Pseudosarcomatous fascitis in children. Cancer 1961;14:1216-22.
7. Allen PW. Nodular fasciitis. Pathology 1972;4:9-26.
8. Bemrich-Stolz CJ, Kelly DR, Muensterer OJ, et al. Single institution series of nodular fasciitis in children. J Pediatr Hematol Oncol 2010;32:354-7.
9. Shimizu S, Hashimoto H, Enjoji M. Nodular fasciitis: an analysis of 250 patients. Pathology 1984;16:161-6.
10. Bernstein KE, Lattes R. Nodular (pseudosarcomatous) fasciitis, a nonrecurrent lesion: clinicopathologic study of 134 cases. Cancer 1982;49:1668-78.
11. Mazura JC, Matrai C, Spigland N, et al. Intramuscular nodular fasciitis of the rectus abdominis muscle in an 11-year-old girl. Skeletal Radiol 2013;42:147-50.

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Case Report

A plastic whistle incarcerated in bronchus diagnosed fourteen years after 'swallowed': a case report

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Abstract: Tracheobronchial foreign body aspiration (FBA) is a common disease in pre-school children but easily overlooked by physicians. In this article, we report a case with bronchial stenosis that is not typical and misdiagnosed for 14 years, in the end bronchoscopy retrieval was successfully performed after adequate preparation. Pitfalls and recommendations in diagnosis and management of FBA are briefly included.

Keywords: Foreign body aspiration (FBA); bronchoscopy; stenosis

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Tracheobronchial foreign body aspiration (FBA) is more often happened in younger children, and it rarely happens in school-age children (1,2). Most FBs are food or food-related, whereas the proportion of plastic FBs in US or worldwide is about 7% and 2% respectively (3). The most frequently reported symptoms, signs, radiological findings and complications may vary due to bronchial foreign bodies. We report a case with latent manifestations of bronchial foreign body in an adult and concerned with the clinical features of this.

A 23-year-old male went to the respiratory clinic of a university hospital with suspicion of FBA as he watched news which reported a child diagnosed FBA 5 years after choking. He had no respiratory history of interest or drug allergies. Upon being questioned further regarding his concerns, he admitted that he got "colds" every two or three months since swallowing a plastic toy 14 years ago. He was evaluated the next day of the incident by a pulmonologist, but chest X-ray and upper gastrointestinal barium meal revealed nothing specific.

Before the diagnostic bronchoscopy, a computed tomography (CT) was performed at local hospital, which only reported slight infection at right lower lob (*Figure 1*). Flexible bronchoscopy revealed inflammatory bronchial stricture of the bronchus intermedius. After the obstructing lesion released, a bronchoscope with 4.8 mm external diameter can pass through and found that the foreign body was embedded in granulation tissue of basal stem bronchus (*Figure 2*). Further treatment was not performed considering the complexity of the case. He was advised to be hospitalized for the retrieval and returned home without any uncomfortable. That night he presented with chills, fever (39 degree centigrade) and cough productive of white sputum. He was admitted to Emergency Room and then transferred to in-patient department. On examination he was febrile, and clubbed-fingers could be observed. Auscultation of the chest revealed rhonchi and wheezing involving right lower lung. Tactile fremitus was decreased at right lower thorax. Pulse oxygen saturation was 97% on room air. An emergency CT scan reported multiple opacities and patchy shadows of right lower lung, and mucous plug was suspected in bronchus (*Figure 3*). Symptoms remitted as intravenous avelox (0.4 g per day) was given to patient. To reduce the oedema of bronchial mucous, 15 mg methylprednisolone per day was given to him.

Retrieval was successfully performed by flexible bronchoscopy (*Figure 4*) under general anesthesia. The foreign body was found to be a pin-shaped plastic whistle with a larger head after the adhesion released. The bronchial stenosis is released by electrocautery, at last the foreign body was removed by forceps after several attempts (*Figure 5*).

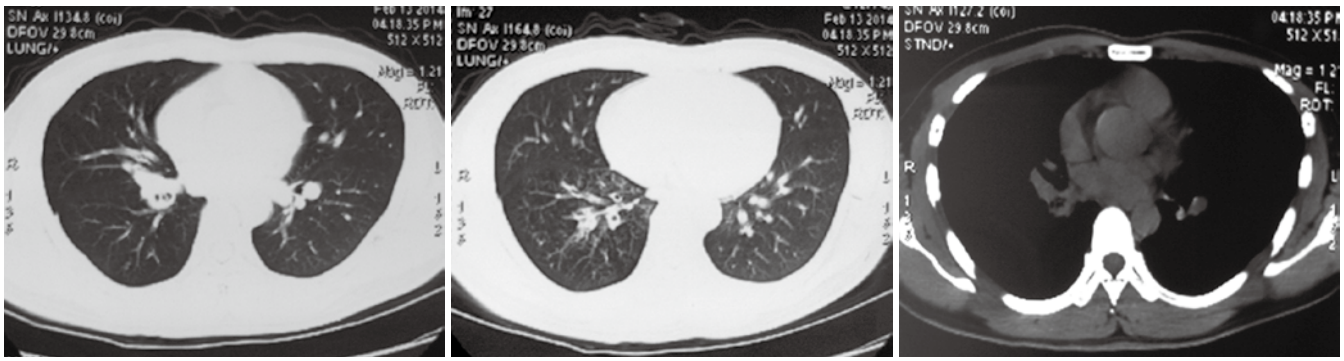

Figure 1 Infection at right lower lob. Under carefully observation, round like shadow can be found in basal stem bronchus.

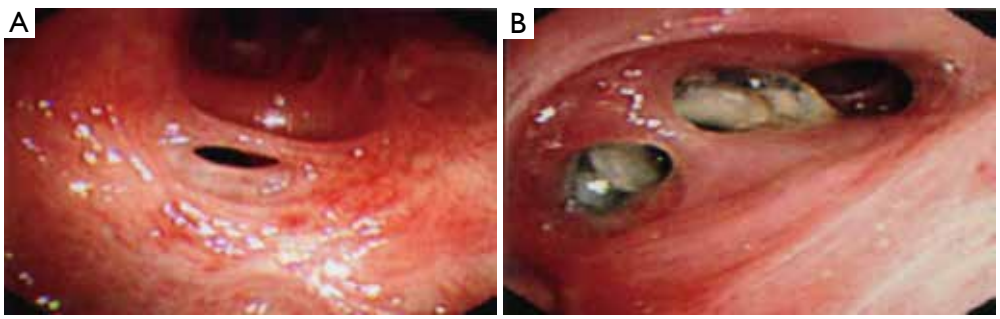

Figure 2 (A) Bronchus intermedius with granulation adhesion; (B) embedded in granulation tissue, a cylindrical foreign body could be found in right basal stem bronchus.

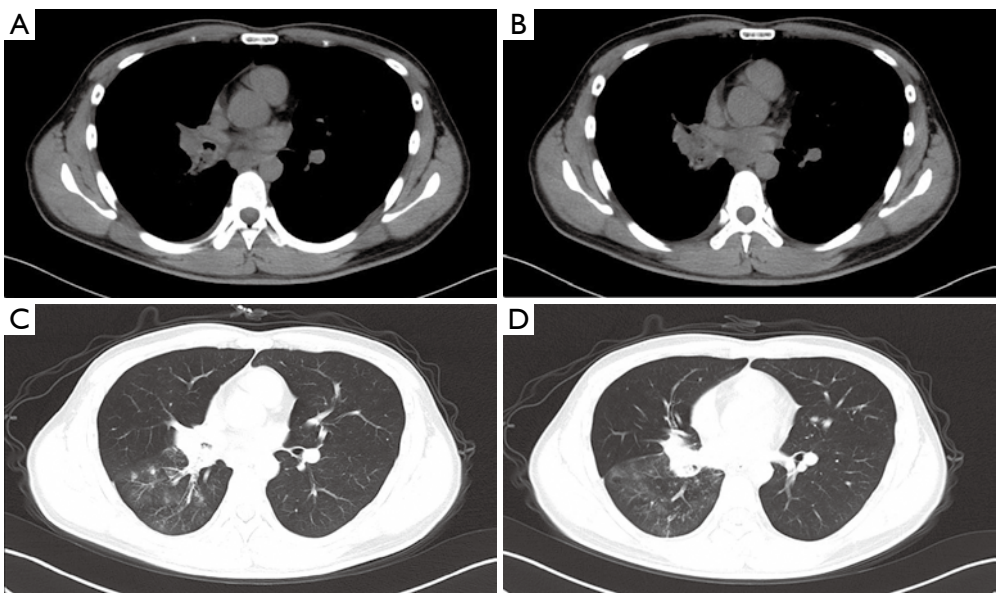

Figure 3 (A,B) Bronchus seems to be partially obstructed by mucous plug on the basis of aspirated foreign body; (C,D) opacities and nodules could be observed, indicating worsened infection after the diagnostic bronchoscopy.

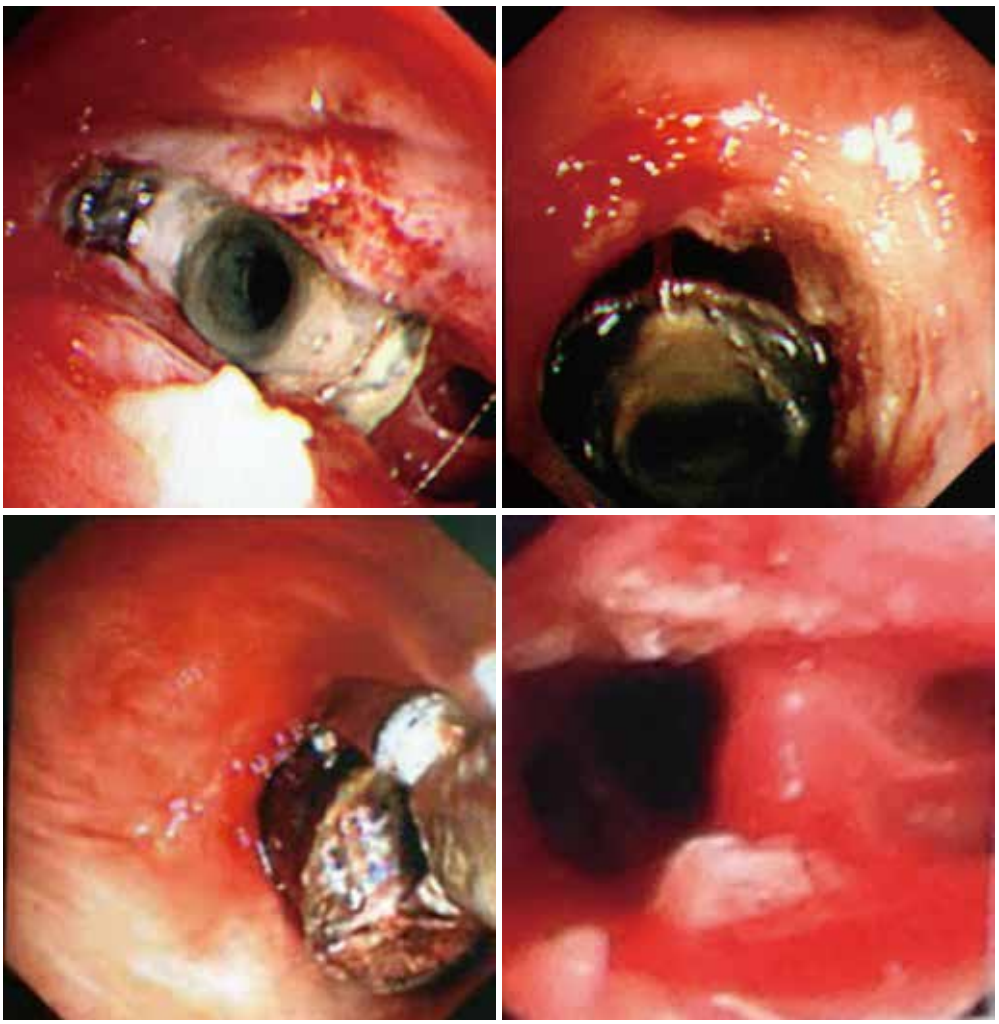

Figure 4 The whistle is in right basal stem segment. Due to the bronchial stricture and pin-shaped foreign body, more efforts were given to the extraction.

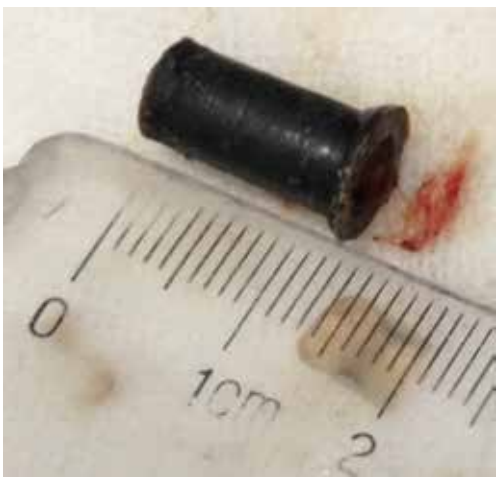

Figure 5 Whistle and its component.

FBA is easily overlooked by physicians when history and manifestations are not typical. In this case, symptoms such as coughing, dyspnea or expectoration are not remarkable after the hollow plastic cylinder aspirated; unlike other FBs, there is no obvious airway obstruction, no respiratory function compromises, but mild bronchus stricture due to a 14-year irritation. Take home message is: plastic foreign body could be overshadowed by mucous secretions or the bronchial wall. Carefully physical examination and scrutinize over CT scans may provide clues of FBA. Flexible fiberoptic bronchoscopy under adequate anesthesia is indicated both in establishing diagnosis and attempting removal of foreign bodies with bronchial stricture. In these compromised patients, the airway might need repeated bronchoscopy to release adhesion and debride secretion, and to some extent surgical

treatment might be needed. To avoid worsened stenosis after bronchoscopy, patient should have regular check-ups to monitor progress, if necessary balloon bronchoplasty should be performed (4). For better diagnosis and clinical management of such rare cases, more cases needed to be reported.

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References

1. Gang W, Zhengxia P, Hongbo L, et al. Diagnosis and treatment of tracheobronchial foreign bodies in 1024 children. J Pediatr Surg 2012;47:2004-10.
2. Huankang Z, Kuanlin X, Xiaolin H, et al. Comparison between tracheal foreign body and bronchial foreign body: a review of 1,007 cases. Int J Pediatr Otorhinolaryngol 2012;76:1719-25.
3. Kaushal P, Brown DJ, Lander L, et al. Aspirated foreign bodies in pediatric patients, 1968-2010: a comparison between the United States and other countries. Int J Pediatr Otorhinolaryngol 2011;75:1322-6.
4. Jimenez Rodriguez BM, de Jesús SC, Merinas López CM, et al. Bronchial stenosis after iron pill aspiration. J Bronchology Interv Pulmonol 2013;20:96-7.

Case Report

Takayasu's arteritis misdiagnosed as mediastinal malignant lymphoma: a case report and review of the literature

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Abstract: Takayasu's arteritis (TA) is a rare chronic large-vessel vasculitis. The early diagnosis is difficult, because of lack of characteristic clinical manifestations. In this paper, we reported a TA case of young female was misdiagnosed as mediastinal malignant lymphoma and mediastinoscope biopsy was performed. The biopsy result demonstrated that thickened tissue adjacent to the aortic arch pathological presentations were in accord with TA. Glucocorticoid was administrated and the condition was greatly improved after treatment. Therefore, we reported this case and review of the pertinent literature in order to help clinicians improve the understanding of TA and PET/CT manifestations of TA at early phase to realize the early diagnosis and treatment of TA, finally reducing the hazards.

Keywords: Takayasu's arteritis (TA); vasculitis; misdiagnosis; mediastinal lymphoma; glucocorticoid

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Takayasu's arteritis (TA) is a rare chronic granulomatous large-vessel vasculitis, predominantly the aorta and its major branches. Due to lack of characteristic clinical manifestations, it's easy to be misdiagnosed or never diagnosed. Recent studies have found that ^{18}F-FDG PET/CT not only could show the morphological changes of artery wall but also indicate the tissue metabolic activity, providing a powerful evidence for the early detection of TA (1). In this paper, we reported a TA case of young female even was misdiagnosed as infectious diseases due to persistent fever for one month, but no response to anti-infection treatment. ^{18}F-FDG PET/CT showed abnormal changes of aorta and mediastinal lymphoma enlarged. She was again misdiagnosed as mediastinal malignant lymphoma, due to insufficient understanding about TA. Mediastinoscope biopsy was performed and found that tissue adjacent to the aortic arch pathological changes were in accord with the TA, the condition was greatly improved after treated with glucocorticoid.

Case report

A 19-year-old female patient was enrolled in a local hospital due to "persistent fever more than one month". The physical examinations on admission were as follows: body temperature 38.7 ℃; pulse 105 times/min; breathing rate 20 times/min; BP 110/73 mmHg (1 mmHg =0.133 kPa). Other physical examinations were normal on admission and medical history and family history were negative. The results of blood tests including, blood biochemistry, immunology and oncology examinations, as well as Weil-Felix and Widal test on admission were normal, except the erythrocyte sedimentation rate was up to 64 mm/h. Infectious diseases was considered firstly and antibiotic with clindamycin, moxifloxacin and cefuroxime were successively administrated, but the fever remained after treated for two weeks. After that, the patient was transferred to a tertiary hospital for further treatment. Medical examination was basically same to before. In laboratory tests, no obvious

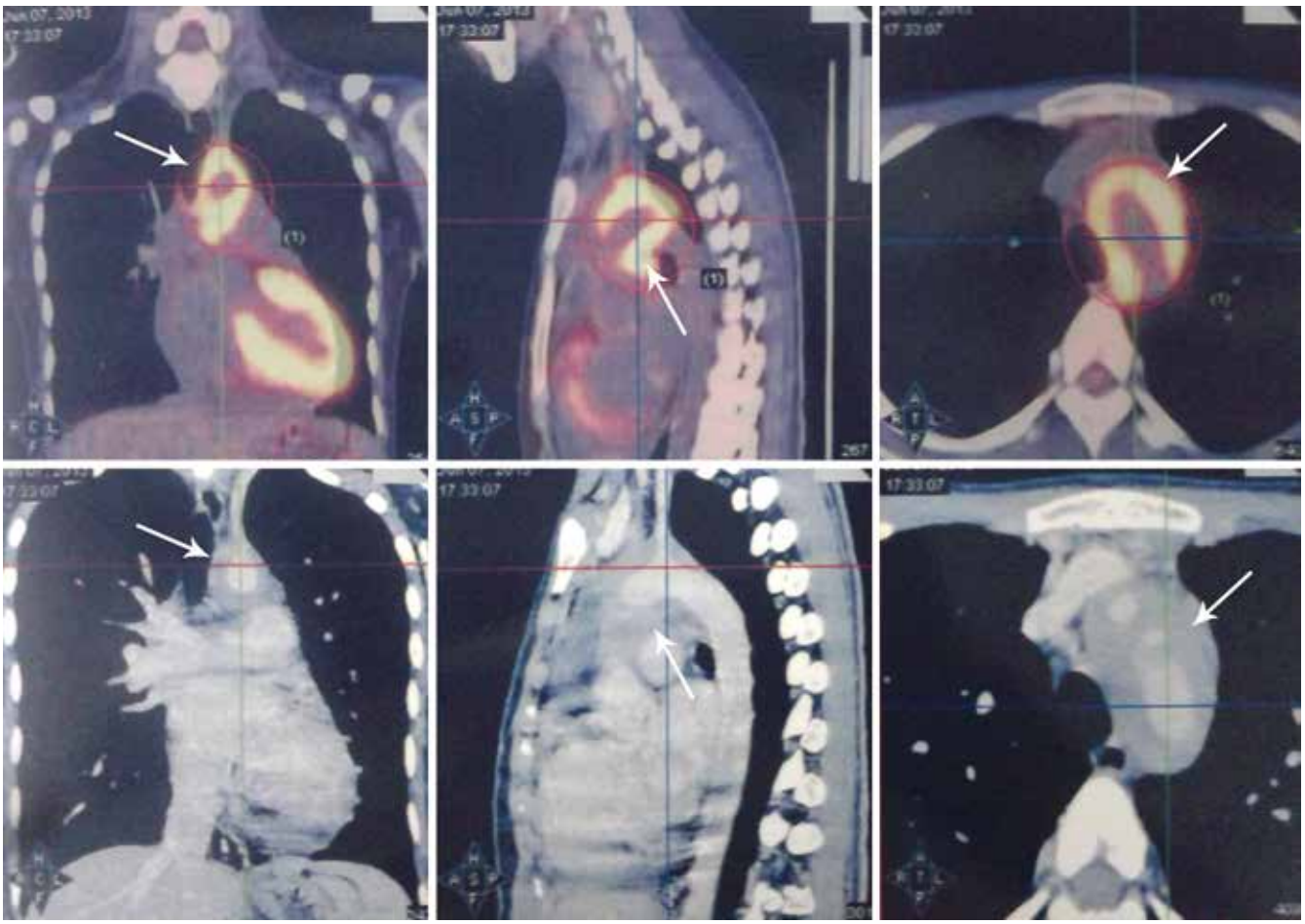

Figure 1 ^{18}F-FDG PET/CT chest image, the right side was coronal image, middle was sagittal image and left was cross sectional image. The above were PET images and below CT images. In CT images, thickening of the blood vessel wall of aortic arch was visible (white arrow), as well as luminal stenosis. In PET images, the thickened soft tissue adjacent to the aortic arch was visible with an active metablism of glucose, whose SUV was 9.4 (white arrow).

abnormities were seen, except the higher erythrocyte sedimentation rate of 89 mm/h and a positive serum mycoplasma antibody. The causes of fever were analyzed including infectious disease (might be infective endocarditic or tuberculosis), tumor disease or connective tissue disease. Advanced antibiotics including imipenem/cilastatin and azithromycin were administrated for the anti-infection treatment, but the fever was still persistent, the highest temperature up to 39 ℃. In order to differentiate from tumor, the ^{18}F-FDG PET/CT was performed and result found that soft tissue thickening adjacent to the aortic arch and descending aorta thickening was visible as well as the lumen of aortic arch narrowed, the standard uptake value (SUV) of thickened soft tissues was 9.4 (*Figure 1*), some enlarged lymph nodes with SUV 3.9 in mediastinal were visible. Mediastinal malignant lymphoma was suspected and mediastinoscopy was carried out to obtain lymph nodes and thickened soft tissue for pathological examination. Biopsy report shown that the lymph node tissue, structure normal, hyperplasia of lymphoid follicles, abnormal lymphocytes no found. Special staining examination including PAS, anti-acid, hexamine silver were all negative, so reactive hyperplasia of enlarged lymph node was considered and malignant diseases were excluded. The patient still had a fever, transferred to our institute for further treatment. The physical examination results on admission were as follows: body temperature 38.5 ℃; BP 113/70 mmHg (upper left limb); 108/68 mmHg (upper right limb); the rest were

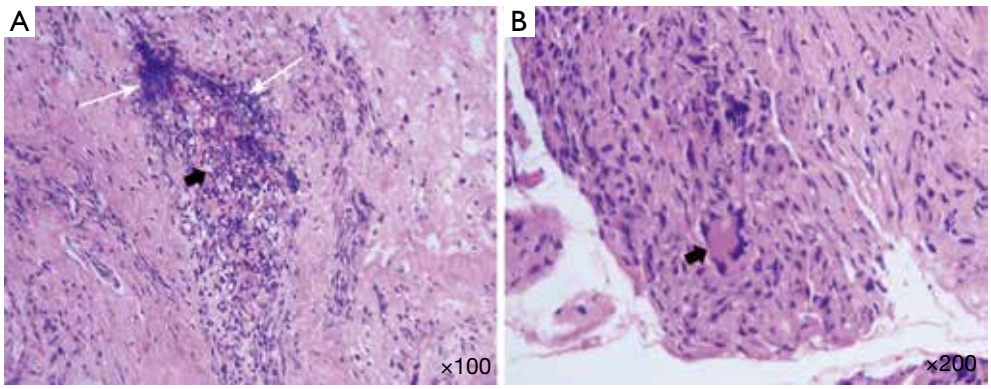

Figure 2 HE staining pathological images of the thickened soft tissue adjacent to the aortic arch. A panel, lymphocytes and plasma cells infiltration were visible in blood vessel wall (white arrow), elastic fiber and smooth muscle fiber of blood vessel wall necrotized (black arrow); B panel multinucleated giant cell granulomatous lesions (black arrow).

negative as before. Reviewing the soft tissue pathological slides again, we found that many lymphocytes and plasma cells infiltration were visible in vessel wall and the elastic fibers were fractured, smooth muscle fibers were necrotized in small vessel. Multinucleated giant cell granulomatous lesions can be visualized. These pathological changes were in accord with arteritis (*Figure 2*). TA was diagnosed and oral 40 mg/d of prednisone was administrated according to the weight of the patient was 45 kg. The body temperature gradually decreased after treated for three days, and became normal after one week, the dose of prednisone was gradually reduced after treated for one month, at present patient is in a stable condition after treated for three months, and in good health. For economic reason, the patient couldn't offer again PET/CT examination.

Discussion and literature review

TA is commonly seen in Japan, Southeast Asia, India and Mexico, which was firstly described in 1905 by Mikito Takayasu. TA commonly occurs in people younger than 30 years old, account for approximately 90%, and less in people older than 40 years old (2). Females are more likely to be affected than males, which was once regarded as "Asian young women disease", but recently founded to occur in men, the morbidity of male:female ratio is about 1:8-9. The prevalence of TA appears to vary greatly in different countries, about 1-2/million in Japan and 0.8/million in Sweden and in the UK (3,4). The etiology is still unknown, may be associated with immune injury caused by infection. Pathological manifestations are as follows: lymphocytes and plasma cells infiltration are visible in the vessel wall at early period, and occasional polymorphonuclear neutrophils and multinucleated giant cell infiltration. Inflammation causes the vessel adventitial thickening, elastic fibers and smooth muscle fiber necrosis of the tunica media, and intimal hyperplasia, resulting in the vessel stenosis and occlusion of lumina. Local aneurysm was formed because elastic fibers were severely damaged in the tunica media (5). Clinical manifestations can be divided into two phases, in the early phase patients may complain of symptoms including fever, weight loss, fatigue, myalgia, headache and other uncharacteristic presentations, vascular stenosis and occlusion are involved with the development of disease, which can be divided into four types according to the anatomical location of the affected arteries: brachiocephalic arterial type, the stenosis and occlusion of carotid and vertebral artery lead to brain ischemia, subclavian artery involvement leads to the unilateral or bilateral upper limb weakness, cold, pain numb, pulse weak or disappeared (pulselessness); thoraco-abdominal arterial type, the thoraco-abdominal aorta involvement is characterized with lower limb weakness, pain and intermittent claudication; Renal arterial type, appears high blood pressure; advanced pulmonary artery involvement appears pulmonary hypertension (2).

TA is easy to be misdiagnosed and early diagnosis is difficult. The mean delay in diagnosis varied from 10 months to 4.9 years (6,7), 91% patients reported seeing more than one physician prior to diagnosis (8). Renal artery vasculits was often misdiagnosed as hypertension (9), aorta vasculitis was often confused with other artery lesions, like syphilitic arteritis, tuberculous arteritis, giant cell arteritis, Bechet's disease and atherosclerosis. In this case, the

patient was a young female, mainly complaining of fever without obvious deficiency in limb artery blood supply, no marked blood pressure different between bilateral upper limbs was found in physical examinations, and no vascular murmur was heard on the subclavian aorta and abdominal aorta. She had been misdiagnosed as infectious diseases, no response to antibiotic treatment. To exclude the possibility of tumor diseases, ^{18}F-FDG PET/CT was performed, the results showed an obvious thickening of soft tissue adjacent to the aortic arch and descending aorta wall, with active metabolism, as well as a mediastinal lymph node enlargement with active metabolism. Due to insufficient understanding of ^{18}F-FDG PET/CT abnormal manifestation about the condition, it's misdiagnosed as malignant lymphoma. Mediastinoscopy was performed to obtain mediastinal lymph nodes and the thickened soft tissues for confirm diagnosis. Pathological results showed that no abnormal lymphocyte was present in lymph node tissue, in the thickened soft tissue the manifestations were in accord with arteritis. Based on the above results, TA was considered. After treated with glucocorticoid, the body temperature gradually decreased to normal, and the condition was improved. For economic reason, the patient couldn't afford a PET/CT review again, but according to the clinical characteristics, PET/CT and pathological results, as well as the glucocorticoid treatment respond, it's in accord with the diagnosis of TA. Although significant blood pressure discrepancy between bilateral upper limbs and ischemic change were often appeared in the aortic arch type TA, but no significant signs were found in the physical examinations of this patient, which might be due to an unobvious stenosis and occlusion in subclavian artery and vertebral artery. TA is one kind of autoimmune disease, corticosteroids or combined with immunosuppressant therapy at early period can relieve the inflammation of blood vessel wall, avoid vascular injury and improve the prognosis. But the early diagnosis of TA is extremely difficult. Recent studies have found that ^{18}F- FDG PET/CT has high sensitivity and specificity for the early diagnosis of TA, but also can judge the curative effect (1,10).

Percutaneous arterial angiography was the gold standard for the diagnosis of TA, but its invasiveness, the damage of X-ray radiation and contrast agent limited the clinical application. More important reason was that angiography indirectly revealed the luminal lesions only through the filling defect of contrast agents, couldn't display the structure and morphology of blood vessel wall, lack of early diagnostic capacity (2). ^{18}F-FDG PET/CT can not only display the anatomical morphological changes of lesions with CT image, but also reflect the cellular FDG metabolic activity. Therefore, it's widely used in the diagnosis of tumor and inflammatory lesions, as well as the judgment of curative effect (11). Some recent studies have shown that ^{18}F-FDG PET/CT have value in early diagnosis of TA. Kobayashi *et al.* (12) found that in 11 cases of acute active TA, 2 cases were characterized with obviously concentrated ^{18}F- FDG in vascular system, whose SUVmax were 2.7 or higher, other 9 cases of mild concentrated, whose SUVmax were between 1.2 to 2.3; for the other 3 cases of inactive TA, SUVmax were 1.2 or less; for the 6 cases of healthy subjects, SUV_{max} were 1.3 or less. Setting SUV_{max} at 1.3 as the cut-off, the sensitivity of ^{18}F-FDG PET/CT for the diagnosis of TA was 90.9%, specificity 88.8%. Research believed that ^{18}F-FDG PET/CT were helpful to the early diagnosis of TA, as well as the confirmation of the location of lesions and scope. Webb M, *et al.* (13) found that the sensitivity and specificity of ^{18}F-FDG PET/CT for diagnosing TA were 92% and 100%, respectively, negative and positive predictive value were 85% and 100%, respectively. Andrews J, *et al.* (14) reported that ^{18}F-FDG PET/CT provided a more favourable basis for the judgment of disease activity than arteriography. In this case, although PET/CT examination had found abnormal manifestations of the aorta, but due to insufficient understand about the rare disease, the attention only focused on the enlarged mediastinum and metabolically active lymph nodes, mediastinal malignant lymphoma was considered, and performed mediastinoscopy, increasing the pain and economic burden of patient.

Through retrospective analysis of this case and review of the pertinent literatures, we hope to improve the understanding of TA in physicians of different clinical departments. In clinical work, attention should be paid to young patients of persistent fever, which failed to antibiotic treatment, as well as young patients with high blood pressure, whose unilateral limb was cold, pain and pulselessness, it should arouse a suspicion of TA. In physical examination, it's requisite to measure the blood pressure of bilateral upper limbs, auscultate the subclavian aorta and abdominal aorta area, also ^{18}F-FDG PET/CT is suggested to visualize the morphology and metabolism of systemic aortas.

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References

1. Lee KH, Cho A, Choi YJ, et al. The role of (18) F-fluorodeoxyglucose-positron emission tomography in the assessment of disease activity in patients with takayasu arteritis. Arthritis Rheum 2012;64:866-75.
2. Johnston SL, Lock RJ, Gompels MM. Takayasu arteritis: a review. J Clin Pathol 2002;55:481-6.
3. Koide K. Takayasu arteritis in Japan. Heart Vessels Suppl 1992;7:48-54.
4. Watts R, Al-Taiar A, Mooney J, et al. The epidemiology of Takayasu arteritis in the UK. Rheumatology (Oxford) 2009;48:1008-11.
5. Mason JC. Takayasu arteritis--advances in diagnosis and management. Nat Rev Rheumatol 2010;6:406-15.
6. Kerr GS, Hallahan CW, Giordano J, et al. Takayasu arteritis. Ann Intern Med 1994;120:919-29.
7. Ishikawa K, Maetani S. Long-term outcome for 120 Japanese patients with Takayasu's disease. Clinical and statistical analyses of related prognostic factors. Circulation 1994;90:1855-60.
8. Abularrage CJ, Slidell MB, Sidawy AN, et al. Quality of life of patients with Takayasu's arteritis. J Vasc Surg 2008;47:131-6; discussion 136-7.
9. Chaudhry MA, Latif F. Takayasu's arteritis and its role in causing renal artery stenosis. Am J Med Sci 2013;346:314-8.
10. Mavrogeni S, Dimitroulas T, Chatziioannou SN, et al. The role of multimodality imaging in the evaluation of Takayasu arteritis. Semin Arthritis Rheum 2013;42:401-12.
11. Jerusalem G, Warland V, Najjar F, et al. Whole-body 18F-FDG PET for the evaluation of patients with Hodgkin's disease and non-Hodgkin's lymphoma. Nucl Med Commun 1999;20:13-20.
12. Kobayashi Y, Ishii K, Oda K, et al. Aortic wall inflammation due to Takayasu arteritis imaged with 18F-FDG PET coregistered with enhanced CT. J Nucl Med 2005;46:917-22.
13. Webb M, Chambers A, AL-Nahhas A, et al. The role of 18F-FDG PET in characterising disease activity in Takayasu arteritis. Eur J Nucl Med Mol Imaging 2004;31:627-34.
14. Andrews J, Al-Nahhas A, Pennell DJ, et al. Non-invasive imaging in the diagnosis and management of Takayasu's arteritis. Ann Rheum Dis 2004;63:995-1000.

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Case Report

Pulmonary sparganosis mansoni: a case report from a non-endemic region

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Abstract: Sparganosis mansoni is a parasitic disease caused by the larva of *Spirometra mansoni*. It occurs worldwide, but only a few patients show pulmonary involvement. Here, we present a case of pulmonary sparganosis mansoni in a non-endemic region. A 32-year-old Chinese woman presented with intermittent bloody phlegm, peripheral blood eosinophilia, and migratory patch shadows in both lungs. She had been misdiagnosed with eosinophilic pneumonia. She had a history of eating raw frogs, and the sparganum mansoni antibody was positive in both her blood and bronchoalveolar lavage fluid. Several *sparganum mansoni* were found in a frog sample that the patient provided. Consequently, she was diagnosed with pulmonary sparganosis mansoni. After two oral courses of praziquantel were administered, her symptoms and radiological lesions improved significantly. To our knowledge, this is the first case of pulmonary sparganosis mansoni occuring in Shanghai. Oral praziquantel is effective for the treatment of sparganosis mansoni, although its course of therapy may need to be repeated.

Keywords: Lung; sparganosis mansoni; diagnosis; treatment

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Introduction

Sparganosis mansoni is an infectious disease caused by the plerocercoid larvae (spargana) of various diphyllobothroid tapeworms belonging to the *genus Spirometra* and occurs in humans and animals (1). Sparganosis mansoni has been reported sporadically worldwide, but its prevalence is higher in several Asian countries, including South Korea, Japan, Thailand, and China (2,3). The cases of eye sparganosis and subcutaneous sparganosis have been reported previously, but very few cases of pulmonary sparganosis mansoni have been reported thus far (4). Here, we presented a case of pulmonary sparganosis mansoni in Shanghai, China.

Case report

The case was of a 32-year-old Chinese woman. She had a steady job in an office and never travelled overseas. In May 2010, the patient complained of fever lasting for 1 month, with a maximum body temperature 38.2 ℃. In June, she reported intermittent bloody phlegm occurring approximately 2-3 times per day. In October 2010, she was admitted to a tertiary hospital. After examinations, she was diagnosed as eosinophilic pneumonia; she was subsequently treated with 40 mg corticosteroid, and the dose was tapered by 5 mg every week. Despite corticosteroid treatment for 2 months, her chest computed tomography (CT) showed recurrent lesions, and she still complained of persistent cough and expectoration. She was therefore transferred to our hospital for a detailed examination. On thoroughly history taking, we found that the patient had a history of eating raw seafood and a preference for raw frogs and bullfrogs. On admission to our hospital, her vital signs were stable. A few inspiratory crackles were noted in the right lower lung lobe. Initial blood analysis showed elevated cells counts: peripheral white blood cell count, 10.5×10^9/L and eosinophils count, 0.69×10^9/L. Administration of oral prednisone was discontinued, and

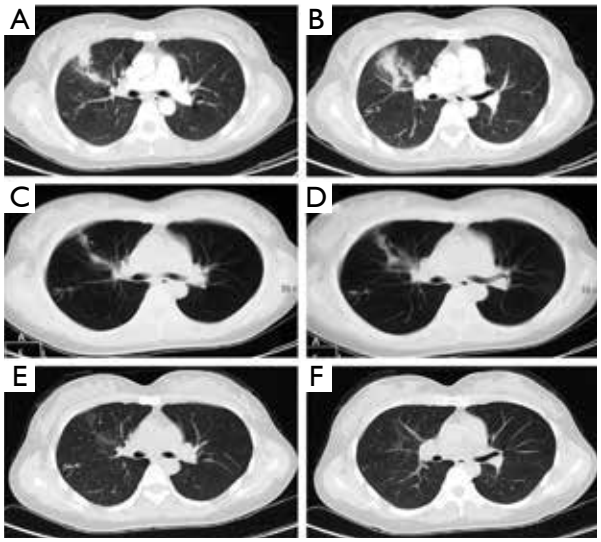

Figure 1 Chest CT scans of the patient. (A,B) Chest CT scans (before praziquantel therapy) reveals scattered patches and nodules in both lungs; (C,D) Chest CT angiogram (5 days after praziquantel therapy) reveals absorption of the right upper lobe shadow (compared to the previous CT scan); (E,F) Chest CT scans (1 month after oral praziquantel tablets) reveals absorption of the right upper lobe shadow (compared to the previous CT scan). CT, computed tomography.

she was started on intravenous cefuroxime (1.5 g bid) and clindamycin (0.6 g bid) for 9 days. Chest CT (10 days after admission) revealed scattered patches and nodules in both lungs (*Figure 1A,B*). Subsequently, bronchoscopy was performed, but there were no remarkable findings. The blood was tested at the Chinese Centre for Disease Control Institute of Parasitic Disease and Parasitology (CDC) to test for the presence of antibodies to any parasites. The results revealed a suspiciously positive result for antibodies against *Sparganum mansoni*. Re-analysis of the blood, and the bronchoalveolar lavage fluid confirmed the presence of IgG antibodies against *Sparganum mansoni*. The brain and other organs such as liver, kidney, heart, and spleen were also examined. Thereafter, the patient was diagnosed with pulmonary sparganosis mansoni and treated with praziquantel tablets (600 mg tid) orally for 5 days. After treatment, the symptoms gradually disappeared. Review of the chest CT (20 days after admission) revealed absorption of the right upper lobe shadow (*Figure 1C,D*). One month later, the patient had no cough and expectoration. Blood analysis showed a normal eosinophil account. However the blood mansoni sparganosis antibody was still positive. In comparison with the previous scan, the chest CT scan showed good absorption of the right upper lobe lesion (*Figure 1E,F*). She received a second treatment dose of praziquantel tablets (600 mg tid) orally for 5 days. Following-up examination conducted 5 months after the treatment showed that the blood IgG antibody against *Sparganum mansoni* was weakly positive, and blood eosinophils account was normal.

It took nearly 6 months to make a final correct diagnosis of pulomary sparganosis mansoni. After two courses of oral praziquantel and 5 months of follow-up, the patient mainly recovered (*Figure 2*).

Discussion

In recent years, with improvements in the standard of living and changes in dietary habits, the incidence of sparganosis mansoni and other food-borne parasitic diseases has increased in China; this is a constant concern to food safety and community health (5). Sparganosis mansoni has been noted in China, especially in Zhejiang, Fujian, Guangdong provinces in the southeast coastal region except in Shanghai. *Sparganum mansoni* is caused by the larvae of *Spirometra mansoni*. The main sources of infection are cats, frogs, dogs, and other animals, which are intermediate hosts. Humans, who are also a second-intermediate host and paratenic hosts, can be infected with spargana by drinking water contaminated with procercoid-infected copepods, eating undercooked meat of snakes or frogs infected with spargana, or using poultices of frog or snake flesh or skin on open wounds (1,6). The current patient was a woman who lived and worked in Shanghai. In our case, we found several *Sparganum mansoni* in a frog sample provided by the patient (*Figure 3*). Thus, infection probably occurred due to consumption of undercooked frogs and bullfrogs.

When humans ingest raw frogs or snakes, they also consume the plerocercoid larvae, which can cause infection. The parasites have strong migration and proliferation abilities and can usually pass through the intestinal wall and the peritoneum, finally reaching the subcutaneous tissues. The plerocercoid larvae rarely affect internal organs such as the lungs, brain, and spinal cord. According to clinical symptoms and location of the parasite, sparganosis mansoni are of 5 types: eye sparganosis, subcutaneous sparganosis, oral and maxillofacial sparganosis, brain sparganosis, and visceral sparganosis (7). Among these, visceral sparganosis is rare, compromising only 1% of all cases. Thus, pulmonary involvement is rarely reported in the literature (4). The

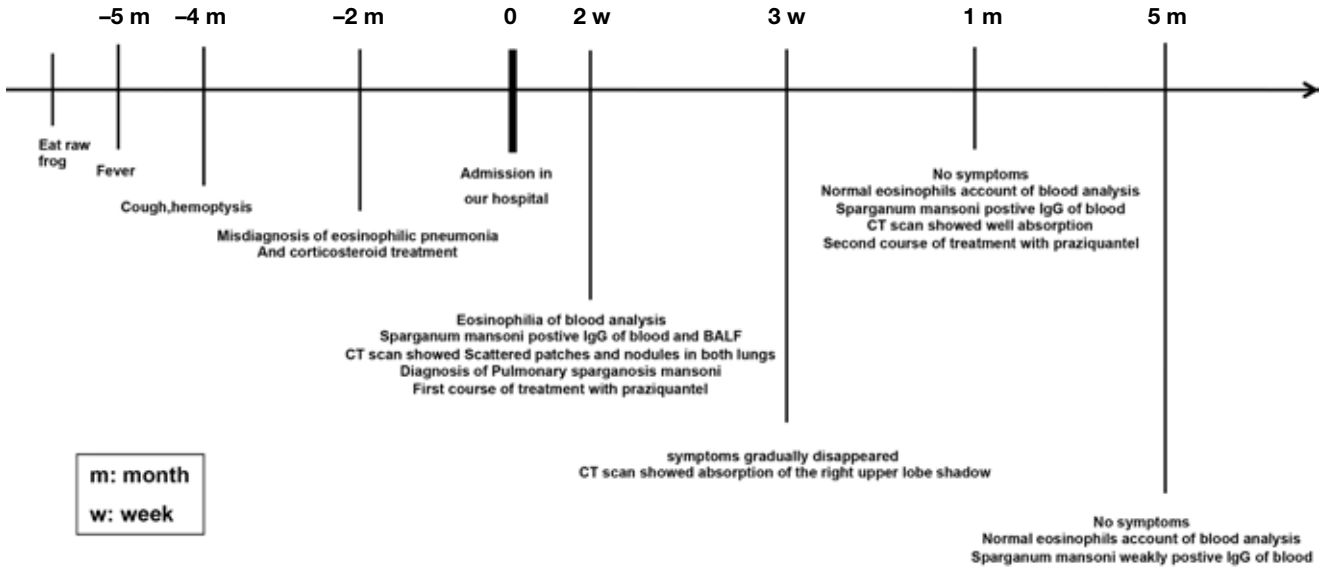

Figure 2 The timeline of diagnosis, treatment and follow-up.

Figure 3 *Sparganum mansonies* in a frog sample. Dissection of a frog from the region where the patient lives shows the presence of *Sparganum mansonies* (arrow).

patient reported here repeatedly presented with intermittent cough, hemoptysis and increased eosinophil count. Chest CT showed migratory patch shadows in both lungs. Thus, the diagnosis was confused with eosinophilic pneumonia due to the clinical features. Physicians should be cautious when corticosteroid therapy shows no effect on lesion absorption in chest radiology.

Considering the small number of cases of sparganosis mansoni in the literature and its confusing clinical features, the diagnostic method used for this infection should be quick and easy, with high sensitivity and specificity. The result should also be verified by using single copy of the gold standard immunoconcentration assay or immunochromatography (8). In our case, considering the patient's increased eosinophils count and food habits, we decided to send her samples to CDC for urgent testing for parasites antibodies. A multiple-dot enzyme-linked immunosorbent assay of the serum and bronchoalveolar lavage fluid showed repeated positive results for the anti-sparganum antibodies. Unfortunately, the patient refused to undergo transbronchial lung biopsy or video-assisted thoracoscopic surgery lung biopsy. Unlike the case reported previously by Iwatani K *et al.* (9), there was no histopathological confirmation in this case. However, considering the epidemiology, clinical features, chest CT findings, and positive blood test results and antibodies, a final clinical diagnosis of pulmonary sparganosis mansoni was made.

Currently, more physicians recommend surgery as the

Table 1 Comparison of the findings before and after correct diagnosis of patient

	History	Symptoms	Signs	Blood analysis	Sparganum mansoni antibody in blood	Sparganum mansoni antibody of BALF	CT of the lung
Before diagnosis	Ingest raw frog	Fever, cough, hemoptysis	A few inspiratory crackles in the right lower lung lobe	Eosinophil count 0.69×10^9/L	Positive	Positive	Scattered patches and nodules in both lungs
After therapy	–	No	No	Normal	Weakly positive	Unknown	Well absorption

BALF, broncho-alveolar lavage fluid; CT, computed tomography.

best treatment for cerebral sparganosis mansoni (10). Since very few reports on pulmonary sparganosis mansoni exist in the literature, it was difficult to find information on the condition. Our patient presented with transmigrating lesions in both lungs. Since surgery was not appropriate, we treated her with two courses of oral praziquantel tablets without corticosteroid therapy. After 1 month, her peripheral blood eosinophil count returned to normal. The patient's symptoms were significantly improved and radiological lesions resolved. Five months later, the *sparganum mansoni* serum antibody was weakly positive. The incidence of lung sparganosis mansoni is rare. It is not well diagnosed and often misdiagnosed as either eosinophilic pneumonia or hypersensitivity pneumonitis owing to its clinical features (*Table 1*). The clinical diagnosis of this disease must be made considering the epidemiology, clinical manifestations, chest imaging findings, and serological or alveolar lavage fluid analysis, relevant antibody testing,as well as patient history.

Conclusions

Pulmonary sparganosis mansoni is a rare condition. To our knowledge, this is the first case of pulmonary sparganosis mansoni reported in east China. Oral praziquantel treatment is effective, although a repeated course of therapy may be required. Epidemiological investigation is important but not necessary when pulmonary parasites are suspected in a patient with lung lesions. Patients should be advised to avoid ingestion of raw frogs or snakes, or exposing any wounds to raw frog meal in order to prevent the disease in endemic and non- endemic region.

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References

1. Li MW, Song HQ, Li C, et al. Sparganosis in mainland China. Int J Infect Dis 2011;15:e154-6.
2. Shin EH, Guk SM, Kim HJ, et al. Trends in parasitic diseases in the Republic of Korea. Trends Parasitol 2008;24:143-50.
3. Anantaphruti MT, Nawa Y, Vanvanitchai Y. Human sparganosis in Thailand: an overview. Acta Trop 2011;118:171-6.
4. Huang F, Gong HY, Lu MH. Pulmonary sparganosis mansoni: a case report. Trop Biomed 2012;29:220-3.
5. Coordinating Office of the National Survey on the Important Human Parasitic Diseases. A national survey on current status of the important parasitic diseases in human population. Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi. 2005;23:332-40.
6. Murata K, Abe T, Gohda M, et al. Difficulty in diagnosing a case with apparent sequel cerebral sparganosis. Surg Neurol 2007;67:409-11; discussion 412.
7. Oh SI, Koh SH, Pyo JY, et al. Sparganosis mimicking an intramedullary tumor of the cervical cord. J Clin Neurosci 2011;18:1128-9.
8. Chung YB, Kong Y, Yang HJ, et al. IgG antibody responses in early experimental sparganosis and IgG subclass responses in human sparganosis. Korean J

Parasitol 2000;38:145-50.
9. Iwatani K, Kubota I, Hirotsu Y, et al. Sparganum mansoni parasitic infection in the lung showing a nodule. Pathol Int 2006;56:674-7.
10. Hong D, Xie H, Zhu M, et al. Cerebral sparganosis in mainland Chinese patients. J Clin Neurosci 2013;20:1514-9.

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ICC COLUMN: The Voice of the Patient

Nobelpharma, a new Japanese pharmaceutical company that only provides medicines for unmet medical needs

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Introduction

When I founded Nobelpharma in 2003. I received the following advice from an older colleague, "When you greet your employees at the start of a new year, don't speak in negative terms such as we live in uncertain times, and nobody can tell what will happen while the world is in a recession, but I still ask each of you to do your best. Even if it might not come to pass, encourage your staff to look forward to a bright future and reflect on the good events of the preceding year". In this article I would like to convey a positive view of the future!

Nobelpharma specializes in R&D of pharmaceuticals for medical treatment, particularly the later phases of clinical trials in Japan, application, corresponding regulatory review, obtaining approval, and following approval, price negotiations and sales. Within the entire field of pharmaceuticals, we only handle drugs for unmet medical needs.

Making only essential drugs

Nobelpharma's clear mission (*Table 1*) is to "Contribute to medical care by providing drugs that are needed but not available, in other words, drugs for unmet medical needs."

Once each quarter of the year all employees gather, and I speak to them about the current business situation and issues, and I always begin by talking about our company's mission. "We will contribute to medical care by developing drugs for unmet medical needs that other companies do not pursue. We will not do anything else." This is what I insist upon, and I am also adamant that when on some occasions we lose track of the appropriate direction, if we return to our mission, we can't go wrong.

Utilizing the strengths of a small number of experienced and highly qualified personnel

"Drugs for unmet medical needs" does not provide a focus on any particular field of treatment, but we concentrate on pediatric, gynecological, and hereditary diseases, and on rare cancers.

We do not seek to become larger because we want to achieve fast decision making in a small organization. We have about 220 employees, about 120 of them are sales personnel, and 50 are working in R&D. We have obtained market approval for eight drug products since the company was established ten years ago. Many of our employees are veterans of major pharmaceutical companies who have a track record of success. They know, based on their experience, how to develop products efficiently in a short period of time. The average age of our employees is high, over 50 years of age.

Nobelpharma's product development

Nobelpharma obtained approvals for three drugs in the first round of development, from 2003 to 2008. We selected three candidate products that we considered would have a high likelihood of obtaining approval. I believed that these three products would offer us the chance to rapidly bring them to market.

The first product that we developed was Nobelzin. This is a treatment for Wilson's disease, a hereditary copper metabolic disorder, and we developed it in response to requests from patients' organizations and related academic medical groups. There are about 1,000 to 3,000 patients in Japan, and we applied for approval after conducting

Table 1 Nobelpharma's mission, policy and action criteria

Our mission
Contribute to medical care by developing, manufacturing & delivering essential medicines that other companies do not pursue
Our policy
Stakeholders, directors and employees should share the mission spirit
Limited number of elite directors and employees is ideal. Scale expansion is not our purpose
Elite should have knowledge and experience, and love their jobs
Maintain an environment where elite can work satisfactorily
As the result of our activities, profit comes to us
Without effort, profit cannot be earned
The earned profit should be shared impartially among stakeholders and retained earnings
Our action criteria
Philosophy: always remember that patients' benefit is the most important when you cannot make a decision; challenge: you don't know unless you try. But, you should never be afraid to cut a loss
Law & ethics
Observe laws, and don't act against ethics
Realize the essence of laws
Speed: due date has the first priority
"Around…" or "Early/late…" are forbidden words→Set deadlines by "Date"
You should do today what you can do today, and should not postpone it until tomorrow
Cost & efficiency
Don't purchase/possess meaningless things. Don't engage in meaningless activities, and don't have anyone else do so
Don't hesitate to buy time. Speed rules success or failure
Procurement from 2 sources is the basic rule. Purchase at a little higher price than the lowest one, and consider consignment
Quality of clinical trial data should be controlled at a logically and scientifically reasonable grade. Don't forget to achieve balance among data quality, cost and speed
Disclosure & transparency
Consideration & report/notice/consultation

phase III trials with 37 patients. We obtained approval in January 2008, the drug price was listed in April, and we launched sales. We obtained orphan drug designation at the beginning of development, and received assistance to cover 21% of development costs. However, post-marketing expenses such as for collection of safety data were significant, and this product has hardly been profitable, but we continue to sell it. Alfresa Pharma handles the drug marketing.

Our second product was Lunabell for gynecological patients. This is a drug to treat dysmenorrhea. Dysmenorrhea can cause unbearable pain and discomfort, significantly reducing the quality of life for young women. There were strong requests to develop this from patients' and related academic groups, and it was even taken up in the Japanese Diet, but since there was a similar drug available at a very low price, no company would pursue it. Even though there was much clinical research reporting on its efficacy, there were no rigorous comparative trials worldwide, so we made our application after conducting a preliminary trial with 36 patients, a placebo-controlled Phase III trial with 96 patients, and a long-term dosage trial with 128 patients. Lunabell was approved in April 2008, the drug price was listed in June, and we launched it in July 2008, marketed by Nippon Shinyaku and Fuji Pharma. This drug is our major product, accounting for a large part of our sales, and we have continued to conduct life cycle management for it after the initial launch, by obtaining approval for additional indications and developing improved drugs to reduce adverse effects that were approved in June 2013. I believe it would not be possible to build a strong foundation for a company in this industry in Japan today with just orphan

drugs and without one commercially successful product like this one.

The third product was Nobelbar, a drug for treatment of pediatric neurological patients. It is an intravenous injection used to treat neonatal seizures and status epilepticus, and it was developed in response to requests from academic neurological and pediatric societies. Prior to Nobelbar there were only agents for muscular injection that were available, which were not really appropriate for infants and newborn babies. Nobelpharma developed this drug on its own, and utilized Japanese government funding for R&D to conduct investigator-initiated trials. It was approved in October 2008, the drug price was listed in December, and then Alfresa Pharma launched the sale of the drug. This treatment for neonatal seizures was given orphan designation early in its development, and 24% of development expenses (other than for clinical trials) up until application were covered by additional aid from the Japanese government. However, this drug is not given repeatedly so sales are limited, and of course it is necessary to collect safety data so it is hardly profitable, but nevertheless patients continue to benefit and sales continue.

Obtaining approval of these three drugs made 2008 a wonderful year for our company. There were articles in the Asahi Shimbun and Nikkei Business about us, and overseas the pharmaceutical journal Script wrote that we had obtained marketing approvals for three products. Surprisingly, Nobelpharma was ranked first in Japan that year among Japanese pharmaceutical companies for the number of approved new drugs!

After that in our second round of development we obtained approval for Fostoin, a treatment for status epilepticus and for prevention of convulsive seizure after surgery, for Gliadel, a drug to treat malignant glioma, for Alabel, a diagnostic agent for visualizing the structure of malignant glioma during surgery, and for Unitalc, a drug to treat malignant pleural effusion. We were proud that our ability to introduce drugs from major established companies like Pfizer (Fostoin) and Eisai (Gliadel) confirmed the recognition of our development capabilities. We could not take on high risk initially when our company was established, so in principle we selected products for development that we saw had potential to be profitable for our company and that had already been approved overseas, but for whatever reason had not yet been approved in Japan. There were some products that did not fit our situation during our early years and which we were unable to introduce, but through perseverance and continuous negotiations, we were able to introduce some of them. Currently we have four products in the application process, and a number in development, including some that are completely new products, having never been approved in Japan or overseas. Some products that we are co-developing with universities were R&D agents that they produced, and we are receiving maximum public aid intended for venture companies in order to minimize development expenses, as part of our constant effort to find ways to be profitable.

We have specialized staff members assigned to discover and select products for development, a considerable number in light of the limited size of our company. These staff members meet regularly to evaluate the progress and status of each candidate product. They each have their own area of expertise and evaluate candidates accordingly, and I believe they do so quickly and accurately. Now we are finding fewer products with a similar low level of risk compared to those we found when the company was newly established, but it is vital for us to find new promising candidate products. I myself am also positively involved in selecting the right products to fulfill our mission, and I work hard to introduce the ones that I decide are right (*Table 2*).

Financing by business investors and from banks

From the very start we did not use venture capital for financing, but obtained funds from Inabata & Co. Ltd. and from a number of banks. Based on the company's mission, the goal was not to grow, but rather to contribute to society, and our management policy was to obtain sales and profits as a means to that end. Our objective was not to have shares listed, and so it was not possible to obtain capital from venture capitalist investors that would look for stock ownership and capital gains on the stock market. Inabata agreed with our company's mission and supported us financially from the very start with both investment and loans. Development Bank of Japan Inc. invested in preferred stock, and various governmental financial institutions, business investors, and commercial banks made long-term loans to us, including The Bank of Tokyo Mitsubishi UFJ Ltd., and Mizuho Bank Ltd.

The attraction of drugs for unmet medical needs

Sometimes I am asked why we develop drugs for unmet medical needs. First, it is the attractiveness of developing drugs that are needed. It is usually the case that there are strong requests from the patients to develop them, and so

Table 2 Approved products of Nobelpharma

Brand name	Indication	Licensor	IND/clinical trial	Approval	Distributor
Lunabell tablets LD; Lunabell tablets ULD	Dysmenorrhea	Janssen	Nov 2004 PIII	Apr 2008; Jun 2013	Nippon Shinyaku/ Fuji Pharma
Nobelzin capsules 25 mg/50 mg	Wilson's disease OD	Teva	Aug 2004 PIII	Jan 2008	Alfresa Pharma
Nobelbar 250 mg for injection	Neonatal seizures OD Status epilepticus	In-house development	Sep 2005 PIII/IIT	Oct 2008	Alfresa Pharma
Fostoin 750 mg for Injection	Status epilepticus Prevention of convulsive seizure after surgery	Pfizer	Mar 2009 PIII	Jul 2011	Eisai
Gliadel 7.7 mg implant	Glioma OD	Eisai	Mar 2009 PI/III (final)	Sep 2012	Eisai
Alabel oral 1.5 g	Diagnosis of malignant glioma OD	SBI Pharma (Medac)	Apr 2010 PIII	Mar 2013	Nobelpharma
Unitalc intrapleural 4 g	Prevention of malignant pleural effusion recurrence	Novatech	Sep 2009 PII/IIT (final)	Sep 2013	Nobelpharma
Foscavir infusion solution 24 mg/mL	Cytomegalovirus retinopathy, viremia and infection OD	Clinigen	Succession of approval	Jan 2012	Nobelpharma
Indacin IV 1 mg	Patent ductus arteriosus of prematurity OD	Lundbeck	Succession of approval	Jan 2013	Nobelpharma
Cosmegen IV injection 0.5 mg	Wilms tumor, chorionepithelioma, pediatric malignant solid tumor, etc.	Lundbeck	Succession of approval	Jan 2013	Nobelpharma

OD, orphan drug designation; IIT, investigator initiated trial.

there are many opportunities to meet the patients directly, and we hear straight from the patients and their families. We always feel our responsibility during development, and when we obtain an approval, the feeling of success is so much greater than for ordinary drug products.

The second reason is that drugs for unmet needs are usually profitable to some degree. However, in Japan the drug price is determined after approval is obtained, and if the sales forecasts were wrong or if the desired drug price is not obtained, then it is possible to lose money.

The third reason is Pharmaceuticals and Medical Devices Agency (PMDA)'s attitude towards drugs for unmet medical needs. PMDA has a different attitude toward the drugs that we are developing than towards ordinary drugs, for which they may not care about their ultimate presence or absence in the market. They recognize that these products are to answer unmet medical needs.

There are examples in the US of companies that succeeded in business as specialists in the medical field, but that is not the case in Japan. Genzyme is a famous example, founded in 1981, and now doing business in 40 countries around the world, with 10,000 employees and sales of around $4.5 billion. It became part of the Sanofi group in 2011.

There are a number of reasons that a company like Genzyme would not be born in Japan, but I think that the biggest are R&D capability, financing capability, and the drug pricing system. Among those reasons, drug pricing stands out. In the US, companies may set their prices freely, but in Japan the government sets the price for the National Health Insurance system after the drug is approved, and even if companies have some room to negotiate, the government has unconditional authority to set the price. This drug pricing system is sometimes unusually callous towards orphan drugs. Despite the fact that these drugs are completely different from those for treating high blood pressure, or hyperlipidemia with nearly ten million patients, and will be very little burden on health insurance finances, they hold prices down to the point where it becomes very difficult to make a profit at all. Consequently, even after working hard to develop an orphan drug, you can't make

enough profit to reinvest in development of the next product. It is because companies in Japan cannot obtain an adequate price for products originally developed here that products are developed in Japan only after they are developed in a country with a freer drug pricing system, especially in the US. It is for these reasons that it is so difficult to establish a company like Genzyme, specializing in orphan drugs, in Japan. While it may be that drug prices are supported by taxes, if you do not allow pricing that rewards excellent innovation, then you will not have people who can create innovative products, and it will be difficult to foster innovation. Currently, new drug products developed in Japan are priced low both in Japan and overseas. The result is that it does not benefit Japan.

Setting higher drug prices for excellent innovation that provides unique benefits to needy patients would mean accepting higher-priced medical care, but in a wealthy modern country like Japan I do not think there are many people opposed to using national finances in such a manner. Our country is poor in natural resources, and in order for us to continue our current prosperity, I believe that our government must amend its way of thinking.

Development overseas

So far most of the drugs our company has had approved were already approved in the US or Europe and were introduced from there, but recently, as I mentioned earlier, we have begun to accept the challenge of developing new drug ideas. It is impossible to avoid some failures in the course of drug development, and so we cooperate with overseas companies to spread the risk, emphasize cooperation with universities, and keep an eye on international development as much as possible. In the near future we will conduct international joint clinical trials, and are thinking about the possibility of Japanese candidate products to obtain approvals in the future in neighboring countries, Europe, or the US.

The kind of people that Nobelpharma requires

Our company exists in order to "develop and sell drugs that must be provided." If we did not have people who had experienced success and who have multiple capabilities, we could not succeed. In addition, more than anything else, we need people who have a strong desire to develop the drug products that are truly required in the medical field to improve patient outcomes.

I work hard to create an environment where employees can work with a good feeling about their job, but our company is only ten years old so compensation is lower than that in major companies, and although I think it is regrettable, sometimes there is a lot of overtime work. However, over these past years there have been very few who have quit working here, and we have a good employee retention ratio.

The wonderful thing about drugs for rare diseases

I am happy that we have developed drugs for rare diseases when patients and doctors express their gratitude. That is the greatest joy for us.

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ICC COLUMN: The Voice of the Patient

Economics that heals

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The article by Shiomura (1) from Nobelpharma concerning a pharmaceutical company that is devoted to healing patients whom other companies ignore deserves careful attention and praise. The humanitarian approach and enlightened business practices that he brings to health care are models for other companies.

It is in stark contrast to many of the large multi-national Pharma companies, which have been found to be guilty of falsification of clinical trial data (2), price gouging (3), bribing physicians to prescribe their drugs (4), concealing side effects of drugs (5), failing to release information from trials of their drugs when the results are not favorable (6), and other practices calculated to harm rather than to heal patients. Such companies are committed to increase profit regardless of adverse patient outcomes. They employ "economics that kill" as Pope Francis has described financial dealings that allow companies to harm people in order to maximize profit (7).

I believe that several aspects of Nobelpharma's mission and business practices deserve attention and affirmation. Their principle of focusing on patients in need with diseases that are being ignored by other drug companies is most important. They do not develop and market "me too" drugs for lucrative conditions for which medications are already available and sell them by false and misleading advertising and by preventing generic alternatives from being made available. This proves their commitment to all patients' welfare.

Second, their focus on diseases for which there is a real need for effective therapy rather than diseases that are already well served or disease indications that have been created by falsified data or by bribing medical specialists to establish new diagnoses of questionable scientific validity (8) is an approach that provides unique benefit to patients in a cost-effective manner.

Finally, Nobelpharma's business practices are patient-oriented. They focus on keeping costs down and expediting the process of bringing the products to patients. Their economic model enables the products to be affordable to patients and not priced at the very highest possible cost as seen in the US and elsewhere. They are not forced to have huge budgets to bribe physicians and politicians or to create false and misleading advertisements to manipulate physicians and patients. In this way, Nobelpharma operates in a cost-effective and humanitarian manner.

All companies in a capitalist economy need to generate profit to exist, but as Nobelpharma shows, this is not incompatible with the cost-effective, humanitarian operations of Pharma. I would also emphasize the personal leadership and frequent personal communication with staff by Nobel's management, which brings all parts of the company into line with its mission and practices. In many of the criminal and unethical actions of large multi-national Pharma cited above, their management claimed that their national operating companies acted in violation of company policy and the company should not be blamed. As a result, the company and its senior management were not held personally responsible, and they continue to commit profitable crimes since the downside is so small and the upside so great.

I believe that the principles of Nobelpharma could be adopted by many of the large Pharma and the results for patients would be life-saving. It would create a Pharma economics that heals rather than the existing Pharma economics that kills. Perhaps their profits would be somewhat diminished, but Pharma should not exist to maximize profits at patients' expense and the cost of patients' lives.

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References

1. Shiomura J. Nobelpharma, a new Japanese pharmaceutical company that only provides medicines for unmet medical needs. J Thorac Dis 2014;6:E125-E129.
2. Forbes Magazine (2013), Diovan data was fabricated says Japanese health minister and university officials. Available online: http://www.forbes.com/sites/larryhusten/2013/07/12/diovan-data-was-fabricated-say-japanese-health-minister-and-university-officials/
3. AIDS Health Foundation (2014). Available online: http://www.aidshealth.org/archives/17736
4. Zhang W, Grouse L. Physician bribes in the US and China. J Thorac Dis 2013;5:711-5.
5. Gupta S. Side-effects of roflumilast. Lancet 2012;379:710-1; author reply 711-2.
6. Kelly J. Randomized Clinical Trials: 1 in 3 Not Reported (2013). Available online: http://www.medscape.com/viewarticle/813447
7. O'Leary N. Pope Francis attacks tyranny of unfettered capitalism, idolatry of money, NBCNews.com, Reuters news service. Available online: http://www.nbcnews.com/news/world/pope-francis-attacks-tyranny-unfettered-capitalism-idolatry-money-v21623507
8. US National Library of Medicine. Available online: http://www.ncbi.nlm.nih.gov/pubmedhealth/behindtheheadlines/news/2013-08-15-controversy-over-dsm-5-new-mental-health-guide/

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Three One Five: a global consumer movement

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During 2014 and 2015, ICC will be promoting patient consumerism through the Three One Five Initiative. San Yao Wu (3 1 5 in Chinese, which refers to March 15) is a global pro-patient and pro-consumer movement. It is highly active all over China and has been active for many years. People can consult with San Yao Wu to see their assessment of what products are unsafe. Due to the booming economy in China, some merchants seek the biggest profit and neglect consumers' health and rights and having a group that asserts consumers' rights is necessary. Consumers' rights activities are needed throughout the world!

The San Yao Wu program is conducted by China Consumers' Association (CCA, http://www.cca.org.cn/english/index.jsp). The topic for groups to promote for 2014 is "New Laws to Protect Consumers: Rights and Responsibilities". In 2013 the topic was "Increase Consumers' Strength". The Chinese government supports this consumer movement through the State Administration for Industry and Commerce (SAIC).

The CCA has branches throughout China that meet with companies to emphasize consumer respect and service. They coordinate between companies and consumers with questions or complaints, and if problems occur then the government through the SAIC has the right to take action against companies.

San Yao Wu/Three One Five is not active in the US or in many western countries. ICC would like to make more health care professionals and patients familiar with its valuable mandate. ICC looks forward to input from its member organizations, which will be writing to ICC about their plans for 2015 patients' rights promotion, and their articles will be published in the ICC Column in JTD. We also look forward to JTD readers' thoughts about patient rights. ICC will be working with our member organizations to work with their governments in passing "New Laws to Protect Consumers" There are many laws that could benefit COPD patients.

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ICC COLUMN: The Voice of the Patient

Open access medical publications

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Overview

Open access journals provide unrestricted, free online access to scholarly articles (1). This new approach to medical publication is revolutionizing medical communications. The rise of open access publishing challenges the long-standing model developed in concert by commercial publishers and medical organizations, a model whose profitability depends on the ability to restrict and sell access the medical information and to use this information for political and financial purposes.

Medical research yields important and valuable information that benefits the people of the world. Communications that facilitate the widest global dissemination of such information are valuable for public health, while those communications methods that restrict the availability of such information limit this benefit. Open access is particularly valuable for developing countries where limited financial resources have historically deprived health care professionals of the latest medical information. The ability of the people of the world to prevent disease and improve their health would be benefited by improved access to reliable medical information.

Academic physicians also have an interest in open access. They are not paid to write the articles that report their research in scholarly journals. Instead, their interest lies in the intellectual impact of their work with their colleagues throughout the world; the broader the dissemination of their work, the more effective they are.

The interest of medical suppliers, which pay organizations to include their advertisements in restricted-access journals, is in getting their products licensed by governments and prescribed by health care professionals. If publication in open access journals means that information about their products is more widely circulated in a credible and effective manner, they would be less likely to provide funds for commercial publishers whose journals have restrictive distributions. Advertisements and other promotional materials will be published wherever they receive the most relevant professional attention.

The purpose of medical communication

It is difficult for commercial publishers to argue that they should have exclusive rights to publish medical research. Much of this research is funded by governments, which use their people's money, and governments exist to serve those citizens. Therefore, the purpose of the communication of medical research should be to benefit patients and not to make physicians, businesses, or governments wealthy.

U.S. medical publishing

In 1905, the American Medical Association (AMA) paved the way for the current medical publishing model in the U.S. by urging physicians not to prescribe drugs that were advertised directly to consumers by pharmaceutical companies. This forced the companies to advertise through the AMA's journals, whose circulation was limited to physicians. Revenues from AMA journal advertisements became the principal source of funds for AMA and the source of the Association's political power (2,3).

Following this model, other U.S. medical professional organizations began to publish journals covering their own specialties or partnered with large international publishers such as Elsevier and Springer Verlag to do so. These journals are available only to members of the medical organization or through expensive subscriptions. These journals provide valuable medical information only for those willing to pay for it.

Commercial publishers

Universities, their libraries, and their academic faculty members are caught in the middle as commercial publishers and medical professional organizations seek to maximize revenue by restricting the dissemination of medical information. Publishers of important medical journals often force medical libraries to pay high prices for access to the journals, and they "bundle" many different journals of much less importance with those that are in great demand, forcing libraries to buy access to many journals that are useless to them in order to get the ones they need.

Competition of commercial and open access publishers

The trend toward open access medical journals has been greeted enthusiastically by academic institutions, medical researchers, libraries, the public, and governments. Medical organizations and commercial publishers have opposed open access journals, but with the widespread and increasing acceptance of such journals, these groups are taking steps to protect their exclusive franchises. In some cases they start new open access journals and attempt to promote their paid subscription journals (4). Other commercial publishers, such as the Nature Publishing Group and John Wiley, work with small internet publishers, such as DeepDyve, and attempt to use five minute glances at digital copies of medical papers of interest to physicians to sell them a download of the papers in order to maintain their revenue (5). Major medical publishers such as the American Medical Association and the New England Journal of Medicine are urgently trying to market and rebrand their services by creating The JAMA Group and the NEJM Group, which attempt to capitalize on their names and reputations to continue to sell their products and maintain their revenue through subscriptions and membership. With many of the major new medical advances now being available through open access and not appearing exclusively in their journals, this will be a difficult challenge for the restricted access journals. Nevertheless, these commercial publishers will continue to find ways to maintain their communications, using various marketing methods.

In the enthusiasm of using new open access journals, we should not ignore the value of existing academic journals and communications that have served physicians and scientists well for many years through restricted access. Hopefully, both commercial publishers and non-profit, open access publishers can succeed in the future.

Governments and academic groups mandate open access

US and European governments are urging their researchers to submit their articles to open access journals whenever possible. Research Councils, UK, the conduit through which the government transmits taxpayers' money to academic researchers in the UK, has mandated that articles be published in open access journals, preferably immediately but certainly within a year (6). This allows the commercial publishers to continue to profit from the materials at least for a short time. Harvard University's Faculty Advisory Council is also urging faculty to submit articles to open-access journals (7). US government physicians and scientists whose work is funded by the government do not have ownership of the materials they produce. Use of the materials is freely permitted by open access journals.

The trend toward open access journals has been even more dramatic in non-medical scientific fields, where sponsorships and advertising play a much smaller role than they do in medical communications.

The costs of medical communication

Although commercial publishers can be criticized for restricting the flow of medical information to colleagues who need it, it should be remembered that having open access on the internet in which the barriers to communication are reduced does not mean that all publishing costs disappear. All publishers have the responsibility to provide access to their publications; they have to obtain rights to the communications, edit them professionally, and provide assurance of their integrity and validity. Open access publishers who wish to develop audiences must also accept these responsibilities; however, they have a challenge to adopt a business model that generates revenue to support their efforts while providing open access.

Currently, open access publications generate necessary revenue in a variety of ways. Some receive grants from charitable foundations or governments. Others charge authors to have their work published; still others are supported by advertising, sponsorships, or the publication of promotional content from proprietary organizations.

Medical requirements for open access

The benefits of open access medical publication in fostering inquiry and the dissemination of medical knowledge are

great and the power of the internet brings this knowledge to the world, including developing countries that have been deprived of this information. However, open access publishing attempts will fail unless it successfully makes medical information valuable and continually available over time. Open access ports must archive their content so that it can be faithfully retrieved when desired; they must diligently strive to ensure their content's accuracy and relevance to physicians and health care professionals, and they must provide safeguards that prevent conflicts of interest, proprietary influence, and political dogmatism or expediency from falsifying scientific and medical facts.

Veracity of published medical articles

Open access journals have been criticized by restricted access journals for deficient peer review and less credibility. In what they term a "spoof" *Science* magazine (a restricted access journal) had bogus articles sent to 305 open access science journals and 157 of them accepted the bogus article for publication. However, it was unclear how they selected these journals from the 8,250 existing open access journals. In addition, they did not include in their "spoof" a control group of restricted access journals that received the bogus article so no inference could be made about the superiority of restricted access journals (8). It was of interest that only 3 of 57 members of the Open Access Scholarly Publishers Association accepted the bogus paper, which suggests that more scholarly peer review would have uncovered the ruse in more of the open access journals.

A much larger flaw in the believability of many published clinical trials of medications and devices that pertains to both restricted and open access medical journals is that the proprietary companies that perform these studies seldom allow access to the actual patient-level data and often select data favorable to their products for publication and falsify and conceal unfavorable data (9-12). Neither restricted nor open access journals appear to be able to prevent these bogus articles from publication. Expensive clinical trials are often not repeated and yet they form the basis for regulatory approval of licensing of products. This is likely one of the reasons that products that are released have many unexpected defects and side effects in practice.

Questions about the dissemination of medical information

As open access medical publication expands, questions about the structure and operation of these new publications need to be addressed. Who owns medical research information? How should it be communicated? What rights do government regulatory organizations have in controlling the development and dissemination of medical information? Who should have access to the primary data generated in medical research? What are the rights of the citizens of the country where the research took place as well as the rights of the people in the rest of the world to have access to medical information? What are the rights of the inventors and patent holders of new therapies and devices to the medical information concerning their inventions that is communicated? Will commercial medical companies be allowed to disseminate biased promotional materials in open access journals? How can continuity of vital information and databases be safely preserved and made available?

These are complex issues, and they should be examined and discussed in an ongoing basis, to ensure that the new open access world of medical communication preserves the value of the old communication models while improving accessibility and reliability of medical information to colleagues and patients around the world.

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References

1. Wolpert AJ. For the sake of inquiry and knowledge--the inevitability of open access. N Engl J Med 2013;368:785-7.
2. Grouse L. Physicians for sale: how medical professional organizations exploit their members. Medscape J Med 2008;10:169.
3. Starr P. The Social Transformation of American Medicine, Harper Colophon Books, 1982:131-4.
4. Solicitation for subscriptions to The Lancet Respiratory Journal disseminated June 13, 2013 by publisher Elsevier. Available online: http://www.sciencedirect.com/science/journal/22132600/open-access
5. Medical Marketing & Media News Brief, Big publishers back startup's "sneak-peek" service, sent June 20, 2013.
6. The Economist, Free-for-all, May 4th, 2013:79.
7. Medical Marketing and Media, Harvard urges faculty to ditch journals, April 24, 2012. Available online: http://chronicle.com/article/Harvard-Faculty-Adopts/40447
8. The Guardian (2013). Acceptance by open access journals of a bogus study. Available online: http://www.theguardian.

com/higher-education-network/2013/oct/04/open-access-journals-fake-paper
9. Nisen P, Rockhold F. Access to patient-level data from GlaxoSmithKline clinical trials. N Engl J Med 2013;369:475-8.
10. Gupta S, Calverley P. Side-effects of roflumilast. Lancet 2013:710-2.
11. Little RJ, D'gostino R, Cohen ML, et al. The prevention and treatment of missing data in clinical trials. N Engl J Med 2012;367:1355-60.
12. Forbes Magazine (2013). Diovan data was fabricated says Japanese health minister and university officials. Available online: http://www.forbes.com/sites/larryhusten/2013/07/12/diovan-data-was-fabricated-say-japanese-health-minister-and-university-officials/

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Chasing on the way of cancer immunotherapy

I would like to share my experience of research in the following story. Based on my background regarding multiple directions in the field of cancer research including antiangiogenesis therapy, specific inhibitors of cancer cell repopulation between cycles of chemotherapy, and immunotherapy, I personally think the most promising approach to cure cancer might be through modulation of the immune system based on my research experience in past two decades.

After graduating from Shandong Medical School in 1980s, I started doing cancer research at the Experimental Oncology Laboratory, Shandong Academy of Medical Sciences, China. At that time there was a fascinating hypothesis "*Starving tumors by terminating their blood supply*" initially proposed by Dr. Folkman from Harvard Medical School. I chased after this novel concept for many years and attempted to demonstrate this hypothesis in our animal tumor models. We established a methodology to screen candidate angiogenesis inhibitors (AI) using the chick embryonic chorioallantoic membrane (CAM) assay, including development from avascular to vascular stages during the early development of chick embryos. In such way, a wide variety of agents were tested, and we successfully developed several AI. Based on our previous studies, anti-angiogenesis therapy was believed to work well to fight against some malignant solid tumors, especially lung, breast and esophageal cancers and also to be effective to cure benign hemangioma. One of the most promising angiogenesis inhibitor, AI-6, was even tested in clinical trials at the Affiliated Tumor Hospital of Shandong Academy of Medical Sciences. The combined therapy of anti-angiogenesis therapy with conventional therapy including surgery, chemotherapy or radiotherapy was demonstrated to have improved efficacy in our clinical trials. However, the trials also showed limited efficacy and relapse could eventually occur, suggesting failure of such a strategy to fight against cancers.

The human steps to fight against cancers would never stop. Since then, we kept searching for new and practical approaches to the treatment against cancers. One day, I accidentally noticed a publication of research paper by Dr. Tannock from Princess Margaret Hospital/Ontario Cancer Institute (PMH/OCI), University of Toronto, Canada. In this paper, the authors reported that cancer cell repopulation during the intervals of treatments with chemoradiation is a neglected area by scientific research, which is most likely to cause treatment failure. This idea was inspiring to me and I contacted him immediately by emails to show my interests. Luckily, Dr. Tannock then provided me an open position in his laboratory as a postdoctoral fellow and a good salary to study cancer cell repopulation between cycles of chemotherapy in mouse models.

Dr. Tannock is the pioneer in the research field of cancer cell repopulation between cycles of chemotherapy. During my postdoc period, we demonstrated that cancer cell repopulation between cycles of chemotherapy speeds up and this process can be suppressed by specific inhibitors. Cancer cell repopulation during the intervals of treatments has been considered an important factor of treatment failure. Targeting this process may be able to improve the outcome of cancer treatment. In the long run, however I notice, these approaches can only slow down tumor growth, but cannot eradicate cancer. My expectation to fight against cancers motivated me to find more powerful strategies that are promising to cure this disease. A close friend of mine who was working at Baylor Institute for Immunology Research (BIIR), Baylor Health Care System, Dallas, Texas, as a principal investigator, one day told me that BIIR was a world-leading institute for dendritic cell (DC)-based immunotherapy. He forwarded to me some of his research slides showing that intratumoral DC vaccination following chemotherapy causes complete disappearance of some tumors in his mouse models. I was so excited at viewing his research findings. It seemed to be promising as an effective cancer treatment.

The immune system has the greatest potential for the specific destruction of tumors with no toxicity to normal tissues. More importantly, the long-term immune memory can prevent cancer relapse. Considerable evidence by immuno-oncological research has indicated that tumors can be recognized by the immune system and their growth can be terminated or controlled by immunosurveillance. The emerging clinical data suggested that cancer immunotherapy is most likely to play a key role in the clinical management of cancers. Fortunately, I got a great opportunity to work at BIIR, as a Research Associate focusing on DC-based immunotherapy against cancers. BIIR, led by Dr. Banchereau, is among the top translational immunology research centers worldwide. Scientists at BIIR particularly concentrate their efforts on the studies of DCs, which are rare cells that turn on and regulate immune responses. Two-year experience of working at BIIR allowed me to study the functions of DC and to translate the research findings into novel approach to treat cancers in animal models.

More encouragingly, Dr. Steiman, Canadian immunologist at Rockefeller University, won the Nobel Prize for Physiology or Medicine in the year of 2011 for "his discovery of DCs and their role in adaptive immunity". He had been diagnosed as having pancreatic cancer and had received DC vaccination at BIIR. This therapy was proved to significantly prolong his life. But unfortunately, he passed away three days before the announcement of his winning the prize from the Nobel Committee.

After completing the 2-year fellowship, I joined the team led by Dr. Marc de Perrot in 2008, as a Research Associate, Latner Thoracic Surgery Laboratories, Toronto General Hospital, University Health Network, University of Toronto, Canada. Dr. de Perrot is the Head of Toronto Mesothelioma Research Program. The focus of our study is to improve the efficacy of mesothelioma treatment by immune modulation. Malignant pleural mesothelioma (MPM) is a rarely found cancer in the general population, but it is rather common among construction and industry workers who have been exposed to asbestos. MPM originates from the lining of the lungs (pleura), and is usually misdiagnosed until its advanced stage when treatment options are very limited and cure is no longer possible. As observed in other cancers, the immunosuppressive components are infiltrated into the tumor microenvironment of MPM patients. Therefore, we employed promising immunotherapy in combination with conventional therapies (i.e., surgery, radiotherapy, or chemotherapy) to treat mesothelioma. We have demonstrated that the number of regulatory T cells infiltrating into the MPM tumor increases over time after tumor challenge in mouse models, and depletion of this population can enhance anti-tumor immune reaction. Blockade of the immune suppressive signals, similarly as release of the brake, can induce specific immunity against tumor. These recent research findings, to my great honor, were presented at the 15th World Lung Cancer Conference held in October, 2013, Sydney, Australia. The abstracts of my two mini oral presentations can be viewed at MO20: Preclinical Therapeutic Models II, MO20.08 and MO20.09, respectively: http://www.2013worldlungcancer.org/documents/WCLC2013-AbstractBook.pdf.

I have worked in the field of cancer research for almost 30 years. On the way of scientific research to fight against cancers, countless scientists, like me, experienced hopes, successes and failures. No matter what the results of our present efforts are, I believe human would definitely win the fight against cancers.

I was very happy to talk with Ms. Grace Lee at the Exhibition Booth on the conference. She introduced about the new column "Between You and Me" in the *Journal of Thoracic Disease*. It was a great opportunity to share my story here with colleagues. I would like to thank you for your kind invitation.

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Hopes versus reality

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We have noticed the publication of a series of small, short-term studies using the ketamine for such varied conditions as suicidal ideation in depression (1), post-traumatic stress disorder (2), and treatment-refractory depression (3). These brief and inconclusive reports have been picked up throughout the internet portraying these uses as "a cure for depression" and "promising therapy for mental illness." Stories about these preliminary findings have been published with an uncritical eye in such large global internet portals as Huffington Post (4), National Public Radio (5), and CBS (6) for the public and MedPage (7) for physicians.

We believe that this widespread, uncritical coverage of ketamine use could be misleading and damaging for patients, particularly since the drug is readily available worldwide as the illegal club drug "Special K". As a result of overly positive reports about the drug in such common conditions, the risks of the epidemic of "Special K" use could be greatly increased. Because of the wide illegal access to the drug, patients could self-medicate and incur the serious side effects and long-term effects of the drug.

"Special K's" active ingredient, the anesthetic ketamine, is an NMDA receptor inhibitor, which still finds some legal use in the US in spite of its prominent hallucinogenic and cognitive impairment side effects; however, by far the broadest use of the drug in the US is from preparations of "Special K" smuggled illegally from Mexico.

We would like to point out an important article concerning "Special K" in a column in the *Journal of Thoracic Disease* (*JTD*) (8), for which we are editors. This column, "Between You and Me", publishes articles from practicing physicians and patients with their observations, thoughts, and experiences concerning medicine.

Dr. Peng Wu of Guangzhou wrote concerning several of her urologic patients who had severe psychiatric and urologic sequelae from long term "Special K" use. His urgent warnings about these problems with the recreational club use of the addictive "Special K" produce a very different view of the widespread, uncritical promotion of small, short-term, inconclusive studies of this agent in situations in which its specific mechanism of action in treating these conditions is certainly not well understood. Since these studies were industry-supported and the authors acknowledge conflicts of interest, the appropriateness of global promotion of the findings at this early stage is questionable.

The purpose of the "Between You and Me" column is to publish such important observations and perspectives of physicians and patients about the realities of medical care and not just the hopes for possible benefit. Dr. Wu's observations have great value for all, and we welcome others' views that will provide valuable information for our readers. Clinical trials are not the only kind of article that needs to be published in a medical journal.

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References

1. Price RB, Iosifescu DV, Murrough JW, et al. Effects of ketamine on explicit and implicit suicidal cognition: a randomized controlled trial in treatment-resistant depression. Depress Anxiety 2014;31:335-43.

2. Feder A, Parides MK, Murrough JW, et al. Efficacy of intravenous ketamine for treatment of chronic posttraumatic stress disorder: a randomized clinical trial. JAMA Psychiatry 2014;71:681-8.
3. Murrough JW, Iosifescu DV, Chang LC, et al. Antidepressant efficacy of ketamine in treatment-resistant major depression: a two-site randomized controlled trial. Am J Psychiatry 2013;170:1134-42.
4. Ketamine Depression Cure? 'Special K' Treats Symptoms Within Hours, Study Reports. Available online: http://www.huffingtonpost.com/2013/05/22/ketamine-cures-depression-study_n_3322006.html
5. Growing Evidence That A Party Drug Can Help Severe Depression. Available online: http://www.npr.org/blogs/health/2014/04/03/298770933/growing-evidence-that-a-party-drug-can-help-severe-depression
6. Ketamine, or "Special K," effectively treats severe depression in study. Available online: http://www.cbsnews.com/news/ketamine-or-special-k-effectively-treats-severe-depression-in-study/
7. IV Ketamine Rapidly Effective in PTSD. Available online: http://www.medpagetoday.com/Psychiatry/AnxietyStress/45314?xid=nl_mpt_guptaguide_2014-04-17&utm_source=guptaguide&utm_medium=email&utm_content=mpt&utm_campaign=04|17|2014&userid=500170&eun=g5625789d10r&email=lgrouse@uw.edu&mu_id=5625789
8. Wu P. Stories of Special K patients. J Thorac Dis 2014;6:E37-8. Available online: http://www.jthoracdis.com/article/view/2169/html

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Stop violence against medical workers in China

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Abstract: The incidence of patient-doctor disputes are alarmingly increasing in China, this article reviews the current status and causes of violence against medical workers in China, six strategies to tackle the daily worrying problems have been proposed and hopefully could improve the medical working environment in China.

Keywords: Violent attacks; threats; assaults; hospitals; medical workers in China

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Introduction

Threats and violence to medical workers is common in every country, but is more common in China (1). Since 2000, the incidence of violence against medical workers has been increasing at about 11% annually (2) (*Figure 1*). Only in 2012, seven medical workers were killed (3) (*Figure 2*), which is approximately half of the total number of deaths in the previous 9 years. In 2013, one Ear, Nose and Throat (ENT) doctor at Wenling Hospital in Zhejiang province was murdered, most medical workers in China were shocked and hundreds of medical staff gathered together to request a safe working environment (*Figure 3*). The government has been tried to stop the violence against medical workers (4-6), unfortunately, daily oral abusive to medical workers occurs in almost every hospital, the number of physical attack to medical workers is still alarmingly rising. Believe it or not, the following violent events were reported in newspapers from January to March 2014 in China, we afraid it continues going on without ending.

Recent ridiculous and unbelievable violence against medical workers

(I) February 17, 2014, at BeiMan TeGang Hospital in Qiqihar city of Heilongjiang province, a perpetrator was not happy with the surgical outcome, and attacked the head of his ENT doctor with an iron pipe and murdered him (7,8);

(II) February 18, 2014, at Yi County Hospital of Hebei province, while Dr. Li, a general surgeon, was consulting a patient, his throat was slashed suddenly by his former patient (8,9);

(III) February 20, 2014, at 2nd Hospital of Zhejiang University Medical College, a female patient and her mother complained the slow process of a pregnant nurse, even they had learned the nurse was pregnant, they still beat the nurse crucially and caused her a mischarge;

(IV) February 25, 2014, at Nanjing Stomatological Hospital, a young female patient was not happy for her ward arranged by a nurse on duty, later her both parents, the government officials, beat the nurse up to paralysis (8);

(V) March 4, 2014, in Chaozhou Central Hospital, a patient had acute alcoholic intoxication due to abuse alcohol and died after prompt treatment. The relatives of deceased patient considered the doctor's treatment lead to a death and called up approximately one hundred people to humiliate the doctor in charge by forcing him to walk in hospital for half an hour until the police breaking it up (*Figure 4*);

(VI) March 4, 2014, in Beijing Union Medical Hospital, being refused of jumping the queue to see a doctor, a female patient and her mother furiously attacked

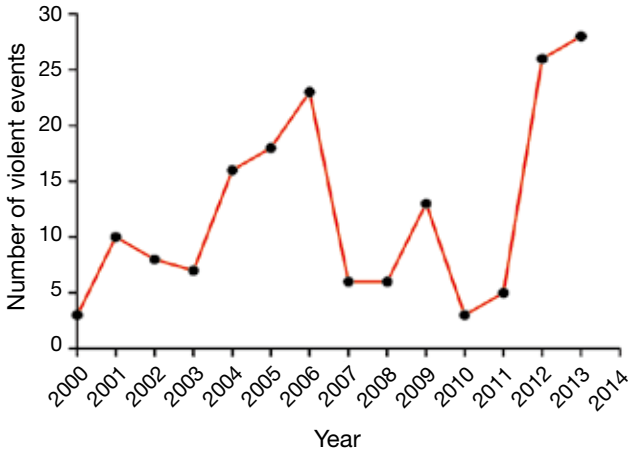

Figure 1 The annual number of violent events reported is increasing.

Figure 2 Medical workers murdered during medical violence.

a nurse and a nursing supervisor, caused the nurse an ocular fundus hemorrhage (10);

(VII) March 4, 2014, a doctor in Laizhou Municipal Hospital was battered to coma by two men with iron rods (11);

(VIII) March 4, 2014, in the early morning, in Ningbo 1st People's Hospital, Dr. Su, a female ENT doctor, was attacked by a patient's relative for one minute and slapped on her face three times leading to facial bleeding (12);

(IX) March 6, 2014, at Beijing Anzhen Hospital, a patient had a ruptured aortic aneurysm and died after aggressive resuscitation; a dozen of patient's family members blocked the ward, attacked the doctor on duty, and interrupted the normal medical service (13);

(X) March 7, 2014, in an elevator of Wenling 1st Hospital in Zhejiang province, Dr. Shan, a thoracic surgeon, was assaulted by a patient's relative. Later the perpetrator claimed, "I just like to attack any doctors who are on white working coat with no reasons (14)".

(XI) March 7, 2014, in 6th Hospital of Beijing University, a doctor who was consulting with a patient was knocked on the back of his head by another patient with a hammer (*Figure 4*). This perpetrator also carried a knife while knocking the doctor;

(XII) March 8, 2014, in 1st Hospital of Hanzhou city, a sick child removed a blocked needle himself

Figure 3 Medical workers in Wenling Hospital of Zhejiang province gathered together automatically to request the right to work with security and mourn Dr. Yujie Wang who was murdered by a patient.

Figure 4 The doctor on duty was humiliated by hundreds of people because he failed to rescue a severe alcoholic patient (left) and the consultant at 6th Hospital of Beijing University was assaulted by another patient (right).

and subsequently caused a bleeding, his father blamed the nurse on duty and hit her head with a bottle, resulting in a cerebral concussion, delayed cerebral hemorrhage, and comminuted nasal bone fracture (15);

(XIII) March 9, 2014, a physician in emergency room at People's Hospital of Bianyang city in Sichuan province ordered CT scan test to exclude cerebrovascular accident for a 80-year old lady who had vomiting, loss of speech, headache, hypertension, diabetes, the CT scan returned as normal, because of normal brain CT imaging, the patient' grandsons, two young relatives of deputy police chief of Bianyang city, considered the order of the CT scan as useless, and assaulted the physician with three punches and kicks, resulted in glasses broken and forehead scratched (16);

(XIV) March 11, 2014, 20 family relatives rushed into Chengdu Municipal Women and Children Central Hospital with iron pipes and assaulted five hospital employees, resulted in head injuries, head hematoma, closed abdominal injuries, renal contusion and bloody urine. The reason was a pregnant woman delivered a dead baby 4 days earlier (17);

(XV) March 13, 2014, a 4-month-old female patient with congenital heart disease and pneumonia had sputum and required suction, after sputum suction, the baby choked with cyanotic mouth on breast feeding, thus, the baby's mother and grandmother in law started to chase and assault the nurse on duty (18);

(XVI) March 21, 2014, at Shanghai 5th People's Hospital, Dr. Cai, a chief surgeon, was consulting with a patient, and was assaulted on the back of his head, his right index and middle fingers were fractured. In addition, another two nurses and one security guard were also injured (19);

(XVII) March 23, 2014, In emergency room at Beijing University of Chinese Medicine Hospital, a patient's male relative with height 1.8 meter beat a nurse to head injury due to her slow reaction (20).

A survey conducted by China hospital association included 8,000 patients and 8,000 medical workers in 316 hospitals at 30 provinces, municipalities, autonomous regions and cities, the results show that the incidence of oral abusive and threaten to medical workers increased from 90% in 2008 to 96% in 2012, whereas the incidence of physical injuries of medical workers escalated from 47.7% in 2008 to 63.7% in 2012. The annual average number of assaults on medical staff per hospital increased from 20.6 assaults in 2008 to 27.3 assaults in 2012.

Statistics results of Chinese hospital association show the number of violence against medical worker is 57 in 2010, 86 in 2011, 99 in 2012 and 130 in 2013. Report from Chinese National Health and Family Plan Commission (NHFPC), formerly the Ministry of Health shows 11 violent events occurred in hospitals in 8 provinces and cities in 2011, resulted in 7 death and 28 injuries including 16 medical workers, 11 patient escorts, and 1 security guard (21). We reviewed the authority reports from January 2011 to April 30, 2014, a total of 88 events of violence against medical workers occurred in 48 cities and 22 provinces, of which 10 medical workers (11.36%) were murdered. The emergency department had the highest incidence (20.5%, 18/88), followed by 120 emergency rescue team (5.7%, 5/88) and intensive care units (5.7%, 5/88). Fortunately, no violent event has been reported in military hospitals, which may reflect their effective restrict management.

Causes of violence

The recent severe violence to medical workers in China reflects the poor related managing systems and little respect to medical workers in current society. The cause of violence against medical worker is not unique. The related legal system and health system are the main roots of deteriorating doctor-patient relationship. In early 1980s, the health care in China has been commercialized. The rapid growing of the medical cost surpasses the patient's affordable capability, and the medical cost is only partially re-imbursed for some people with health insurance. A severe disease may cost the lifetime earnings of patient's family. Some patients and their family have been demanding greatly for a favorable outcome of any intervention, believe that the money they spent must translate into excellent or even full recovery of the health, even for untreatable advanced disorders. Once a patient died, the related family members will blame the doctors and try to fight for huge amount of compensation from the doctor as much as possible. They do not go legal process to sue the doctors, as it takes a long time and go through a very complicated process, and finally get a little compensation; in addition, they perceived that a legal channel has been in favor of medical establishment.

Yinao, a patient-doctor dispute, is an illegal interruption of normal activity at hospital conducted by a patient, relatives of patient or those who aimed for money. Yinao gangs have been walking around the hospital, and find

the way to provide illegal support for patients such as intimidation, threats, and violence to medical staff. It is common to find the scattered small cards advertising and providing illegal support for any patients who are unhappy with a doctor, saying call me if you are not satisfying with any medical services and need help.

Once a severe violence occurs, the reaction of police to the request of help from hospital is notoriously slow. They arrive in delay or just stand by observing the dispute or violence, and explain that this kind of issue belongs to doctor-patient relationship rather than the criminal issue and therefore they could do nothing but watching and waiting the order from their supervisor (22). For the media, it usually focuses on reporting the severity of medical worker, and ignores how the perpetrators were punished. The hospital administrators usually try to negotiate with perpetrator and to spent money for the settlement of dispute. Some injured medical staffs usually do not know how to protect themselves, and in fact, no effective avenue to protect medical staff is available. Therefore, they hesitate to report the assaulted events but just suffering emotionally and physically with heart in tears. Otherwise, unfortunately, the situation may get worse, the injured medical staff may be unfairly criticized or punished by the hospital governors. All these factors cultivate the growth of the violence in hospitals. As a proverb says, gaining big money after a big violence, winning small money after a small violence, and having no money for no violence. The medical workers have no sense of security in practice medicine and 86% medical staff do not expect their children to study medicine any more in the future (23). Nearly 40 percent of surveyed medical staff plan to give up the profession due to medical violence (23). Recent survey from Dxy.cn including 3,360 medical workers and 565 medical students shows 90% medical workers experienced or witnessed medical disputes, 50% had depression and 40% suffered from anxiety (21).

Six strategies for violence

Six solutions should be emphasized in order to ensure the security of medical care environment. First, the law must be reformed to tackle the violence in hospital as that in public area. Currently, the hospital is considered a special place rather than a public area, and therefore, the procedure in control the violence in hospital is rather slower and less effective than in public area. The perpetrator in hospital is punished much less than outside hospital, even outside the door of hospital. The slow response and delayed arrival of police to any urgent request from the medical workers and no reaction of police for the violence in hospital are a well-known phenomenon and should be solved. Second, any biased report, unfair or even false report from media must be stopped and punished seriously and severely. Free speech and free report on medical issue do not mean false or unfair report. Some reporters have little knowledge of medicine, but interested in reporting medical dispute or controversial events incorrectly for gaining public attention and generating the best-selling of the newspapers, they completely ignored the negative impact on the society and medical workers (7). Third, a neutral and fair judicial system to evaluate the medical dispute must be established, which should process independently and promptly, and should not be controlled by medical administrator or government. Fourth, the government should raise the salary of medical workers and encourage them to work with no worries about their living conditions. The aim of the hospital should be focused on the health of patients rather than hospital financial performance. The government should not evaluate the hospital by the index of financial income. Only in this way can the president of hospital, the director of department and medical workers concentrate on the quality of medical care. The medical workers should be guided to strive and compare the medical achievement of health care. Fifth, the suspected violent patients should be listed in the computer system and warned the medical workers to treat them cautiously. Sixth and finally, the medical workers should improve communication ability besides the medicine and know how to communicate with potential violent patients. And more importantly, the medical workers, especially the surgeons have to observe the guidelines of medical practice and evidenced medicine, and should always ask yourself in mind when practice medicine, "could I do it in this way if the patient in front were myself, my mom or my dad?"

Overall, the dream of medical workers in China is to deliver medical care in a safety working environment without assault. We expect that the six strategies above could be performed successfully, a friendly mutually trusted patient-doctor relationship can be rebuilt up and the quality of medical care will be improved further in China. In the end, hopefully, the dream of medical worker in China will become true.

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Dr. Ren designed and wrote this article.

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References

1. Violence against doctors: Why China? Why now? What next? Lancet 2014;383:1013.
2. Zhu L, Xu C. Practice on strengthening hospital connotation construction and reducing medical disputes. Chinese Hospitals 2013;2:1-3.
3. The National Health and Family Planning Commission of China. Guideline on strengthening the security and protection system construction in hospitals, issued by the National Health and Family Planning Commission of China, and the Ministry of Public Security. 2013. Available online: http://www.moh.gov.cn/yzygj/s3590/201310/e0a558aeb9d34700ba6dd1cfdfe93164.shtml. Accessed 5 May 2014.
4. Hesketh T, Wu D, Mao L, et al. Violence against doctors in China. BMJ 2012;345:e5730.
5. Wang XQ, Wang XT, Zheng JJ. How to end violence against doctors in China. Lancet 2012;380:647-8.
6. Ending violence against doctors in China. Lancet 2012;379:1764.
7. Jie J. 17 cases of violence against medical workers in 2013. Beijing Youth Newspaper Nov 1, 2013. Available online: http://wwwfarmercomcn/sh/jk/201311/t20131101_905793htm
8. BBC News-China sees wave of violence against hospital staff. Feb 28, 2014. Available online: www.bbc.co.uk/news/world-asia-china-26364133. Accessed May 17, 2014.
9. Xinhua News Agency. Doctor's throat slashed: violence to doctor in Yi county in Hebei province. Information Times. Available online: http://newshexuncom/2014-02-20/162316857html. Accessed Feb 20, 2014.
10. Luo J. Two nurses of Beijng union hospital injuried. Beijing youth newspaper. March 6, 2014. Available online: http://wwwdxycn/bbs/thread/27693431#27693431
11. He X. Yantai evening news two men beat laizhou doctor to coma. March 6, 2014. Available online: http://zhaoxy06h-ceocom/article/read/21/2332html
12. He JY. He Jiangyong Ningbo xianshan ENT doctor was assaulted for one minute. Chinanews.com. March 7, 2014. Available online: http://wwwdxycn/bbs/thread/27693431#27693431
13. Ning M. Doctor in Anzhen hospital was assaulted March 7, 2014. WWW CMT.com.cn. Available online: http://humcmtcomcn/detail/450861html
14. Dxy.cn. Thoracic surgeon was beaten in elevator. March 7, 2014. Available online: http://www.dxy.cn/bbs/thread/27684077#27684077
15. Youth times. A nurse in Hangzhou was attacked and resulted in nasal bone fracture. March 9, 2014. Available online: http://www.xsnet.cn/news/hz/2014_3/2029898.shtml
16. Xinjing News. Relatives of deputy police chief of Bianyang city in Sichuan assaulted physician. March 10, 2014. Available online: http://news.qq.com/a/20140310/001472.htm
17. Chengdu Evening News. 20 family relatives assaulted 5 hospital staff. March 13, 2014. Available online: http://news.sina.com.cn/c/2014-03-13/050029694285.shtml
18. Two relatives of patient chased and assaulted a nurse who sucked sputum for the patient. March 17, 2014. Available online: http://news.sina.com.cn/s/2014-03-17/142529726830.shtml
19. Sun GG, Sun WY. A surgeon in shanghai was attacked. Medical Forum Internet. March 24, 2014. Available online: http://newsxinhuanetcom/local/2014-03/21/c_119890241htm. Assessed May 17, 2014.
20. Beijing morning news. March 23, 2014. A man assaulted a nurse to brain injury due to her slow response. Available online: http://health.people.com.cn/n/2014/0323/c14739-24710062.html
21. High incidence of depression in medical workers and silence of alliance of zero tolerance to violence against medical workers. South Municipal Newspaper. Available online: http://news39net/yltx/140507/4384415html. Accessed May 17, 2014.
22. Southern Metropolis Daily. Timely response of police on call to stop violence. April 1, 2014. Available online: http://epaper.oeeee.com/A/html/2014-04/01/content_2046604.htm
23. Violence against doctors on the rise: survey. Aug 16, 2013. Chinadaily.com.cn. Available online: http://wwwchinadailycomcn/china/2013-08/16/content_16897958htm

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Erratum

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Erratum to: J Thorac Dis 2014;6:S70-7
Erratum to: J Thorac Dis 2014;6:S32-8
Erratum to: J Thorac Dis 2014;6:S173-9

No.1 Left atrial appendage exclusion—Where do we stand?

In the above article that appeared on Page 70-77 (1) of the Focused Issue of March 2014 in the *Journal of Thoracic Disease* (*JTD*), there was an error in the author's name "Thomas Beslevis". The correct name is "Thomas Beleveslis".

No.2 Thirteen years follow-up of heart myxoma operated patients: what is the appropriate surgical technique?

In the above article that appeared on Page 32-38 (2) of the Focused Issue of March 2014 in the *Journal of Thoracic Disease* (*JTD*), there were errors in the following authors' names. The corrections are as follows:
"Alexandra Kakourou" should be corrected as "Artemisia Kakourou";
"Alexandra Batistatou" should be corrected as "Anna Batistatou";
"Stelios Sismanidis" should be corrected as "Sokratis Sismanidis";
"Theodora Syminelaki" should be corrected as "Thalia Syminelaki";
"Eleftherios Apostolakis" should be corrected as "Efstratios Apostolakis".

No. 3 Surgical management of cardiac implantable electronic device infections

In the above article that appeared on Page 173-179 (3) of the Focused Issue of March 2014 in the *Journal of Thoracic Disease* (*JTD*), there were errors in the following authors' names. The corrections are as follows:
"Alexandra Petrou" should be corrected as" Anastasios Petrou";
"Eleftheria Piavali" should be corrected as "Helen Priavali";
"Eleftheria Gesouli" should be corrected as "Helen Gesouli";
"Eleftheria Apostolakis" should be corrected as "Efstratios Apostolakis".

References

1. Sakellaridis T, Argiriou M, Charitos C, et al. Left atrial appendage exclusion—Where do we stand? J Thorac Dis 2014;6:S70-7.
2. Siminelakis S, Kakourou A, Batistatou A, et al. Thirteen years follow-up of heart myxoma operated patients: what is the appropriate surgical technique? J Thorac Dis 2014;6:S32-8.
3. Koutentakis M, Siminelakis S, Korantzopoulos P, et al. Surgical management of cardiac implantable electronic device infections. J Thorac Dis 2014;6:S173-9.

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Retraction

Retraction: Application status of MALDI-TOF mass spectrometry in the identification and drug resistance of Mycobacterium tuberculosis

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Retraction to: J Thorac Dis 2014;6:512-516

The article "Application status of MALDI-TOF mass spectrometry in the identification and drug resistance of Mycobacterium tuberculosis" (doi: 10.3978/j.issn.2072-1439.2014.02.19) that appeared on page 512-516 of the May 2014 issue of the *Journal of Thoracic Disease* needs to be withdrawn due to some misconduct in the manuscript. We are sorry for the inconvenience caused.

Cite this article as: Retraction: Application status of MALDI-TOF mass spectrometry in the identification and drug resistance of Mycobacterium tuberculosis. J Thorac Dis 2014;6(6):E147. doi: 10.3978/j.issn.2072-1439.2014.06.33