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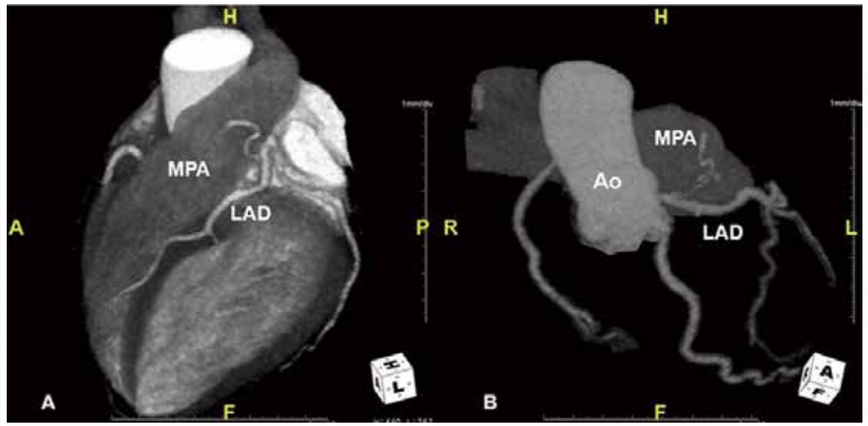

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Cover image:

The VR image from MDCT angiography demonstrating the fistulous communication to the LAD and the pulmonary artery (A,B). (See E29 in this issue).

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Red cell distribution width: the crystal ball in the hands of intensivists?

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Critically ill patients admitted to intensive care unit (ICU) are at a high risk of morbidity and mortality (1), with significantly increased medical costs. In the meanwhile, intensive care resources are limited, especially in developing countries such as China (2). As a result, rational allocation of limited intensive care resources to those patients who are more likely to benefit from intensive care may depend upon the accurate assessment of clinical prognosis. Therefore, many investigators have developed different prognostic tools, in the form of single parameter, composite indices, and even complicated scores (3-5), in order to assess the severity of illness, predict prognosis, or benchmarking different ICUs. Despite all these efforts, ideal prognostic tools are still lacking, so that investigators are trying to explore new prognostic indices that prove to be accurate, cheap, and readily available.

As a quantitative measurement of variability of red cells or red blood cell volume, red cell distribution width (RDW) has been traditionally considered useful in the differential diagnosis of anemia (6). Elevated RDW has been shown to exert prognostic value in healthy adults 45 years or older (7-9), and chronic conditions (such as coronary heart disease, heart failure, cancer, chronic lower respiratory tract disease, and inflammatory bowel disease) (9-13). During recent years, increased RDW has also been found associated with mortality in acute illness, including acute kidney injury, community-acquired pneumonia, and acute heart failure (14-16). To make the issue more complicated, RDW may be affected by genetic factors, thyroid diseases, renal or hepatic dysfunction, inflammatory disease, nutritional deficiency, and medications (17).

In a recent issue of the *Journal of Thoracic Disease*, Zhang *et al.* (18) reported that, in a retrospective study involving 1,539 critically ill patients admitted to a general ICU during 38-month study period, elevated RDW was associated with risk-adjusted hospital mortality, with odds ratio of 1.11 (95% confidence interval 1.04 to 1.18, P=0.001). However, the prognostic performance of RDW was rather poor [area under the receiver operating characteristic curve (AU-ROC) 0.6202], followed by albumin (AU-ROC 0.6134), C-reactive protein (CRP) (AU-ROC 0.6093), and Charlson index (AU-ROC 0.5980). In addition, RDW changes up to 20 days after ICU admission did not correlate with hospital mortality.

Why does RDW correlate with clinical prognosis in critically ill patients? One possible explanation is that such a correlation might indicate cause and effect relationship. However, current data suggest that this hypothesis is unlikely to be true. More importantly, even if increased RDW might be the direct cause of hospital mortality in ICU patients, we still lack the definitive intervention(s) to reduce RDW. Another possibility is that RDW merely represents a surrogate marker of severity of illness in the early phase of acute illness, and therefore correlates with clinical prognosis. In other words, increased RDW and high mortality are two apples on the same tree, which may be inflammation, poor nutrition, or oxidative stress (9,11,19).

The question remains that why do we need more prognostic indices (such as RDW), acknowledging that we already have a bunch of them, such as acute physiology and chronic health evaluation (APACHE), sequential organ failure assessment (SOFA), serum albumin level, platelet count, and others. Clinical evidence suggests that the old ones exert good, if not excellent, calibration. Calibration measures how much the prognostic estimation of a

predictive model matches the real outcome probability (i.e., the observed proportion of the event), and can be measured by Hosmoer-Lemeshow's χ^2-statistic. Likewise, Zhang *et al.* reported a fair calibration for RDW, as suggested by a Hosmer-Lemeshow's χ^2 of 11.17 (P=0.19) (18). However, most prognostic tools often exhibit poor discrimination. By definition, discrimination reflects the ability of a given prognostic index to distinguish a status (died/survived, event/non-event), and can be measured by AU-ROC. Zhang *et al.* reported a poor AU-ROC for RDW, with 0.6202 for RDW alone, and 0.6618 for composite prognostic indices including RDW, CRP, albumin, and Charlson index (18). One major limitation of the research by Zhang *et al.* is that no comparison of RDW and APACHE has been made. As a result, it still remains unclear whether RDW shows superior prognostic performance than APACHE, the most commonly used and validated prognostic model. Moreover, we do not know whether addition of RDW in the previous prognostic model (such as APACHE) may improve prognostic accuracy. All these questions might be answered by further investigation.

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Original Article

The evaluation of different treatment protocols for trauma-induced lung injury in rats

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Background: Lung contusion is an important factor that affects mortality and morbidity of lung injury after blunt chest trauma (BCT). The present study aims to evaluate the effectiveness of different treatment regimens on BCT-induced lung injury.

Methods: A total of 35 Sprague Dawley rats were divided into five experimental groups (n=7): sham, control; BCT; BCT + MP, BCT group treated with methylprednisolone (MP; 30 mg/kg on first day and 3 mg/kg/d on the following days); BCT + Q, BCT group treated with quercetin (Q; 50 mg/kg/d for seven days); and BCT + MP + Q, BCT group treated with the same doses of MP and Q. Serum Clara Cell Protein-16 (CC-16), thiobarbituric acid reactive substances (TBARS), and superoxide dismutase (SOD) levels were analyzed to determine histopathological changes in the lung tissues.

Results: Elevated serum CC-16 and TBARS levels and reduced serum SOD levels were found in the BCT group compared to the Sham group. There was a significant change in the serum CC-16 levels in the BCT + MP group compared to the Sham group. Serum TBARS levels were significantly lower in the BCT + MP and BCT + Q group compared to the BCT group. The combined therapy regimen yielded significantly decreased CC-16 levels and increased serum SOD levels compared to the individual treatment groups. Serum TBARS levels did not significantly differ between the BCT + MP + Q group and the other treatment groups. Compared to the BCT + MP + Q group, the BCT + MP group showed significantly lower alveolar edema (AED) and alveolar exudate (AEX) scores, while the BCT + Q group showed significantly lower peribronchial inflammatory cell infiltration (PICI) and AED scores.

Conclusions: The combined usage of quercetin and low dose MP treatment after initial high dose MP at the early stage of lung injury after BCT is more effective.

Keywords: Blunt; chest; trauma; quercetin; steroid

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Introduction

Traumas injury are the leading cause of death for young people (<45 years) and the fourth leading cause of death in western countries (1). Blunt chest trauma (BCT), responsible for 25% of trauma-related deaths, is seen in patients with approximately 10% (1-3). The most common type of injury in BCT, which is often associated with other systemic injuries, is pulmonary contusion; its incidence varies between 17% and 25% (3,4).

BCT-induced lung injury depends on trauma severity and alveolocapillar membrane damage (4,5). A better understanding of the pathophysiological mechanisms and cellular events in lung contusion can help in determining

the prognosis and treatment methods for this important clinical condition (4). Therefore, many studies have been performed to clarify the pathophysiological mechanisms, cellular changes, and inflammatory processes occuring in lung consiont (4-8).

Imbalance between defense mechanisms involving antioxidants such as superoxide dismutase (SOD), catalase, and glutathione peroxidase with free radical formation and increased levels of oxygen metabolites during inflammatory processes play an important role in lung damage. In other words, oxidative stress is an important pathophysiological event leading to tissue damage (9,10). The end product of lipid peroxidation, thiobarbituric acid reactive substances (TBARS), is used as a common marker of oxidative stress (11). Several studies have indicated that a significant relationship exists between TBARS levels and the extent of tissue damage (12,13). Moreover, Clara Cell Protein-16 (CC-16) is also a marker of acute lung injury (ALI). This protein which is secreted from the tracheobronchial tree and particularly from the terminal bronchioles plays role as an anti-inflammatory effect on lung tissue after inflammatuar process (10).

In these previous studies, effective anti-inflammatory agents preventing and/or reducing inflammation in lung tissues and medicines with antioxidant and histological healing effects on damaged tissues were used for the treatment of ALI. Steroids are known as the strongest anti-inflammatory agents; they function via intracellular receptors by affecting prostaglandin synthesis (14). In particular, administration of high doses of steroids in the early stage of lung injury was found to be effective (15). Another therapeutic agent with anti-inflammatory and antioxidant properties is quercetin (Q). Several studies have shown that Q prevents tissue damage because of its specific effect on the pathophysiological processes induced by free radical formation (16-18).

The purpose of this study is to determine the effects of the antioxidant and anti-inflammatory properties of Q and methylprednisolone (MP) on damaged lung tissue in a lung contusion model of rats and evaluate whether their combined use improves treatment efficacy.

Materials and methods

Experimental protocol

This study was approved by the Ethics Committee of Ondokuz Mayis University for Experimental Animal Studies. A total of 35 healthy female Sprague Dawley rats weighing 250-300 g were included. Food and tap water were provided ad libitum. All rats were kept in windowless animal quarters with temperature automatically maintained at 24 ℃ and controlled lighting (12 h light/12 h dark cycle) and humidity (55-60%).

The rats were divided into five groups as follows: Sham (control); BCT; BCT + MP, BCT group treated with i.p. MP (20 mg Prednol-L; Mustafa Nevzat, Turkey); BCT + Q, BCT group that received oral gavage administration of Q (Sigma Chemical Co., St. Louis, MO, USA); and BCT + MP + Q, BCT group treated with MP and Q. Each group had seven animals.

The rats in the BCT groups were anesthetized with ketamine hydrochloride (100 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) and subjected to chest trauma with 1.96 J of impact energy as described by Raghavendran K. *et al.* (4). The impact energy (*E*) of the falling weight was calculated from the following equation: $E = m\ (0.4\ kg) \times g\ (9.8\ m/s^2) \times h\ (50\ cm)$ (19). The rats in the BCT + MP group were injected with MP i.p. once a day (30 mg/kg injected 5 min after the trauma on day 1 and 3 mg/kg from days 2 to 7) (15). The rats in the BCT + Q group were administered Q once a day orally (50 mg/kg administered 5 min after the trauma on day 1 and 50 mg/kg from days 2 to 7) (20). The rats in the BCT + MP + Q group were administered the same doses of MP and Q for the same duration. All rats were kept under observation until they recovered from the experimental procedure.

After seven days of treatment, all rats were killed with i.p. ketamine hydrochloride and xylazine injections. Their lungs were removed from the thorax for histopathological and immunohistochemical analyses.

Histopathological evaluation

Lung tissue samples were immersed in neutral-buffered 10% formalin solution for 24 h and then embedded in paraffin. For histopathological analysis, 5-μm-thick tissue sections were stained with the hematoxylin-eosin (HE) stain. Pathologists analyzing the samples were blinded, and the samples from all study groups were examined microscopically to determine the extent of peribronchial inflammatory cell infiltration (PICI), alveolar septal infiltration (ASI), alveolar edema (AED), alveolar exudate (AEX), alveolar histiocytes (AHI), and interstitial fibrosis (IF) formation using a 4-point scale (*Table 1*) (21).

Table 1 All parametres for histopathologic evaluation (4 point scale)

	0	1	2	3
PICI	No	Prominent germinal centers of lymphoid follicules	Infiltration between lymphoid follicules	Confluent bandlike form
ASI	No	Minimal	Moderate	Severe, impending of lumen
AED	No	Focal	In multiple alveoli	Widespread, involving lobules
AEX	No	Focal	In multiple alveoli	Prominent, widespread
AHI	No	Scattered ina few alveoli	Forming clusters in alveolar spaces	Filling the alveolar spaces
IF	No	Focal, minimal	Focal, prominent fibrous thickening	Widespread, prominent fibrous thickening

Abbreviations: PICI, peribronchial inflammatory cell infiltration; ASI, alveolar septal infiltration; AED, alveolar edema; AEX, alveolar exudate; AHI, alveolar histiocytes; IF, interstitial fibrosis.

Biochemical analysis

Sample preparation

After the rats were sacrificed, blood samples were collected in sterile test tubes. Whole blood was allowed to clot at room temperature for 30 min. Then, the samples were centrifuged at 3,000 ×*g* for ten minutes at 4 ℃. Following centrifugation, the serum was removed and transfered into a clean tube. All samples were stored at –80 ℃ until analysis. A day before the study, all samples were dissolved at 2-8 ℃.

Measurement of serum CC-16 levels

CC-16 levels were determined using a sandwich ELISA plate as per the manufacturer's instructions (Uscn Life Science Inc., Wuhan, China). The plate was pre-coated with an antibody specific to CC-16. Then, samples with a biotin-conjugated antibody specific to CC-16 were added to the wells. Next, avidin-conjugated to horseradish peroxidase was added to the wells and the plate was incubated. After the TMB substrate solution was added, the enzyme-substrate reaction was terminated by adding sulphuric acid solution, and the CC-16 levels were determined colorimetrically at 450 nm. The concentration of CC-16 concentration was then determined from the standard curve, and the results have been presented in picograms per milliliter.

Measurement of serum TBARS levels

TBARS were measured using the TBARS Assay Kit (Catalog No. 10009055, Cayman Chemical Company, Ann Arbor, MI, USA). The principle of the test is the formation of the Malondialdehyde-Thiobarbituric acid (MDA-TBA) adduct by the reaction of MDA and TBA under high temperature (90-100 ℃) and acidic conditions. The concentration of the MDA-TBA adduct was measured colorimetrically at 530 and 540 nm. The results have been presented in micromoles per liter.

Measurement of serum SOD levels

SOD levels were measured using the Superoxide Dismutase Assay Kit (Catalog No. 706002, Cayman Chemical Company, Ann Arbor, MI, USA). The principle of the test is based on the utilization of a tetrazolium salt for the detection of superoxide radicals generated by xanthine oxidase and hypoxanthine. One unit of SOD is defined as the amount of enzyme needed to cause 50% dismutation of superoxide radicals. The results have been presented in units per milliliter.

Statistical analysis

Biochemical results and histopathological scores were analyzed using IBM SPSS 21.0 for Windows. The results were presented as median (minimum/maximum) or mean ± standard deviation. All values were evaluated with the non-parametric Mann-Whitney *U* test. Differences were considered significant at $P<0.05$.

Results

Biochemical examination

Increased serum CC-16 and TBARS levels were found in the BCT group compared to the Sham group (P=0.001 and P=0.001, respectively) (*Table 2*). However, serum SOD levels were decreased in the BCT group compared to the Sham group (P=0.001). With MP and Q treatment, serum CC-16 levels were decreased in the BCT group compared to the Sham group, but only the BCT + MP group showed a significant difference ($P<0.05$). Serum TBARS levels were significantly lower in the BCT + MP and BCT + Q group than the BCT group (P=0.001 and P=0.001, respectively). However, serum SOD levels were not significantly difference

Table 2 The comparison of serum troponin I and TNF-α levels in all experimental groups

	Sham	BCT	BCT + MP	BCT + Q	BCT + MP + Q
CC-16 (pg/mL)	24.86±2.75	72.79±16.29†	50.98±14.00‡	58.08±11.70	29.97±6.30$^{\gamma,\#,\beta}$
SOD (U/mL)	1.28±0.06	1.02±0.13†	1.11±0.05	1.12±0.05	1.24±0.07$^{\&,\#}$
TBARS (μmol/mL)	6.72±1.76	19.11±1.71†	12.61±1.61‡‡	11.49±2.46§	9.77±5.33$^{\&}$

The values were represented as mean ± SD. Sham, control; BCT, blunt chest trauma; BCT + MP, BCT group treated with methylprednisolone; BCT + Q, BCT group treated with quercetin; BCT + MP + Q, BCT group treated with methylprednisolone and quercetin; CC-16, Clara Cell Protein-16; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances; MP, methylprednisolone; Q, quercetin. †, P=0.001 compared to Sham group; ‡, P<0.05 compared to BCT group; ‡‡, P=0.001 compared to BCT group; §, P=0.001 compared to BCT group; $^{\gamma}$, P=0.001 compared to BCT group; $^{\&}$, P<0.01 compared to BCT group; $^{\#}$, P<0.01 compared to BCT + MP group; $^{\beta}$, P=0.001 compared to BCT + Q group.

between these treatment groups (BCT + MP and BCT + Q) (*Table 2*). In the BCT + MP + Q group, serum CC-16 and TBARS levels were decreased compared to the BCT group (P=0.001 and P<0.01, respectively). On the other hand, serum SOD levels were the highest in the BCT + MP + Q group among the BCT groups (P<0.01) (*Table 2*). The combined therapy regimen significantly decreased CC-16 levels compared to the individual treatments with MP and Q (P<0.01 and P=0.001, respectively). Similarly, combined MP and Q treatment significantly increased the serum SOD levels compared to the other two treatment groups (P<0.01 and P<0.05, respectively) (*Table 2*). Serum TBARS levels were not significantly different in the BCT + MP + Q group compared to the other treatment groups (*Table 2*).

Histopathological findings

Images of histopathological sections from all study groups are shown in *Figure 1*. All histopathological scores including PICI, ASI, AED, AEX, AHI, and IF were higher in the BCT group than in the Sham group (P<0.01, P=0.001, P=0.001, P<0.01, P<0.01, and P=0.001, respectively) (*Figures 1,2*).

The MP caused a significant decrease in the PICI, ASI, AED, and IF scores compared to the BCT group (P<0.05, P<0.05, P=0.001, and P=0.001, respectively) (*Figures 1,2*). However, Q treatment only caused a significant decrease in the AED and IF scores in BCT + Q group compared to the BCT group (P=0.001 and P<0.01, respectively) (*Figure 2*). On the other hand, combined MP and Q combined treatment caused a significant decrease in the scores of all the above histopathological parameters (*Figure 2*).

Compared to all treatment groups; the combined treatment group (BCT + MP + Q) showed significantly lower AED and AEX scores compared to the BCT+MP group (P<0.05 and P<0.05, respectively) (*Figure 2*). In addition, PICI and AED scores in combined therapy group showed significantly lower than the BCT + Q group (P<0.05 and P<0.05, respectively). There was no significant difference in the histopathological scores between the BCT + MP and BCT + Q groups (*Figure 2*).

Discussion

In this study, the effects of the antioxidant and anti-inflammatory properties of Q and MP on damaged lung tissue in a lung contusion model of rats were and evaluated. Combined MP and Q treatment significantly reduced AED and AEX formation, which is particularly important in the progression of ALI to acute respiratory distress syndrome (ARDS) according to alone usage of MP. In addition, this combined therapy significantly reduced PICI and AED, which is regarded as an important marker of inflammatory reaction according to alone treatment of Q. Apart from its histopathological effectiveness, the ability of combined therapy to reduce serum CC-16 and to increase SOD levels are considered particularly effective in alleviating lung injury according to the other treatment groups.

ALI developing after BCT is among the most important factors that determine prognosis. This injury occurs in about 50% to 60% of patients with BCT (22). Today, despite the widespread availability of intensive care facilities and advanced respiratory devices, the BCT-related mortality rate is 15.5%, and BCT is the third most common cause of death among young people (23,24).

Lung contusion developing after BCT is a clinical condition accompanied by histopathological findings such as alveolar congestion, alveolar hemorrhage, AED, leukocyte infiltration and ventilation-perfusion defects

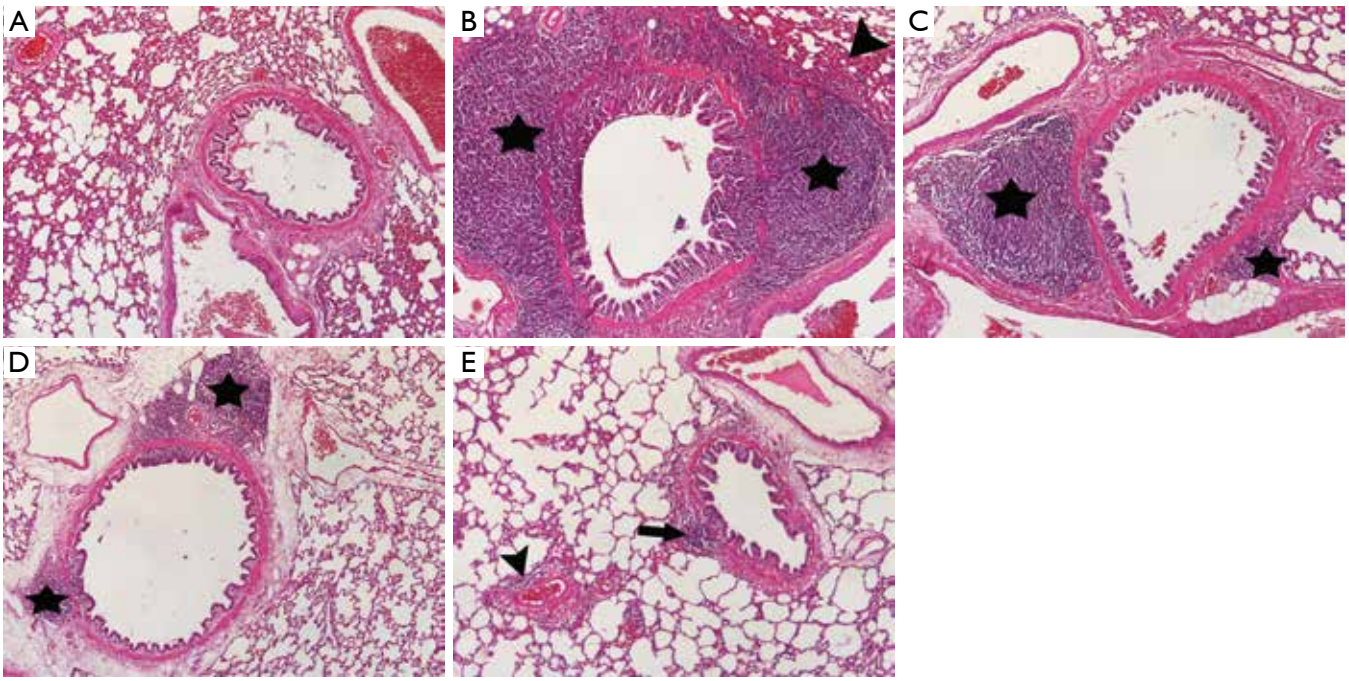

Figure 1 Histopathological evaluation of lung tissues. (A) Sham group, there is no peribronchial inflammatory cell infiltration and alveoler septal infiltration HE ×4; (B) BCT group, severe peribronchial inflamatory cell infiltration showing infiltration between lymphoid follicules, (stars) and severe alveolar septal infiltration collapsing the lumen of the alveols (arrow head) HE ×4; (C) BCT + Q group, there is weak germinal centres of lymphoid follicules (stars) HE ×4; (D) BCT + MP group, moderate peribronchial inflammatory cell infiltration (stars) HE ×4; (E) BCT + MP + Q group, weak peribronchial inflammatory cell infiltration (star) and weak mononuclear cell infiltration in the perivasculer area (arrow head). Sham, control; BCT, blunt chest trauma; BCT + MP, BCT group treated with methylprednisolone; BCT + Q, BCT group treated with quercetin; BCT + MP + Q, BCT group treated with MP and Q.

caused by pathological changes, hypoxemia, hypercapnia caused by intrapulmonary shunt development and reduction in compliance, and increased respiratory workload with variable weight and duration (25,26). ARDS occurs because of the disruption of endothelial and epithelial cell functions as a result of lung injury developing after BCT. The most significant histopathological event in the lung tissues of ARDS patients is noncardiogenic high-permeable pulmonary edema (27). Alveolocapillar damage, occurring with fibrosis following structural disorder of type 2 alveolar pnomosit and increase in myofibroblasts after edema in the lungs, leads to ARDS (28). In our study, similar to the histopathological findings mentioned in the literature, there was a significant increase in the PICI, ASI, AED, AEX, AHI, and IF scores after BCT. We also found that MP and Q combination therapy was more effective than MP or Q alone in reducing AED, which is accepted as a trigger in the process leading to ARDS after lung contusion.

The underlying molecular and physiological changes in lung injury developing after BCT have still not been clearly elucidated. However, bacterial toxins often increase free oxygen radical release by activating inflammatory cells, proteases, and microembolies, thus leading to expansion of lung pores and increased permeability. Further, apoptosis causes pulmonary edema, which is accepted as the basic mechanism underlying ALI (27,29). Under normal physiological conditions, there is a balance between free oxygen radicals, which are effective in pathophysiology, and the antioxidant enzyme system (SOD, catalase, GSH-Px, glutathione, etc.). An imbalance between oxidants and antioxidants causes DNA and RNA damage due to oxidation of structural lipids, proteins, and polysaccharides at the cellular level (9). Increased TBARS (end product of lipid peroxidation) levels in the damaged tissue is recognized as a marker of oxidative damage (12,13). An experimental study performed by Mokra *et al.* showed that serum TBARS levels correlated with the severity of lung injury and increased significantly in lung injury occurring after meconium aspiration (30). Further, Goraca *et al.* demostrated that TBARS levels in the BAL fluid increased after oxidative

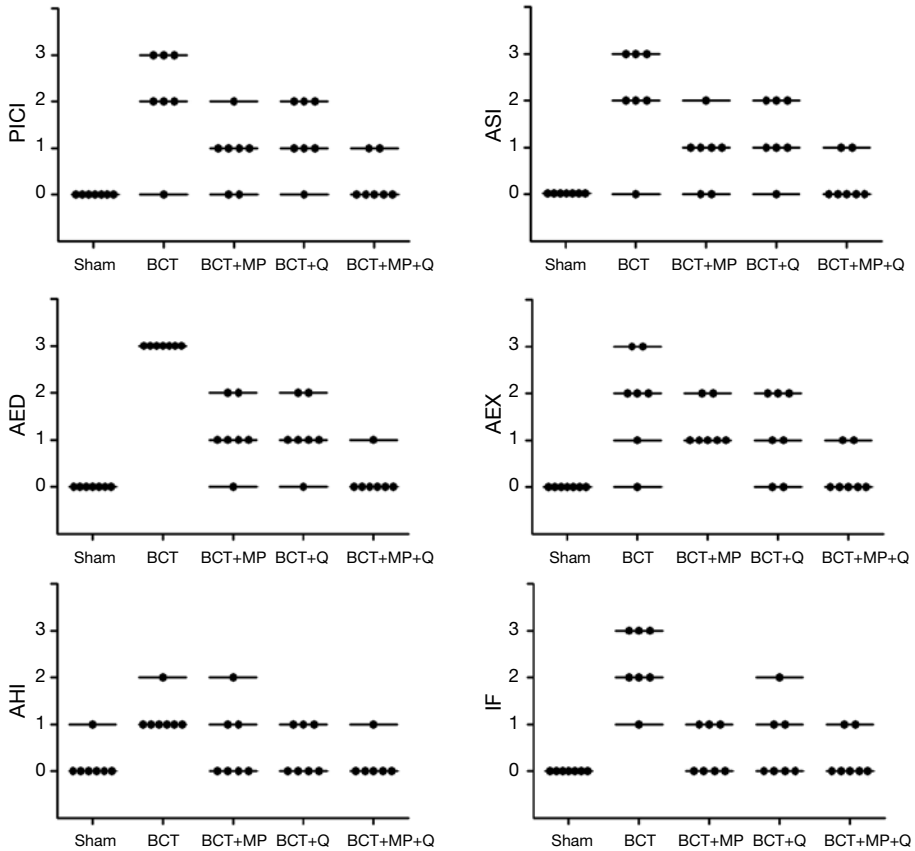

Figure 2 All histopathological scores in study groups were represented. PICI, peribronchial inflammatory cell infiltration; ASI, alveolar septal infiltration; AED, alveolar edema; AEX, alveolar exudate; AHI, alveolar histiocytes; IF, interstitial fibrosis; Sham, control; BCT, blunt chest trauma; BCT + MP, BCT group treated with ethylprednisolone; BCT + Q, BCT group treated with quercetin; BCT + MP + Q, BCT group treated with methylprednisolone and quercetin.

stress and decreased after treatment with alpha-lipoic acid in one of their studies (31). In our study, we measured serum TBARS and SOD levels for determining the oxidant-antioxidant balance. Significant decrease was found in the level of serum SOD after BCT, while there was an increase in serum TBARS levels. We found that while MP and Q therapies when used alone did not cause significant changes in serum SOD level, they only caused significant increase in TBARS levels. We also found that the combined use of MP and Q was the most effective treatment for maintaining the balance of oxidants and antioxidants. This result demonstrated that the combined therapy was significantly effective in reducing oxidative damage and particularly effective in maintaining the oxidant-antioxidant balance.

CC-16 is a protein secreted from tracheobronchial tree and particularly from the terminal bronchioles where the Clara cells reside and plays a protective role against oxidative stress and inflammatory response in the respiratory tract (10,32). It is considered as a biomarker to determine the severity of lung injury (10,33). In a study performed by Wutzler *et al.*, it has been reported that there was a significant correlation between the level of serum CC-16 and increased respiratory complications after chest trauma (33). In our study, in accordance with histopathological findings, significant increase in serum CC-16 levels were detected after contusion. Moreover, it was determined that the combined therapy of MP and Q decreased serum CC-16 level significantly compared to the other treatment groups.

The pathophysiological mechanism of lung contusion has not been clearly elucidated. Symptomatic and supportive approach is usually considered in the treatment of BCT. These treatment strategies are respiratory failure treatment, post-traumatic pain management, chest physiotherapy, and fluid support (34). In addition to these treatment strategies, medications such as antioxidants, steroids diuretics, and surfactants are used in reducing lung injury (3,5,34-36). The

beneficial effect of steroid administration in lung injury has been found to reduce proinflammatory cytokine production, expression of leukocyte adhesion proteins, and prevention of excessive collagen accumulation in alveolar tissues because of antifibrotic activity (27). Some studies have reported that early administration of high-dose steroids after BCT significantly reduced lung injury and in-hospital mortality; the incidence of post-traumatic complications was also reduced in patients treated with steroids (37,38). Glucocorticoids and antioxidants were used together for treating lung and heart lesions in a variety of experimental studies (39,40).

In conclusion, high-dose MP therapy should be administered in the early stage of lung injury developed after BCT. The initial high-dose MP therapy should be maintained on lower doses. In addition, we suggest that the use of combined quercetin and this MP treatment regimen is more effective for treating lung injury after BCT.

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Original Article

Prolonged pleural catheters in the management of pleural effusions due to breast cancer

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Background: Breast cancer is the second most common etiologic cause in malignant pleural effusions (MPE). The aim of this study was to investigate the efficacy of long term pleural catheters in inducing self sclerosis in pleural effusions of breast cancer patients.

Methods: In this study, 26 patients with breast cancer relapleural effusions that occurred between January 2011 and July 2013, who were considered not to undergo any other treatments and managed with prolonged pleural catheters (Jackson-Pratt silicone flat drain), were retrospectively analyzed. Thirty pleural catheters were inserted in 26 patients. All patients were female, mean age was 52 (range, 37-66) years old. Drainage over 1,500 mL per day was not allowed in order to avoid a lung edema. The catheters were removed in patients who had restoration of lung expansion and drainage under 50 mL/day.

Results: The histologic subtypes in pleural effusions were invasive ductal carcinoma in 18 patients, ductal carcinoma *in situ* in 4, invasive lobular carcinoma in 2, tubular carcinoma in 1, and medullary carcinoma in 1. Three of the 26 patients underwent bilateral catheter insertion, and one patient underwent a reinsertion of the catheter into the same hemithorax due to a recurrence. The catheters were retained for a mean period of 18 days (range, 11-38 days). In one patient with invasive ductal carcinoma and paramalignant pleural effusion (PMPE) (3.8%), a recurrent pleural effusion was seen 34 days after removal of the catheter. There were no complications. One patient died while the catheter was in place.

Conclusions: Prolonged catheters for the management of pleural effusions in selected patients have become more popular than other treatment alternatives due to a shorter length of stay and lower costs. We recommend the use of Jackson Pratt (JP) silicone flat drains which in our opinion provide effective pleurodesis in addition to easy application in recurrent effusions caused by breast cancer.

Keywords: Breast cancer; pleural effusion; prolonged pleural catheter; pleurodesis

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Introduction

Malignancy related effusions are classified as malignant pleural effusions (MPE) and paramalignant pleural effusions (PMPE). MPE is a sign of an advanced or generalized tumor stage (1). MPE is identified based on the proof of malignant cells in the pleural fluid or tissue, and it is detected in most of the patients with malignant tumors (approximately 50%) (1,2). Effusions in which there is a malignancy but no direct infiltration of the pleura are named PMPE (3). Almost all malignant tumors in advanced stage may affect the pleura, and may thereby result in pleural effusion or pleural carcinosis (4). The second most common etiologic cause of MPE after lung cancer is breast cancer (5-7). Moreover, there is a close relationship between breast cancer and MPE, 2-11% of the patients with breast cancer develop MPE during the course of the disease (8,9). Approximately 80% of the patients with pleural recurrences develop MPE

within five years of primary breast cancer surgery, whereas MPE is rare beyond ten years after the surgery (10,11).

The clinical presentations of pleural effusions due to breast cancer are similar to that of other other pleural effusions; they are characterized with progressive dyspnea, cough, and pleuritic type chest pain. In addition, a poor general condition and symptoms of breast cancer may be present. The clinical presentation is related to the size (width) of the effusion, time to development, and the physical condition of the patient (4). Physical examination may show decreased lung sounds and dullness on percussion.

The most common and easiest method for investigating the presence of a pleural effusion in breast cancer patients is a chest X-ray (posteroanterior and lateral). Pleural ultrasonography (USG) may be used for the diagnosis of smaller quantities. USG is also helpful in determining the site of thoracentesis, especially when there is a small amount of collection (4).

The first step after detection of pleural fluid collection is pleural drainage performed by diagnostic and therapeutic thoracentesis (12). However in pleural effusions following breast cancer, the pleural fluid often reaccumulates after simple aspiration (10). Reaccumulation of pleural effusion ipsilateral or contralateral to the site of previous drainage is named recurrent pleural effusion. MPEs due to breast cancer also develop recurrent pleural effusions (10). There are numerous management alternatives in recurrent MPE and PMPE, pleurodesis is the most commonly used. It can be often applied with pleural catheter, tube thoracostomy, and videothoracoscopy (13,14). Some studies have advocated the use of permanent pleural drainage catheters due their easy application and use; the method has advantages compared to repeated thoracenteses or pleurodeses, such as successful alleviation of symptoms, decreased mortality, lower costs, and decreased length of stay (7,15-17).

In our study, we evaluated 26 patients with recurrent pleural effusions caused by breast cancer who underwent pleural drainage with Jackson Pratt (JP) silicone drains® because of the easy application and also lower costs of these drains.

Methods

In this study 26 patients who developed breast cancer related pleural effusions between January 2011 and July 2013, in whom no further treatments were considered and who accepted the JP drain treatment after being informed on other treatment alternatives were retrospectively analyzed. The patients included into the study had symptomatic or asymptomatic pleural effusions that did not respond to treatment or that recurred, and therefore received the indication for drainage. Patients diagnosed with pleural effusions underwent insertion of 10 F JP® catheters which could be applied in a tunneled manner easily under local anesthesia.

The patients were referred to our unit mostly from the medical oncology departments after detection of pleural effusion with radiologic studies including posteroanterior chest X-ray, chest tomography, ultrasound, and also laboratory studies.

The insertion sites for the prolonged pleural catheters (JP® Drain) were determined with thoracic ultrasound prior to the procedure in order to find a position for easy access. The catheters were inserted from the lateral chest wall in all patients. Prior to the procedure, complete blood counts, bleeding and coagulation times, a PTT, and PT measurements were carried out. When the insertion sites could be determined by posteroanterior chest X-rays or chest CTs the catheters were inserted directly, in the remaining patients, especially in the presence of loculated effusions the insertion site was marked with a thoracic USG (*Figures 1*,*2*). Mean procedure time for catheter insertion was 15-20 minutes. The location of the catheter inside the thoracic cavity was confirmed with a postoperative chest X-ray (*Figure 3*). Daily drainage was restricted to 1,500 mL to avoid lung edema, drainage was also stopped when an abnormal cough disturbing to the patient began. Ambulatory patients in good general condition were discharged either home or to the referring unit after receiving instructions on the use of catheters. The catheters were removed once drainage decreased to less 50 mL/day and lung expansion was achieved.

The patients were evaluated with respect to age, sex, breast cancer cell type, and the period the catheter remained in place.

Results

Thirty prolonged pleural catheters were inserted in 26 patients with MPE and PMPE due to breast cancer. All patients were female, mean age was 52. Twelve of the patients were diagnosed as MPE due to presence of tumor cells in the aspirate, the remaining 14 patients were diagnosed as PMPE due to their absence.

The most common histologic subtype of breast cancer

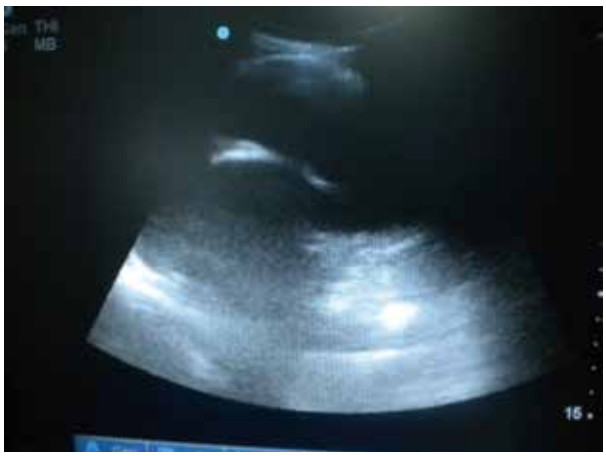

Figure 1 Ultrasonographic appearance of the pleural effusion.

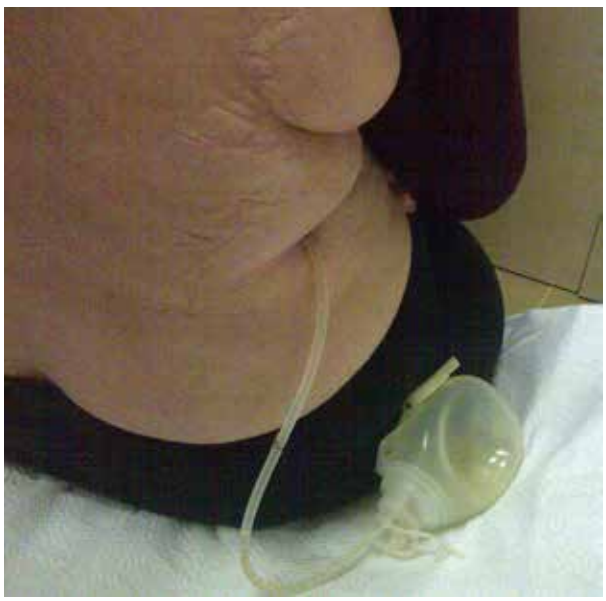

Figure 2 Appearance of the Jackson Pratt (JP) catheter after the procedure.

was invasive ductal carcinoma, others were ductal carcinoma *in situ*, invasive lobular carcinoma, tubular carcinoma, and medullary carcinoma. The distribution of breast cancer according to cell type is shown on *Table 1*. Three patients underwent bilateral catheter insertion due to bilateral effusion. One patient developed a recurrent ipsilateral effusion 34 days after removal of the initial catheter (*Table 1*), and a new JP drain was inserted on the same side. This patient with recurrent effusion had invasive ductal carcinoma, and was diagnosed as PMPE.

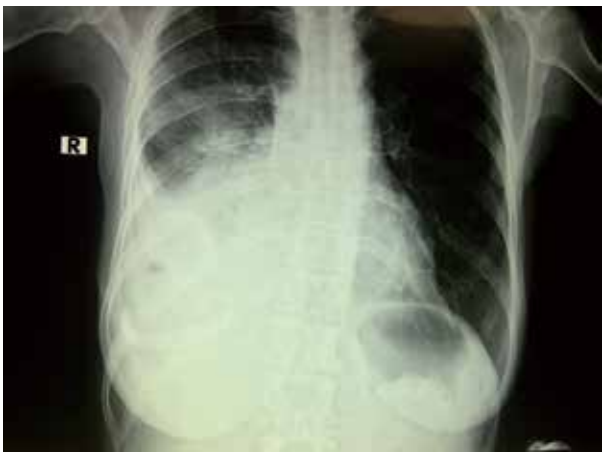

Figure 3 Postoperative control radiograph of the Jackson Pratt (JP) drain.

Table 1 Characteristics of the patients

		n [%]
Age (years)	52 [37-66]	26 [100.0]
Sex	F	26 [100.0]
ER/PR (+)		20 [77.0]
HER2 (+)		7 [26.0]
Triple (–)		4 [15.0]
The distribution of breast cancer related pleural effusions	MPE	12 [46.1]
	PMPE	14 [53.8]
Duration of the catheter (days)	10-15	7 [23.3]
	15-20	15 [50.0]
	20-30	6 [20.0]
	Over 30	2 [6.6]
Histopathology of pleural fluid	Invasive ductal carcinoma	18 [69.0]
	Ductal carcinoma *in situ*	4 [15.3]
	Invasive lobuler carcinoma	2 [7.6]
	Tubular carcinoma	1 [3.8]
	Medullary carcinoma	1 [3.8]
Number of recurrent effusions		1 [3.8]
Complications		0 [0]

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; HER2, human epitelial growth receptor 2; F, female; MPE, malignant pleural effusion; PMPE, paramalignant pleural effusion.

One of the patients having invasive ductal carcinoma died while the catheter was in place. There were no preoperative or postoperative complications related to the procedure (*Table 1*).

Discussion

MPEs are associated with a poor prognosis. Median survival in breast cancer patients is 5 to 13 months after pleural fluid accumulation (7,10,18). Many patients with malignant effusions experience dyspnea, additional symptoms include weight loss, anorexia, malaise and fatigue, which negatively affect the quality of life. Therefore, management of MPEs is important to improve the quality of life of patients, inadequate management results in deterioration in respiratory function that can shorten the expected survival time.

Therapeutic thoracentesis is the initial approach for patients with respiratory symptoms including dyspnea. However, accumulation of pleural fluid usually recurs after a simple aspiration (10). There is currently no standard treatment approach in recurrent pleural effusions. Options include pleurodesis, pleuroperitoneal shunt, repeated thoracenteses, and pleural catheters.

Pleurodesis requires a chest tube and videothoracoscopic methods which prolong the length of stay in the hospital and increase the costs. Pleural catheters on the other hand cost less, require shorter hospitalizations, along with pleurodesis success rates reaching as high as 81% especially in MPE (19).

A pleuroperitoneal shunt can be used in recurrent pleural effusions, however a disadvantage with respect to practicality is requirement that the patient needs to use the pump, and approximately 1-2 cc liquid is transferred to the peritoneal cavity with each use of the pump (20).

The British Thoracic Society (BTS) report concluded that PPC was an effective alternative in the control of malignant effusions, minimized the length of stay and enabled avoidance of hospital admission (21). Davies *et al.* found that both talc pleurodesis and indwelling pleural catheters were effective treatments for relieving dyspnea and improving patients' quality of life, but indwelling catheter were nor superior to talc pleurodesis for these outcomes (22). We preferred the PPCs due to their easy availability, lower costs, lower likelihood of obstruction provided by the constant negative pressure, and practical use by the patients.

Some studies have advocated the use of prolonged pleural catheters in especially MPE. The most commonly used tunneled pleural catheter is Pleurx (Denver Biomaterials, Golden CO, USA), which is a 15.5 Fr diameter silicone tube that measures 24 inches long, has a perforated distal edge, and bears a valve at the proximal edge for drainage (7,16,19,23). It has been shown that the use of this catheter lowered the length of stay and costs (16,19).

Other simple and smaller diameter catheters may be also used in recurrent pleural effusions, however they have disadvantages such as a higher risk of extrusion compared to tunneled catheters and also inappropriateness for long term use. As a result of shorter stay in the body, they may have lower infection rates, which was measured to be around 5% in a previous study (19). There were no infections in our study.

The waiting period in the hospital is less for patients undergoing prolonged catheters, therefore all patients in this study were discharged on the same day without any complications. A previous study reported a 9.9% recurrence rate on the ipsilateral side (7). One of our patients (1/26, 3.8%) developed an ipsilateral collection one month after removal of the catheter.

Prolonged pleural catheters are suggested as the first line treatment in patients with trapped lung. The lung cannot expand in these patients and pleurodesis fails to prevent the recurrent pleural effusion. A decortication procedure will be a major invasive procedure, therefore such patients are suggested to undergo a prolonged pleural catheter or pleuroperitoneal shunt (20,24). None of the patients in our study had trapped lung syndrome.

An advantage of breast cancer compared to other malignancies is the efficacy of systemic treatment (cytotoxic and endocrine management) in decreasing the pleural fluid and treating dyspnea (25). All of our patients had received systemic treatment before and after catheter insertion. We believe that in our patients systemic treatment contributed to the high self sclerosis success rates provided by the catheter itself, and self sclerosis reached 96.2%.

Prolonged pleural catheter is a reliable and effective treatment method in recurrent pleural effusions (16,21). Self sclerosis rates as high as 81% were reported, with higher rates in gynecologic malignant effusions (19,26).

Our study has some limitations. First, it is a retrospective study and the sample size is small. Also, we did not perform a comparison with other alternatives regarding costs and length of stay.

The use of prolonged pleural catheters has become a widely accepted treatment recently for the management of especially MPE. We believe that in addition to its easy usage and success in providing effective pleurodesis, the JP® drain is effective in providing self sclerosis in breast cancer related pleural effusions.

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Original Article

A study of weekly docetaxel and carboplatin as first-line chemotherapy for advanced non-small cell lung cancer

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Background: Weekly docetaxel demonstrated similar efficacy but better tolerability than standard triweekly docetaxel, and carboplatin was less nephrotoxic, neurotoxic and emetogenic than cisplatin. This study aimed to evaluate the efficacy and safety of weekly docetaxel with carboplatin as first-line chemotherapy for advanced non-small cell lung cancer (NSCLC).

Methods: Forty-three Chinese patients have been included. Patients were administered docetaxel at a dose of 35 mg/m^2 on days 1, 8, 15 and carboplatin at an area under the curve (AUC) 5 on day 1 every 28-day cycle (maximum six cycles).

Results: Of the 43 eligible patients, the assessed overall response rate (RR) was 30.2% with 30.2% partial response (PR) in 13 patients, 48.8% stable disease (SD) in 21 patients and 20.9% progressive disease (PD) in 9 patients. The estimated median progression free survival and median overall survival (OS) time were respectively, 120 days (95% CI: 80-160 days) and 340 days (95% CI: 224-456 days) with the patients surviving of 46.5% (95% CI: 31.6-61.4%) at one year and 20.0% (95% CI: 7.1-33.3%) at two years. The major grade 3/4 hematological toxicities were included leucocytopenia in 6 patients (13.9%) and neutropenia in 8 patients (18.6%). One patient (2.3%) suffered grade 1 febrile neutropenia. All grade of the nonhematological toxicities, such as nausea, vomiting, alopecia and fatigue held the proportion of 48.8% (grade 3/4 4.6%), 27.9%, 55.8% and 53.5% (grade 3/4 9.3%), respectively.

Conclusions: The combination of weekly docetaxel and carboplatin showed feasible efficacy with acceptable hematologic toxicities for advanced lung cancer.

Keywords: Chemotherapy; docetaxel; carboplatin; non-small cell lung cancer (NSCLC)

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Introduction

Throughout the world, most of the cancer-related deaths are resulted from lung cancer, among which non-small cell lung cancer (NSCLC) holds a constitution of approximately 80% of all cases (1-3). And locally advanced inoperable (stage IIIB) or metastatic (stage IV) disease influences almost 70% of the patients with NSCLC (4). The combinations of platinum and some third-generation new drugs, such as taxanes, gemcitabine and vinorelbine have been regarded as the standard treatment for advanced NSCLC (5), which provides a better survival advantage over the best supportive care alone in patients with a good Eastern Cooperative Oncology Group (ECOG) performance status (PS) (6-10).

The combination of docetaxel with cisplatin, exerts a significant effect on NSCLC, with response rate (RR) ranging from 35% to 45%, and overall median survival reaching 12 months (9). However, the clinical use of

Table 1 Clinical characteristics of 43 baseline patients with advanced NSCLC

	No.	%
No. included	43	
Male/female	30/13	69.8/30.2
Age		
Median age [years], 60 [29-75]		
<60 years	20	46.5
≥60 years	23	53.5
ECOG performance status		
0	4	9.3
1	33	76.7
2	6	14.0
Stage		
IIIB	13	30.2
IV	30	69.8
Histoogy		
Adenocarcinoma	31	72.1
Squamous	11	25.6
Others	1	2.3
Sites of metastasses		
Lung	13	30.2
Liver	3	7.0
Bone	10	23.3
Nodes	26	60.5
Other	11	25.6
No. of involved sites		
1	24	55.8
2	15	34.9
3	2	4.7
≥4	2	4.7

NSCLC, non-small cell lung cancer.

cisplatin is sometimes hampered by its severe toxicity, such as nephrotoxicity, gastrointestinal toxicity including nausea and vomiting, and neurotoxicity which requires hydration to release renal damage. Carboplatin, a second-generation platinum-containing compound, is much less nephrotoxic, neurotoxic and emetogenic than its parent compound cisplatin. A recent meta-analysis for advanced NSCLC reported that the rate of ≥ grade 3 nausea and vomiting for cisplatin-based therapy was higher than carboplatin-based therapy (11,12). And, although cisplatin-based chemotherapy produced a higher RR, it brought no improvement in survival compared with carboplatin-based therapy. So, carboplatin may be a good alternative choice for NSCLC.

The traditional 3-weekly schedule of docetaxel is 75 mg/m^2 afflicted by severe grade myelotoxicity (13,14), for example 12% to 16% patients were observed experiencing with febrile, which had been a major limitation, particularly in the elder patients, and patients with a poor PS. A weekly docetaxel, been studied as a single agent in second line NSCLC, improved the toxicity profile of the drug, particularly neutropenia and febrile neutropenia, and showed even more significant benefits than 3-weekly regimen (15,16). So, weekly administration of docetaxel seems to be safe and effective.

Carboplatin and docetaxel perform differently in antitumor and most of their toxicities do not overlap, their combination may yield higher efficacy than either alone. Other studies have observed that docetaxel plus carboplatin had RRs of 39% and 43% and tolerable toxicity (9,17). However controversy still exists regarding which schedule and dosage for the combination of docetaxel and carboplatin results in the optimum clinical outcome while minimizing hematologic and nonhematologic toxicities.

Therefore, we designed this phase II trial to evaluate the efficacy, safety of a weekly docetaxel and carboplatin in NSCLC.

Methods

Patients' eligibility (Table 1)

The patients enrolled from 18 to 75 years old, were confirmed cytologically or histologically of stage IIIB or stage IV NSCLC, with a measurable tumor examined clinically and/or radiologically. The patients were also required to have an ECOG PS of 0 to 2 and a life expectancy of more than three months. Laboratory requirements included hemoglobin 9 g/dL, white blood cell count 4,000/mm^3, neutrophils 2,000/mm^3, platelets 100,000/mm^3, total bilirubin 1.5 mg/dL, transaminase 1.5 times the institutional upper limit of the normal value, serum creatinine 1.5 mg/dL, and partial pressure of oxygen in artery (PaO_2) 60 mmHg. Patients were excluded if they were founded have symptomatic brain metastases, active double cancer, or a severe comorbidity, included symptomatic cardiovascular disease, uncontrolled diabetes, pulmonary fibrosis obvious in a chest X-ray, or a sever infectious disease. The study protocol was approved by the Institutional Review Board

at First Affiliated Hospital, College of Medicine, Zhejiang University. Every patient signed a written informed consent before participating in the study

Drug administration and modification

Patients were administered docetaxel intravenously at a dose of 35 mg/m^2 on days 1, 8 and 15 with carboplatin at an area under the curve (AUC) 5 on day 1 every 28-day cycle. The Cockroft-Gault equation was used to evaluate the creatinine clearance. It is necessary that patients were injected antiemetic agents and antagonist before they received the anticancer agents.

The National Cancer Institute Common Toxicity Criteria version 3.0 was regarded as the standard to estimate the toxicities during the administration of antitumor agents. If the neutrophil count and the platelet count were more than 1,000/mm^3 and 75,000/mm^3, respectively, the administration of docetaxel was permitted on days 8 and 15. On the contrary, dose reduction happened to caboplatin to AUC 4 on condition of febrile neutropenia less than 1,000/mm^3, thrombocytopenia less than 20,000/mm^3, or the demand of platelet transfusion. When a major nonhematological toxicity was at least grade 3, dose reduction was required to eliminate anorexia or nausea. If these kinds of toxicities were continued, the dose of docetaxel was to be decreased in the following cycle.

All patients were required to receive at least two cycles of the treatment, excepting the circumstances of their disease progression, unacceptable toxicities, the patients' refusal of further treatment or the physicians' decision of terminating the treatment.

Treatment assessment

patients were asked to accept the following baseline assessment items: a complete medical history and physical examination, complete blood cell counts, hepatic and renal function tests, urinalysis, 12-lead electrocardiograph, and PS. Method of measurement for visible and palpable tumors at baseline included chest X-ray, chest and upper abdominal computed tomography (CT), brain CT or magnetic resonance imaging, a radionuclide bone scan, and other diagnostic procedures were performed as clinically indicated. It is significant to monitor weekly the medical history, physical examination, weight, vital signs, PS, toxicity, complete blood cell counts and blood chemistry. Patients' response to the treatment was assessed by chest and upper abdominal CT every two cycles and sooner, if required, to document disease progression. According to the new Health Organization criteria (REECIST criteria), the unidirectional items were defined as: complete response (CR)—the disappearance of all lesions, partial response (PR)—a decrease of at least 30% in the sum of the longest diameter of the tumor, progressive disease (PD)—an increase of at least 20% of the longest diameter of the tumor or the appearance of any new lesions, and stable disease (SD)—any response other than CR, PR, or PD. The RR was defined as the proportion of the patients who attained CR or PR to the number of enrolled patients. Overall survival (OS) and time to progression (TTP) were assessed from the start of therapy until death or progression, respectively, or until the last follow-up. Toxicities were estimated in accordance with Common Terminology Criteria for Adverse Events (CTCAE) version 3.0.

Study design and statistical analysis of the trial

The primary objective of this study was to evaluate the overall RR, as well as the secondary were TTP, OS, toxicities and safety. The Optimal Simon two-stage phase II design performed as the determination of the sample size and interim analysis was implemented as the first 13 eligible patients had been recruited (11). If more than three responses were observed, 30 patients were to be enrolled, or the study was to be terminated. Withal, when more than 12 responses were to be found in the 43 patients, it was reasonable to regard the regimen as a sufficient and active one with a significance level of 5%. In addition, power of 80% was to be referred for further estimation. The Kaplan-Meier method was considered as the appropriate one to analyze TTP and OS, which were updated to 1 January 2009. On the basis of SPSS (version 10.0), the study undertook a series of statistical computations.

Results

Patient characteristics (Table 1)

Between December 2005 and July 2007, 43 advanced NSCLC Chinese patients were enrolled with 30 males and 13 females. The characteristics of these patients were showed in *Table 1*. Among them, 76.7% patients had PS 1, and 6 patients had PS 2. Their median age was 60 years (range, 29-75 years) and 21% of the patients were aged over 70. In the respect of histology, adenocarcinoma (72.1%)

held a predominant position. Thirteen (30%) patients were at the stage IIIB and other 30 (70%) stage IV.

Tumor response and survival (Table 2) (Figure 1)

In total, 153 chemotherapy cycles were administrated for 43 eligible patients, with a median of 4 cycles per patient (range, 2-6 cycles). Of the 43 eligible patients, the overall RR was 30.2% (95% CI: 17-46%) and the best response to treatment was the PR in 13 patients (30.2%). SD was observed in 21 patients (48.8%) and PD in 9 patients (20.9%).

Survival analysis was implemented at the close-out date (Jan 1, 2009), at which 9 patients (20.9%) were still alive and other 34 patients (79.1%) had died. Thus, the median OS was 340 days (95% CI: 224-456 days). A percent of 46.5 patients survived at one year and 20.0% at two years. Furthermore, as at the close-out date, 2 patients were observed alive without disease progression but 9 patients (20.9%) had progressed. The median progression-free survival time was 120 days (95% CI: 80-160 days), with 9.35% patients (95% CI: 1.76-20.5%) estimated without progression.

Table 2 Best overall response (N=43)

	No.	%
Complete response (CR)	0	0
Partial response (PR)	13	30.2
Objective response rate (RR)	13	30.2
Stable disease (SD)	21	48.8
Progressive disease (PD)	9	20.9
Not assessable (NA)	0	0

Toxicity (Tables 3,4)

There was the evaluation of toxicity for all patients. In total, 16 (37.2%) received 2 cycles, 2 (4.7%) 3 cycles, 15 (34.9%) 4 cycles, 5 (11.6%) 5 cycles and 5 (11.6%) 6 cycles. About the treatment delay, 4 cycles (19%) in 4 patients were more than seven days, of which, 18 cycles for toxicity reasons.

Neutropenia, the most common hematological grade 3/4 adverse event, was observed in 8 patients (18.6%). Leucocytopenia was in 6 patients (13.9%). There was only one (2.3%) patients founded suffering with febrile neutropenia. Additionally, nonhematological toxicities were relatively moderate. All grade of the nonhematological toxicities, nausea, vomiting, alopecia and fatigue held the proportion of 48.8% (grade 3/4 4.6%), 27.9%, 55.8% and 53.5 % (grade 3/4 9.3%), respectively. Of the total 153 cycles, grade 3/4 neutropenia occurred in 20 (13.1%) cycles and grade 3/4 leucocytopenia in 13 cycles (8.5%). Grade 3 nonhematological toxicities were observed in 6 cycles (3.9%).

Discussion

In this phase II study, we investigated the activity and safety of weekly docetaxel plus carboplatin in patients with advanced NSCLC. The results showed that the regimen was effective for advanced NSCLC. Of the 43 eligible patients, we observed an overall objective RR of 30.2% (95% CI: 17-

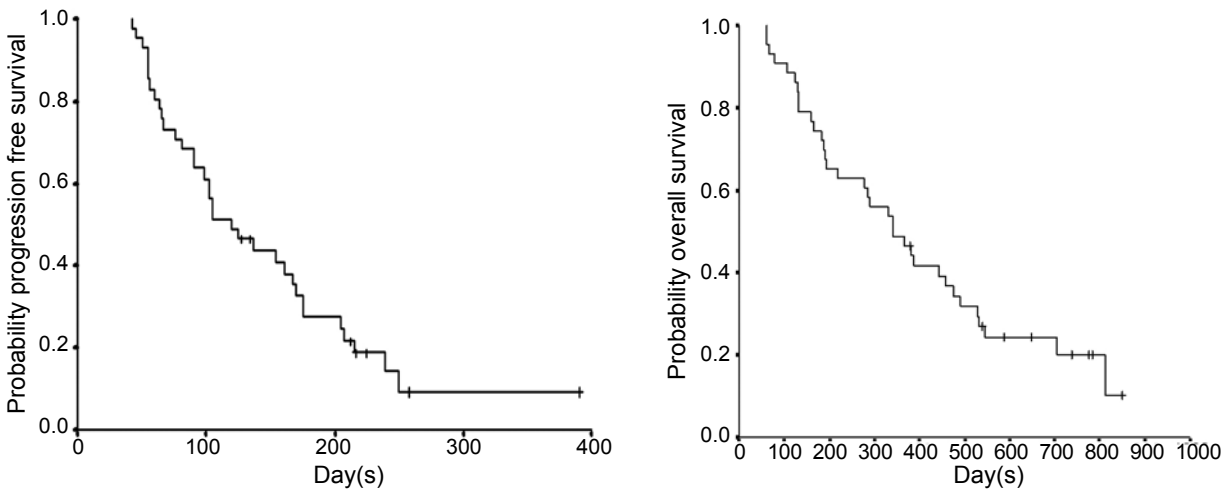

Figure 1 Kaplan-Meier curve for progression free survival and overall survival.

Table 3 Hematological and nonhematological toxicities (by patient)

	NCI-CTC grade (n=43)				
	Grade 1 (%)	Grade 2 (%)	Grade 3 (%)	Grade 4 (%)	Grade 3-4 (%)
Leucocytopenia	10 (23.2)	5 (11.6)	4 (9.3)	2 (4.6)	6 (13.9)
Neutropenia	5 (11.6)	5 (11.6)	4 (9.3)	4 (9.3)	8 (18.6)
Febrile neutropenia	1 (2.3)	0	0	0	0
Anemia	5 (11.6)	8 (18.6)	0	0	0
Thrombocytopenia	5 (11.6)	2 (4.6)	0	0	0
Nausea	17 (39.5)	2 (4.6)	2 (4.6)	0	2 (4.6)
Vomiting	10 (23.3)	2 (4.6)	0	0	0
Diarrhea	2 (4.6)	1 (2.3)	0	0	0
Constipation	2 (4.6)	0	0	0	0
Increased AST	5 (11.6)	1 (2.3)	0	0	0
Increased creatinine	1 (2.3)	0	0	0	0
Alopecia	11 (25.6)	13 (30.2)	0	0	0
Neurological toxicity	2 (4.6)	0	0	0	0
Mucositis	3 (7.0)	0	0	0	0
Fatigue	3 (7.0)	16 (37.2)	4 (9.3)	0	4 (9.3)

NCI-CTC, National Cancer Institute Common Toxicity Criteria.

Table 4 Hematological and nonhematological toxicities (by cycle)

	NCI-CTC grade (n=153)				
	Grade 1 (%)	Grade 2 (%)	Grade 3 (%)	Grade 4 (%)	Grade 3-4 (%)
Leucocytopenia	27 (17.6)	31 (20.3)	11 (7.2)	2 (1.3)	13 (8.5)
Neutropenia	24 (15.7)	15 (9.8)	13 (8.5)	7 (4.6)	20 (13.1)
Febrile neutropenia	2 (1.3)	0	0	0	0
Anemia	18 (11.8)	27 (17.6)	0	0	0
Thrombocytopenia	11 (7.2)	5 (3.3)	0	0	0
Nausea	49 (32.0)	10 (6.5)	6 (3.9)	0	6 (3.9)
Vomiting	26 (17.0)	13 (8.5)	0	0	0
Diarrhea	4 (2.6)	3 (2.0)	0	0	0
Constipation	7 (4.6)	0	0	0	0
Increased AST	16 (10.4)	2 (1.3)	0	0	0
Increased creatinine	4 (2.6)	0	0	0	0
Alopecia	58 (37.9)	57 (37.3)	0	0	0
Neurological toxicitity	10 (6.5)	0	0	0	0
Mucositis	5 (3.2)	0	0	0	0
Fatigue	10 (6.5)	58 (37.9)	15 (9.8)	0	0

NCI-CTC, National Cancer Institute Common Toxicity Criteria.

46%), the 1-year survival rates of 46.5%, PFS of 120 days (95% CI: 80-160 days), and OS of 340 days (95% CI: 224-456 days), respectively. In previous phase II study, triweekly docetaxel plus carboplatin showed the overall RRs were 42% to 44%, the 1-year survival rates was 50% to 53% and the median OS was 12.0-15.0 months (17,18). In recent phase III study, docetaxel plus carboplatin was observed that the overall RR was 24% and the 1-year survival rate was 38% (19). In another phase II trial, biweekly docetaxel combined with carboplatin showed the overall RR of 30%, the 1-year survival rate of 50%, and the median OS of 11.8 months (9). Furthermore, our data confirmed the previous observations that weekly docetaxel and carboplatin showed the overall RR of 36.7%, the 1-year survival rate of 37.6%, TTP of 5.2 and OS of 10.4 months (20). So, our weekly regimen was comparable with those studies in the aspect of efficacy and the survival results.

As regarding hematological toxicity, our patients experienced the more moderate adverse effects than that of triweekly docetaxel plus carboplatin in which 70% of patients were observed with neutropenia, and 15% with febrile neutropenia (9,11,17,19). Comparatively, in our weekly regimen, the grade 3 or 4 neutropenia was observed in eight (18.6%) patients and there was only one (2.3%) patients suffered with febrile neutropenia, which suggested that our weekly regimen might be safer. So, weekly docetaxel was more advantageous in hematological toxicity than that of 3-weekly schedule (21,22).

For nonhematological toxicities, they were mild and moderate to be managed. Grade 3 or 4 nonhematological toxicities were rare in this study (4.6% nausea, and 9.3% fatigue). Contrasted to previous findings, severe diarrhea or pulmonary toxicity was barely observed in the administration of our weekly regimen (9,23).

The use of weekly docetaxel, rather than traditional 3-weekly, has been investigated in elderly patients, for reducing toxicity of myelosuppression (24). In our study, the elderly population holds a proportion of 53.5%, and there is no difference in the patient characteristics, such as PS or stage between younger patients and elderly patients. However, our study was not terminated because of these more than half of the elderly patients. Moreover, weekly docetaxel with carboplatin illustrated significant efficacy. So, weekly docetaxel and carboplatin might also be a good choice for elderly patients; however it needed further large, randomized studies to evaluate this result.

In conclusion, the combination of weekly docetaxel and carboplatin showed feasible efficacy with acceptable hematologic toxicities for advanced NSCLC.

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Original Article

Is video-assisted thoracic surgery a versatile treatment for both simple and complex pulmonary aspergilloma?

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Background: Pulmonary aspergilloma (PA) is a common fugal infectious disease mostly occurred in developing countries. This study aims to evaluate the outcomes of video-assisted thoracic surgery (VATS) treatment for simple pulmonary aspergilloma (SPA) and complex pulmonary aspergilloma (CPA).

Methods: From October 2009 to March 2013, 16 patients were treated by VATS for PA in our department. The patients were divided into SPA group and CPA group. Records were retrospectively reviewed and data were collected and compared.

Results: Patients had a median age of 52.8 years [95% confidence interval (CI): 47.8-57.9 years]. The most common symptom was hemoptysis (68.7%) in our patients. The underlying lung diseases were tuberculosis (31.1%), bronchiectasis (12.5%) and pneumatocele (6.2%). All patients received successful lesion resection by VATS, none was converted to thoracotomy. No significant difference was found in terms of sex and age. Patients with CPA tent to have larger lesion (P=0.001) and more intraoperative findings (P=0.003), they also needed longer operative time (P=0.016) and more blood loss (P=0.003). In addition, CPA patients had more volume of drainage after surgery (P=0.005), longer duration of drainage ((P=0.007) and length of stay in hospital (P=0.004). No difference was found in postoperative complications between the two groups.

Conclusions: SPA patients are the best candidates for VATS, but comprehensive measure should be taken for the overall benefit of CPA patients before conducting VATS.

Keywords: Pulmonary aspergilloma (PA); video-assisted thoracic surgery (VATS); outcomes

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Introduction

Decision to preform surgical resection has been controversial since pulmonary aspergilloma (PA) was classified into simple pulmonary aspergilloma (SPA) and complex pulmonary aspergilloma (CPA) by Belcher and Pulmmer (1). Generally, surgical treatment for SPA is acceptable because the surgical risk is minimal. In contrast, resection is limited to low-risk patients in CPA because surgery for CPA patients had been reported with more complication and higher mortality (2-4). Over the past few years, video-assisted thoracic surgery (VATS) has been increasingly applied for both benign and malignant pulmonary disease because of its optimal results such as less morbidity and rapid recovery. Although Qian-Kun Chen had reported that VATS was feasible for PA (5), the risk of massive hemorrhage and high level of complication still exist in CPA patients treated by VATS, further studies are still necessary before VATS can be widely accepted as a standard treatment for PA patients, particularly the CPA patients. Here, we performed VATS for 16 patients with PA, surgical outcomes of both SPA and CPA were compared in this study.

Patients and methods

Between October 2009 and March 2013, 16 patients were consecutively treated by VATS for PA in our department. Record of each patient was retrospectively reviewed

Table 1 Symptoms and underlying lung disease in the studied patients

Symptoms and underlying lung disease	n	%
Symptom		
Hemoptysis	11	68.70
Blood-tinged sputum	5	31.30
Fever	2	12.50
Pharyngalgia	1	6.20
Underlying lung disease		
Tuberculosis	5	31.30
Bronchiectasis	2	12.50
Pneumatocele	1	6.20

regarding the preoperative, perioperative and postoperative information. This study was approved by the first affiliated hospital of Zhejiang University medical school review board of clinical research. The need for informed consent from patients was waived because of the retrospective design of the study.

Diagnosis of aspergilloma was made on the basis of medical history, clinical symptoms and computed tomography (CT)-scan image. Sputum cultures were not routinely performed preoperatively. Fiberoptic bronchoscopy was not recommended to perform because it may cause server cough and aggravate hemoptysis. All specimens from resected lung had pathologic confirmation of PA.

The types of aspergilloma were classified retrospectively based on CT-scan image findings and the report of Belcher and Plumme (1): aspergilloma is considered as SPA when it develops in isolated thin-walled cysts (≤3 mm) lined by ciliated epithelium with little or no surrounding parenchymal disease. In contrast, CPA has a thick-walled (>3 mm) cyst with surrounding parenchymal disease such as tuberculosis, bronchiectasis, chronic lung abscess and greater pleural thickening. Our criteria for the selection of patients who received the VATS is based on the following two conditions: (I) young patients (≤65 years) without severe pulmonary parenchyma disease; (II) there is no severe pleural thickness which may suggest obvious pleural adhesions. In addition, patients with invasive aspergilloma and calcified lymph nodes near pulmonary arteries and veins were excluded.

All surgeries were performed by the same experienced surgeon in our department. Patients were placed in lateral decubitus and all procedures were conducted under general anesthesia with single-lung ventilation. Exploratory VATS using two lower thoracoscopic ports was performed, one 7 mm and two 1 cm access incisions were made on the lesion side at the fourth intercostal space on the anterior axillary line, the seventh intercostal space on the midaxillary line and the eighth or ninth intercostal space on the posterior axillary line. In general, lesion and all major pulmonary vessels and bronchi in the affected lobe were resected using endoscopic staplers. One of the incisions was extended approximately to 2.0-3.5 cm in length to facilitate the removal of the resected lobe from the thoracic cavity with a retrieval bag. One or two chest tubes were placed at the end of the procedure depending on hemorrhage level of each patient, the tubes were removed if there is no air leak and the daily output was less than 200 mL. Patients showed no main complication or no obvious pneumothorax and chest fluid by X-rays were discharged from the hospital. In our series, antifungal agents were not used preoperatively but all patients were told to take antifungal agent (Fluconazole) for two weeks with the dosage of 500 mg per day when they are discharged from hospital.

Follow-up data were obtained from the outpatient clinic chart reviews and telephone call to patients or families. The follow-up process ended in October 2013. Data were analyzed using SPSS version 20.0 (SPSS Inc. Chicago, III, USA). The association between variables was analyzed by either the chi-square or *t*-test. Statistical significance was defined as P value is less than 0.05. Categorical variables were analyzed using Fisher exact test.

Results

The patients included ten men and six women with a median age of 52.8 years (95% CI: 47.8-57.9 years). Among the 16 patients, one patient had systematic underlying diseases of hypertension and diabetes. The most common symptom was hemoptysis (≥100 mL, 68.7%) followed by blood-tinged sputum (<100 mL) (31.3%). There was no severe attack of hemoptysis in either group. Four patients had medical history of diagnosis of aspergilloma, ten patients had a preoperative diagnosis of aspergilloma regarding their CT-scan manifestation of air crescent sign which was considered as the characteristic of fungus ball. In addition, all specimens from resected lung had pathologic confirmation of PA. In our patients, nine patients (56%) were classified into the SPA group without any underlying disease; seven patients (44%) were classified into the CPA group. The underlying lung diseases were shown in *Table 1*: tuberculosis (five cases, 31%) represented the main underlying disease in CPA

Table 2 Preoperative characteristics of patients and perioperative clinical data

Characteristics	SPA (n=9)	CPA (n=7)	P
Sex (female/male)	3/6	3/4	0.549
Age (years)	52.4	53.3	0.867
Hemoptysis/blood-tinged sputum	5/4	6/1	0.308
Size of lesion (cm)	1.9	3.2	0.001
Intraoperative findings (Yes/No)	2/7	7/0	0.003
Procedure of operation (L/W)	4/5	7/0	0.034
Duration of operation (min)	101.3	187.9	0.016
Blood loss (mL)	55.6	150	0.003
Chest drainage (mL)	523.3	1020.7	0.005
Duration of chest drainage (days)	3.9	9.9	0.007
Postoperative hospital stay (days)	5	11	0.004
Complication (Yes/No)	5/4	3/4	0.500

SPA, simple pulmonary aspergilloma; CPA, complex pulmonary aspergilloma; L, lobectomy; WR, wedge resection.

Table 3 Postoperative complications in patients

Postoperative complication	n	%
Pneumonia	4	25.0
Pneumothorax	3	18.8
Hydrothorax	1	6.3
Hydropneumothorax	1	6.3
Emphysema	1	6.3

patients, the rest were bronchiectasis (two cases, 13%) and pneumatocele (one case, 6%).

Comparison of the baseline demographic characteristics and perioperative clinical findings between patients with SPA and CPA are shown in *Table 2*. Five patients (55.6%) in SPA group and six patients (85.7%) in CPA group presented the symptom of hemoptysis. Operations were performed electively. Intra-operative finding included varying degrees of pleural adhesions and pleural wall thickening, pleural adhesion was observed in six patients (85.7%) and pleural thickening was found in two patients (28.6%). No patients was converted to thoracotomy, all lesion were resected successfully.

There was no difference in terms of gender, age and occurrence of hemoptysis. However, the patients in CPA group had larger size of lesion (mean =3.2 cm, 95% CI: 2.7-3.7 cm, P=0.001) and intra-operative findings (P=0.003) (*Table 2*) compared with those in the SPA group. In addition, data indicated that CPA patients were more likely to be selected to conduct lobectomy and operation time was relatively longer (mean =187.9 min, 95% CI: 158.2-217.5 min, P=0.016) with more blood loss (mean =150 mL, 95% CI: 72.8-227.2 mL, P=0.003) (*Table 2*). The amount of chest drainage was significantly less in SPA group (mean =523.3 mL, 95% CI: 386.6-660.1 mL, P=0.005) coupled with relatively shorter duration of chest drainage (mean =3.9 days, 95% CI: 2.8-4.9 days, P=0.007) and postoperative hospital stay (mean =5 days, 95% CI: 4.0-6.0 days, P=0.004) (*Table 2*).

There was no perioperative deaths in either group, ten complications developed in eight patients (50%): four pneumonia, three pneumothorax, one hydrothorax, one hydropneumothorax, and one emphysema (*Table 3*), no significance difference was found in the occurrence of complications. The mean follow-up period was 21.6 months (95% CI: 16.1-26.7 months). One patient was lost to follow-up in SPA group because of the changed telephone number. Satisfyingly, further complications, recurrence and PA-related death were not found among the other patients.

Discussion

PA generates from inhalation of aspergillus-reproduced conidia through airways. These aspergillus organisms mainly colonize in pre-existing pulmonary cavities of aspergillus fumigatus. CPA usually develops in benign cavitary pulmonary disease such as tuberculosis, bronchiectasis and lung abscess. The most common cavitary lesion in all series is tuberculosis (3,6,7), consistent with our experience: tuberculosis was the main cause of cavitary lung lesions in 31% of our cases. It is reported that the most common symptom

associated with aspergilloma is hemoptysis (8). Consistently, our data demonstrated that hemoptysis (≥100 mL) serves as the leading symptoms (68.7%) followed by blood-tinged sputum (<100 mL, 31.2%) (*Table 1*). No significance difference was found between SPA and CPA group in number of patients who developed hemoptysis and blood-tinged sputum, and there was no occurrence of emergency hemoptysis attack in CPA group, indicating that hemoptysis cannot be considered as a characteristic feature to distinguish CPA from SPA in our study.

Lesion excision is considered as the mainstay treatment of PA, it not only reduce the incidence of life-threating hemoptysis but also result in a likelihood of a permanent cure (3,9,10). The decision of surgery procedure of VATS depends on the balance between the risk of life-threating hemorrhage in surgery and benefit of rapid postoperative recovery. Wedge resections under VATS have been widely used for patients with benign and malignant lung disease due to its safety, minimal time consumption and reliability. It only requires 2-3 small, 1-1.5 cm long incisions. Whereas lobectomy, another procedure which causes more injury, is inevitable for the limitation of lesion site and size in some cases. In our practice, we take wedge resection as priority in case of SPA with peripheral and smaller lesion, because the surgical risk is minimal. Shirakusa wrote that wedge resection can be performed in patients when aspergilloma is sufficient small and located in the healthy lung periphery (11). However, we must evaluate the patients and only recommended wedge resection for low-risk patients without infiltration of the hilum, no matter SPA or CPA patients, because dissection of lesion and calcified lymph node near pulmonary arteries is of enormous jeopardy. Based on that, four patients in SPA group were performed wedge resection but none of CPA patients was candidate for wedge resection.

PA lesions are more difficult to be surgically dissected in patients with CPA. The dense fibrosis and diseased pulmonary surrounded the enlarged cavity made resection of PA lesion via VATS more technically challenging. Besides, obliteration of pleural space and fissures increased the risk of surgical bleeding. Adhesions accompanied with more proliferative vessels made the surgical field bloody and blurred during dissection, although the interchangeable use of the electrocoagulation hook and ultrasonic dissector effectively kept the surgical field clean, the separation of pleural adhesions definitely accounted for longer time consuming and more blood loss, and prolonged postoperative chest drainage and hospital stay.

As for postoperative complications, no significant differences were found between the two groups. In SPA group, three patients had pneumonia, and they were managed by antibiotics. One patient had pneumothorax and one patient had hydrothorax, they were managed by prolonged chest tube drainage. In CPA group, two patients had pneumothorax and one patient had hydropneumothorax combined with emphysema, this patient is a 49 aged male with tuberculosis and recurrent pneumothorax. This woman was managed by conservative comprehensive treatment.

Complications such as wound infection and empyema is caused mainly by contamination of pleural cavity and wound during resection of infected or purulent lesions. Some researcher wrote that the routine usage of neomycin sulfate solution before closing would effectively avoid the intraoperative dissemination (5). In our institute, we use a mass of 0.5% povidone-iodine to clean the cavity and incision to avoid contamination, usage of antibiotic rinse is not recommended because of the possible generation of antibiotic resistance, which makes the treatment of unpredictable infection or recurrence of PA thornier.

None of our patients experienced bronchopleural fistula, which was considered as one of the most serve complications and happened in 1.6% to 15.8% of PA patients in previous studies treated by surgeries (12-14) . Our favorable outcomes probably attributed to the usage of intercostal muscle, pleural flaps, or a pericardial fat pad to cover the bronchial stump. Moreover, avoiding of excessive dissection is highly recommended to preserve perfusion for healing of the bronchial stump.

Good underlying pulmonary condition and postoperative care are essential for preventing postoperative mortality, because most deaths are caused by chronic respiratory failure or pneumonia (15). In our study, mortality was not found in either group during follow-up period. Previous studies reported high mortality ranging from 1.4% to 34.3% of patients with CPA treated by thoracotomy (2). In the literate reports about VATS for PA, only one CPA patient died after operation, and that patient is a 70-year-old male with a history of surgeries for esophageal cancer (2,5). The minimal invasive approach for lung resection and the early postoperative recovery of VATS are considered to attribute to the favorable mortality results. The different severity of underlying disease and a more stringent patient selection in this study may explain the improved results as well, we must declare that our patients were relatively young and there was no existing of serious systematic underlying disease or local pulmonary disease such as lung and esophageal cancer in our patients.

There are several limitations to this study because of its retrospective design. The first limitation of this study concerns the relatively small cases volume. More patients in China will choose thoracotomy because of the higher expense of VATS, and some records of patients are lost during the transformation of the medical record system in our hospital. The second limitation lies in the long time span of this study, the treatment effect might be different with the increased experience of surgeon performing VATS as time goes on.

Conclusions

SPA patients with small and peripheral lesion are best candidates for wedge resection by VATS. Although the mortality of CPA patients treated by VATS is not inferior to thoracotomy, comprehensive measure should be taken for the overall benefit of CPA patients before conducting VATS. Application of VATS should not be pursued as something trendy, accumulation of the further cases are necessary before VATS can be widely accepted as a standard treatment for CPA patients.

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Disclosure: The authors declare no conflict of interest.

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Original Article

Expression and association of *CD44v6* with prognosis in T2-3N0M0 esophageal squamous cell carcinoma

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Aim: To investigate the expression of *CD44v6* in stage T2-3N0M0 esophageal squamous cell carcinoma (ESCC) and its prognostic significance.

Methods: The expression of *CD44v6* in a series of 227 ESCC specimens was evaluated by immunohistochemistry (IHC). A reproducible semiquantitative method which took both staining percentage and intensity into account was applied for IHC scoring, and receiver operating characteristic (ROC) curve analysis was utilized to select the cut-off score for high or low IHC reactivity. Then, the correlations of *CD44v6* expression with clinicopathological features of patients and its prognostic relevance were determined.

Results: In the present study, the proportion of low *CD44v6* expression was found significantly lower in Grade 3 of ESCC, than that of Grade 1 and Grade 2 of ESCC. There are no significant correlations between *CD44v6* expression and other clinicopathological parameters including gender, age, tumor size, tumor location, depth of invasion and pathological stage. The Kaplan-Meier survival curves showed that up-regulated expression of *CD44v6* indicated a poorer post-operative survival for ESCC patients of stage T2-3N0M0 (P=0.009), especially for those with T2 lesions (P=0.044) or with stage IIB diseases (P=0.005). Multivariate analysis also confirmed that *CD44v6* expression [relative risk, 1.639; 95% confidence interval (CI): 1.142-2.354, P=0.007] and depth of invasion (relative risk, 1.487; 95% CI: 1.063-2.080, P=0.020) were independent prognostic factors.

Conclusions: Elevated *CD44v6* expression may be an adverse prognostic indicator for patients with stage T2-3N0M0 ESCC, especially for those with T2 lesions or stage IIB diseases.

Keywords: Esophageal squamous cell carcinoma (ESCC); *CD44v6*; prognosis; receiver operating characteristic curve; immunohistochemistry (IHC)

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Introduction

Esophageal cancer is one of the most common lethal malignancies in the world (1-3), causing more than 400,000 deaths per year (4). In Asian countries, esophageal squamous cell carcinomas (ESCC) accounts for over 90% of esophageal cancers (5). Despite tremendous advances in diagnosis and multimodality therapy, the prognosis of ESCC remains poor and the overall 5-year survival is still less than 15% (1,6). More and more evidence has revealed that classical staging criteria provide insufficient prognostic characterization of ESCC patients, and molecular tumor analysis may provide an effective and feasible means of better defining prognosis (7,8). Therefore, to further improve the survival rate in patients with ESCC, novel prognostic and molecular targets for therapeutic intervention are critically needed for patients with ESCC.

CD44 forms a family of cell surface proteins involved in cell migration, cell adhesion, tumor progression and metastatic spread (9-11). The CD44 gene is 50-60 kDa in size, located on chromosome 11p13, and is known to be composed of at least 20 exons. *CD44v6* is an important isoform of CD44 family (12-14). Related studies have shown that the expression of *CD44v6* correlates with tumor progression and metastasis development in several human malignancies, such as lung cancer, gastrointestinal cancer, breast cancer, and so on (10,13,15-17). However, reports on the relationship between *CD44v6* expression and prognosis are contradictory. Some authors have reported that the *CD44v6* is an independent prognostic factor (15,18), but others have failed to draw a similar conclusion (19,20). Therefore, we examined the expression of *CD44v6* in T2-3N0M0 ESCC to elucidate its significance in clinical prognosis.

Materials and methods

Patients and tissues

The study was approved by the Research Ethics Committee of the Cancer Center of Sun Yat-Sen University. From January 1993 to August 2004, we enrolled 227 consecutive patients with stage IB-IIB node-negative ESCC who received surgical treatment with curative intent, and the resected specimens were assessed for *CD44v6* expression level via immunohistochemistry (IHC). Patients were followed prospectively and their survival data was recorded through October 2009. Patients were included in this study based on the following eligibility criteria: (I) histopathologically proven ESCC; (II) disease stage of T2-3N0M0 based on the seventh edition of the American Joint Committee on Cancer (AJCC) staging system for esophageal cancer (21); (III) at least 15 lymph nodes to be removed for pathological evaluation; (IV) age at least 18 years; (V) no evidence of metastatic disease as determined by history, physical examination, and blood chemistry analysis or routine computed tomography; and (VI) no history of adjuvant therapy. Patients were excluded based on the following criteria: (I) history of previously treated cancer other than basal or squamous cell carcinoma of the skin; (II) history of preoperative chemotherapy and/or radiotherapy; (III) unknown cause of death in follow-up.

Immunohistochemistry (IHC)

Immunoperoxidase stain for *CD44v6* (1:50 dilution; Novocastra Laboratories, Ltd., Newcastle upon Tyne, United Kingdom) was done on 4-μm-thick paraffin sections. The slides were deparaffinized in xylene then hydrated prior to antigen retrieval by microwaving in sodium citrate buffer (pH 6.0). The slides were then incubated with a peroxidase block and then the primary antibody. After a PBS wash, the slides were incubated first with the secondary antibody and then with 3,3'-diaminobenzidine, then counterstained with hematoxylin (Hematoxylin 7211; Richard-Allen Scientific, Kalamazoo, Michigan, United States of America). The peroxidase block, secondary antibody and 3,3'-diaminobenzidine were all obtained from the DakoCytomation EnVision System (Glostrup, Denmark). After a hematoxylin counterstain (Hematoxylin 7211; Richard-Allen ScientiWc, Kalamazoo, MI), the slides were coverslipped.

Immunohistochemical scoring

The immunohistochemical scoring of *CD44v6* was performed using a semiquantitative system as demonstrated previously (7,15,22). Each slide was assigned a score: the score of tumor cell staining multiplied by the score of staining intensity. Tumor cell staining was assigned a score using a semiquantitative six-category grading system: 0, no tumor cell staining; 1, 1% to 10% of tumor cells staining; 2, 11% to 25% of tumor cells staining; 3, 26% to 50% of tumor cells staining; 4, 51% to 75% of tumor cells staining; 5, more than 75% of tumor cells staining. Stain intensity was assigned a score using a semiquantitative four-category grading system: 0, no staining; 1, weak staining; 2, moderate staining; 3, strong staining. Two experienced pathologists independently scored 400 ESCC samples including the cases used in this study blinded to clinical follow-up data. The complete score agreement between these two pathologists is 87% of cases, indicating that the scoring method was reasonably reproducible. A third blinded pathologist intervened and evaluated cases with different IHC scores. If the third pathologist agreed with one of the previous scorings, that score was used for analysis. For the cases in which three different scores were obtained, the three pathologists were asked to reach agreement on the case.

Selection of cut-off score

The cut-off scores for *CD44v6* expression were selected based on receiver operating characteristic (ROC) curve

Table 1 Characteristics of esophageal squamous cell carcinoma (ESCC) patients and tumors

Characteristic	Patients with ESCC (N=227)
Sex (%)	
Male	165 (72.7)
Female	62 (27.3)
Age (years)	
Median	58
Range	33-77
Tumor size (cm)	4.92±2.046
Tumor grade (%)	
Grade 1	57 (25.1)
Grade 2	114 (50.2)
Grade 3	56 (24.7)
Tumor location (%)	
Upper	25 (11.0)
Middle	152 (67.0)
Lower	50 (22.0)
Depth of invasion (%)	
T2	88 (38.8)
T3	139 (61.2)
AJCC staging system (7^{th} edition) (%)	
IB	12 (5.3)
IIA	83 (36.6)
IIB	132 (58.1)

AJCC, American Joint Committee on Cancer.

analysis. The sensitivity and specificity for the outcome under study were plotted, thus generating a ROC curve. The score closest to the point with both maximum sensitivity and specificity [i.e., the point (0.0, 1.0) on the curve] was selected as the cut-off score leading to the greatest number of tumors classified correctly as having or not having the clinical outcome. The area under the ROC curve (AUC) was calculated to estimate the discriminatory power of *CD44v6* over the entire range of scores for overall survival. Both generation and analysis of the ROC curve were performed by a MedCalc statistical software package 11.0.1 (MedCalc Software bvba, Belgium).

Statistical analysis

Associations between categorical variables were analyzed using the Chi-square test. Survival curves were calculated by the Kaplan-Meier method and were compared by the log-rank test. Time to event (death) was calculated from date of surgery to date of event. In event-free subjects, the time variable was censored at date of last follow-up. Multivariate analysis of prognostic factors was performed using Cox's regression model. A significant difference was defined as a two-tailed P-value of less than 0.05. All of the statistical analyses were performed using the SPSS 13.0 for Windows software system (SPSS Inc, Chicago, IL, USA).

Results

Characteristics of all patients and expression of CD44v6

Table 1 provided all the patients' clinicopathological features. Among the 227 patients included in this study, 162 (72.7%) were male patients and 62 (27.3%) were female patients. The median age was 58 years (range from 33 to 77 years). In cancer cells, it was generally the cytoplasm and membrane that revealed various intensity of positive reaction for *CD44v6* (*Figure 1*). According to the ROC curve in our study (*Figure 2*), threshold value of 14 was the closest to the point with both maximum sensitivity and specificity, and thereby selected as the cut-off score. The AUC of our ROC curve analysis was 0.550 [95% confidence interval (CI): 0.483-0.616]. Patients were thus divided into two groups, namely high expression group (n=50) and low expression group (n=177).

Correlation between the expression of CD44v6 and clinicopathological features

The association between *CD44v6* expression and clinicopathological variables of ESCC patients is shown in *Table 2*. Low *CD44v6* expression was found in 66.1% of Grade 3 of ESCC, significantly lower than that of Grade 1 and Grade 2 of ESCC. There are no significant correlations between *CD44v6* expression and other clinicopathological parameters including gender, age, tumor size, tumor location, depth of invasion and pathological stage based on the seventh edition of AJCC staging system (21).

CD44v6 expression and survival

At the time of data analysis (October 2009), with a median follow-up of 32 months (range, 5-138 months), 72 patients (31.7%) remained alive and 155 patients (68.3%) died from cancer-related causes. The 1-, 3- and 5-year overall survival

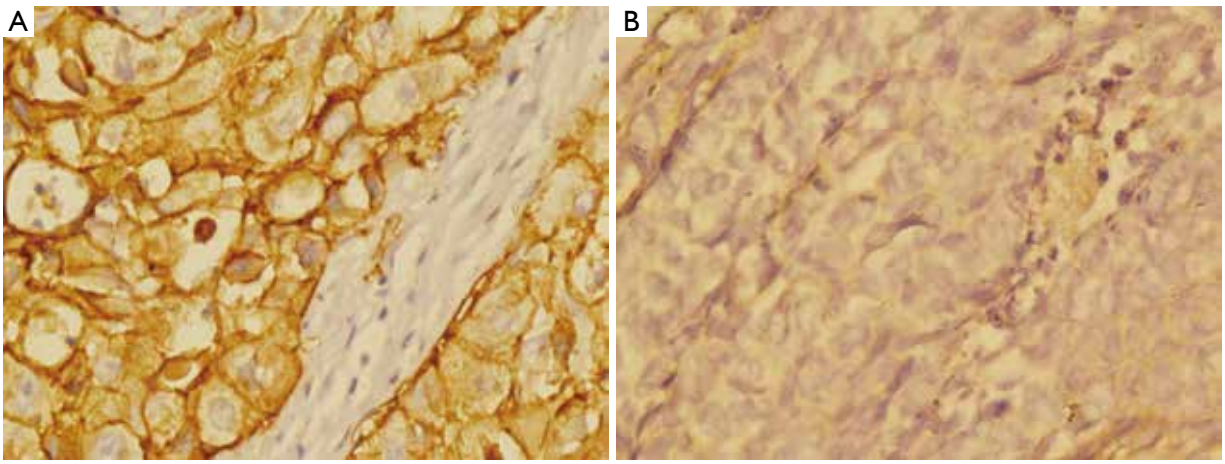

Figure 1 Expression of *CD44v6* in esophageal squamous cell carcinoma (ESCC) tissue (Envision, ×400). (A) Immunohistochemical staining of *CD44v6* in cytoplasm and membrane (IHC score: 15); (B) Immunohistochemical staining of *CD44v6* in cytoplasm (IHC score: 3). IHC, immunohistochemistry.

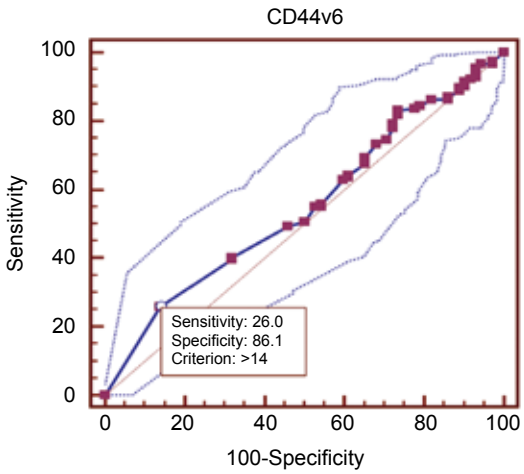

Figure 2 Receiver operating characteristic (ROC) curve analysis for *CD44v6* and the selection of cut-off score.

probabilities for the entire group were 58%, 39% and 33%, respectively.

The Kaplan-Meier survival curves (*Figure 3*) showed that patients with low *CD44v6* expression experienced significantly better post-operative survival than those with high *CD44v6* expression (P=0.009). In further stratified analysis split by depth of invasion (*Figure 4*), *CD44v6* expression had a statistically significant influence on the survival of patients with T2 diseases (P=0.044) rather than on the survival of those with T3 lesions (P=0.056). Furthermore, the stratified analysis split by pathological stage based on the new staging system (*Figure 5*) revealed that the prognostic significance of *CD44v6* expression remained on the survival of patients with stage IIB ESCC (P=0.005) but not on the survival of those with IIA diseases (P=0.421). Patients with stage IB ESCC were not included in this analysis in view of the small sample size.

We performed analysis using the Cox proportional hazards model to identify factors involved in OS of T2-3N0M0 ESCC patients (*Table 3*). The univariate analysis showed that depth of invasion and *CD44v6* expression were significant prognostic indicators for OS, and thereby selected as the parameters to be included in the same Cox regression model. Further multivariate analysis also confirmed that *CD44v6* expression (relative risk, 1.639; 95% CI: 1.142-2.354, P=0.007) and depth of invasion (relative risk, 1.487; 95% CI: 1.063-2.080, P=0.020) were independent prognostic factors.

Discussion

Numerous studies have been performed in order to elucidate the role of *CD44v6* in ESCC; however, data for this prognostic marker in ESCC are quite controversial (15,18-20). One problem faced by researchers is the determination of the extent of tumor IHC positivity for *CD44v6* which is clinically and biologically relevant. Several previous studies had applied different scoring systems and predetermined cut-off scores which might be set arbitrarily. This may primarily be responsible for the contradictory results of these studies evaluating *CD44v6* and its prognostic

Table 2 Correlation of *CD44v6* with clinicopathological variables

	n	*CD44v6*		P-value
		Low n (%)	High n (%)	
Sex				
Male	165	127 (77.0)	38 (23.0)	0.552
Female	62	50 (80.6)	12 (19.4)	
Age				
≤60 years	138	104 (75.4)	34 (24.6)	0.237
>60 years	89	73 (82.0)	16 (18.0)	
Tumor size (cm)				
≤5.0	155	123 (79.4)	32 (20.6)	0.461
>5.0	72	54 (75.0)	18 (25.0)	
Tumor grade				
Grade 1	57	48 (84.2)	9 (15.8)	
Grade 2	114	92 (80.7)	22 (19.3)	0.041[a]
Grade 3	56	37 (66.1)	19 (33.9)	
Tumor location				
Upper	25	15 (60.0)	10 (40.0)	
Middle	152	121 (79.6)	31 (20.4)	0.067
Lower	50	41 (82.0)	9 (18.0)	
Depth of invasion				
T2	88	67 (76.1)	21 (23.9)	0.595
T3	139	110 (79.1)	29 (20.9)	
AJCC staging system (7th edition)				
IB	12	12 (100.0)	0 (0)	
IIA	83	65 (78.3)	18 (21.7)	0.152
IIB	132	100 (75.8)	32 (24.2)	

AJCC, American Joint Committee on Cancer; [a]P<0.05.

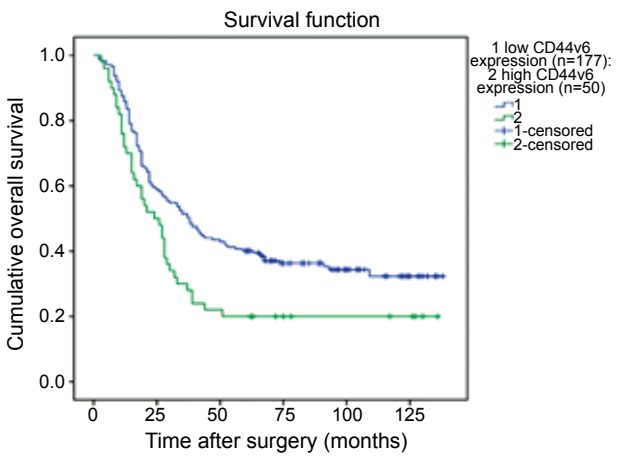

Figure 3 Kaplan-Meier survival function for patients with T2-3N0M0 esophageal squamous cell carcinoma (ESCC) according to *CD44v6* expression.

value in ESCC. Therefore, our study utilized a reproducible scoring method which took both staining percentage and intensity into account, and selected the cut-off score based on ROC curve analysis for *CD44v6* expression evaluation, where the score closest to the point with both maximum sensitivity and specificity was chosen. This method allowed the greatest number of tumors to be correctly classified as carrying or not carrying the clinical outcome (23). In addition, all patients contained in our series were all free of lymph node metastasis and received no other therapy before or after the resection.

In the present study of T2-3N0M0 ESCC patients, high expression of *CD44v6* was significantly correlated with the worse post-operative survival rate, both by univariate and multivariate analysis, which is consistent with the reported findings. Nozoe *et al.* (24) reported that the over-expression

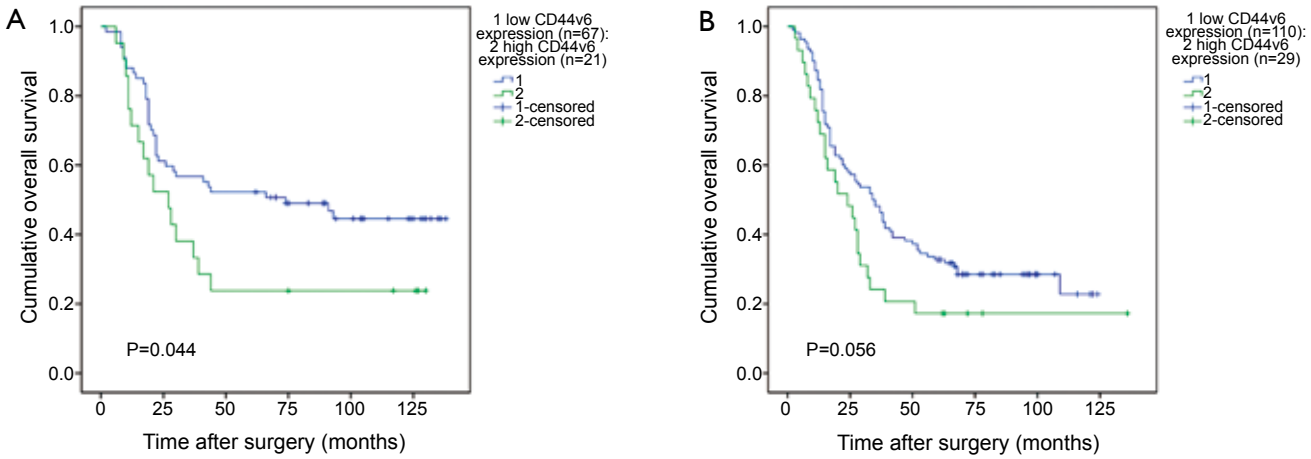

Figure 4 Kaplan-Meier survival curves for patients with esophageal squamous cell carcinoma (ESCC) stratified for depth of invasion according to *CD44v6* expression. (A) Correlation between *CD44v6* expression and post-operative survival of patients with T2 lesions; (B) Correlation between *CD44v6* expression and post-operative survival of patients with T3 lesions.

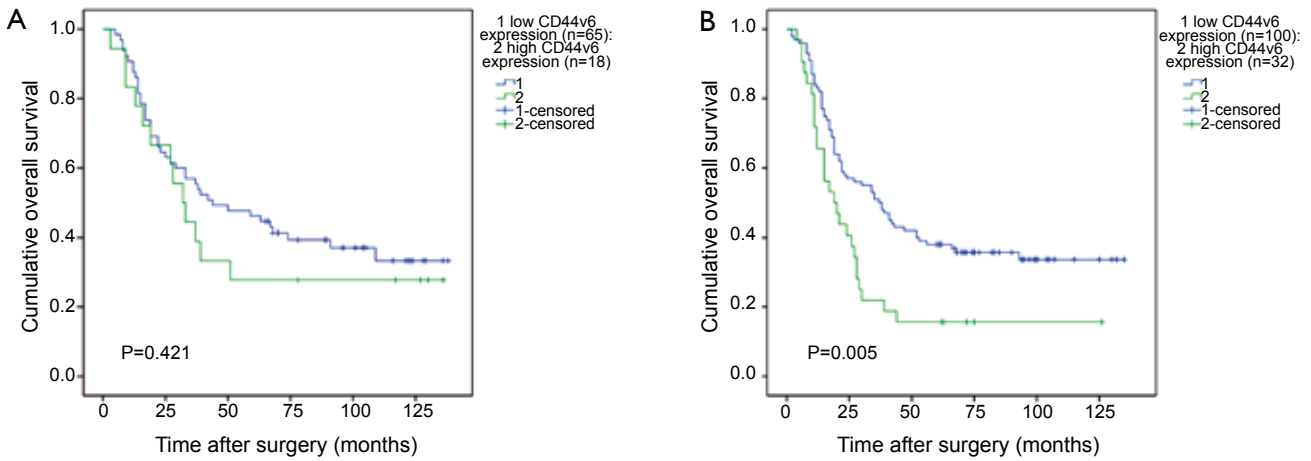

Figure 5 Kaplan-Meier survival curves for patients with esophageal squamous cell carcinoma (ESCC) stratified for pathological stage according to *CD44v6* expression. (A) Correlation between *CD44v6* expression and post-operative survival of patients with IIA diseases; (B) Correlation between *CD44v6* expression and post-operative survival of patients with IIB diseases.

level of *CD44v6* in ESCC patients is significantly correlated with the higher proportions of the incidence of lymph node metastasis, lymphatic permeation, blood vessel invation, more advanced stage and therefore poorer prognosis. Similarly, Shen *et al.* (25) received a comparable conclusion that *CD44v6* expression in ESCC was significantly higher than that in adjacent normal tissue and was correlated with distant metastasis and TNM stage.

It has been known that well differentiation of ESCC which is deeply related to apoptosis to the tumor cells is likely to influence the less invasive potential of ESCC (26,27). Several investigations suggest the correlation of *CD44v6* with the histologic grade in SCC of the head and neck (28,29). In our study, we draw a similar conclusion that the proportion of low *CD44v6* expression was found significantly lower in Grade 3 of ESCC, than that of Grade 1 and Grade 2 of ESCC, which was consistent with Shen *et al.* (25).

Moreover, *CD44v6* expression has been reported to be an indicator to predict the sensitivity to neoadjuvant chemotherapy in cervical carcinoma (30); hence, an assessment of an IHC *CD44v6* expression might possibly be an auxiliary information regarding the response of ESCC to the adjuvant therapies. Also, Koyama *et al.* (31) reported that the expression of *CD44v6* was significantly reduced

Table 3 Univariate and multivariate overall survival analysis of ESCC patients by Cox proportional hazards model

Variable	Univariate analysis			Multivariate analysis		
	Hazard ratio	95% CI	P-value	Hazard ratio	95% CI	P-value
Age						
≤60 *vs.* >60 yrs	1.023	0.741-1.414	0.889			
Gender						
Male *vs.* female	0.691	0.474-1.008	0.055			
Tumor size						
≤5 *vs.* >5 cm	0.987	0.704-1.386	0.941			
Grade						
G1 *vs.* G2&3	1.145	0.788-1.664	0.477			
Tumor location						
Upper & middle *vs.* lower	0.953	0.657-1.384	0.802			
Depth of invasion						
T2 *vs.* T3	1.462	1.046-2.044	0.026[a]	1.487	1.063-2.080	0.020[a]
AJCC staging system (7th edition)						
IB & IIA *vs.* IIB	1.193	0.865-1.644	0.282			
CD44v6						
Low *vs.* high	1.608	1.120-2.308	0.010[a]	1.639	1.142-2.354	0.007[a]

AJCC, American Joint Committee on Cancer; CI, confidence interval; [a]P<0.05.

in the irradiated primary ESCC, which might imply that *CD44v6* could be a factor on the response of ESCC to radiotherapies.

In conclusion, the study elucidated that *CD44v6* might be correlated with the histologic grade and its high expression may be a potential worse prognosis indictor for the patients with T2-3N0M0 ESCC, especially for the patients with T2 lesions or stage IIB diseases. However, there are still many unanswered questions regarding *CD44v6* expression in ESCC. Additional studies with randomization and longer follow-up are needed for the establishment of more effective management plan, which will aid in improving prognosis.

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Original Article

Efficacy of combination therapy of triazole and echinocandin in treatment of invasive aspergillosis: a systematic review of animal and human studies

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Objective: The effectiveness of the combination therapy of triazole and echinocandin in treatment of invasive aspergillosis (IA) remains controversial. The objective of this systematic review was to assess the efficacy of combination therapy of triazole and echinocandin in treatment of IA.

Methods: Relevant articles on the combination therapy of triazole and echinocandin in IA, including the animal studies and clinical studies from January 1966 to October 2013, were searched on Web of Science, PubMed and Cochrane Library. The prolongation of survival of the combination therapy of triazole and echinocandin in IA was performed as risk ratio (RR) with 95% confidence interval (95% CI).

Results: Nine animal studies with a total of 1,582 animals and five clinical trials totaling 872 patients were included. The survival of the included animal studies with combination therapy was significantly prolonged compared with echinocandin alone [RR =2.26, (95% CI, 1.79-2.87; P<0.00001)], but no statistical difference compared with monotherapy of triazole [RR =1.19, (95% CI, 0.98-1.44; P=0.08)]. Of the four human cohort studies, two studies observed that the combination therapy of triazole and echinocandin was associated with a significant reduction in mortality compared with other treatments, and one study might be considered as a preferable therapy [HR =0.58, (95% CI, 0.3-1.14; P=0.117)]. While another study revealed that there was no significant difference among the combination therapy of triazole and echinocandin and either of the monotherapy. In the randomized clinical trial (RCT), of the 135 patients who received the combination therapy, 39 died, while 55 died out of 142 patients who received monotherapy (P=0.08, 95% CI, –21.4, 1.09) by week 12.

Conclusions: The combination therapy of triazole and echinocandin in treating IA results in a trend towards improved overall survival in animals' studies and clinical studies. Well-designed RCTs and further improved clinical trials are necessary to study the effectiveness of the combination therapy.

Keywords: Triazole; echinocandin; invasive aspergillosis (IA); systematic review

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Introduction

Invasive aspergillosis (IA) is an opportunistic infection caused by fungi of the genus *Aspergillus*. Due to increasing number of people with compromised immunity (most as a results of AIDS and organ transplantation), IA has been on a sharp rise for the past few decades. *Aspergillus fumigatus* is widely present in environment and the most common species recovered from cases of IA among which 90% are involved into the lung (1). Other commonly recovered species are *Aspergillus flavus*, *Aspergillus niger*, and *Aspergillus terreus*.

Invasive pulmonary aspergillosis (IPA) is a life-threatening infection associated with severe mortality. Voriconazole is considered to be the primary therapy for IPA based on the results of randomized clinical trials (RCTs) (2,3) and alternatives are liposomal amphotericin B, amphotericin B lipid complex, caspofungin, micafungin, posaconazole and

itraconazole. Despite these treatment options, the outcomes of IPA remain poor, with mortality rates of 25% to 35% 12 weeks after diagnosis (4).

The target of triazole is at cell-membrane, and the target of echinocandin is at cell-wall (2), so that the combination therapy of triazole and echinocandin may result in synergistic function against *Aspergillus spp*. strains with a wider spectrum of efficacy and lower toxicity (5-7). However, some studies showed that the combination therapy of triazole and echinocandin did not significantly improve the therapeutic outcome (8), or they might even be potentially antagonistic to each other (9). Furthermore, the combination of antifungal drugs for primary therapy of IPA is not routinely recommended by the Infectious Diseases Society of America due to lack of enough clinical data (2). Therefore, our objective was to evaluate the evidences for the combination therapy of triazole and echinocandin in treatment of IA in animal and clinical studies.

Materials and methods

Literature search

Relevant articles from January 1966 to October 2013 were searched on Web of Science, PubMed and Cochrane Library by two researchers. Keywords or text words in medical subjects heading (MeSH) included: "invasive aspergillosis" OR "invasive pulmonary aspergillosis", "triazole" OR "itraconazole" OR "voriconazole" OR "posaconazole" OR "ravuconazole", "echinocandin" OR "caspofungin" OR "micafungin" OR "anidulafungin". We also did hand searching of reviews, guidelines and citations of all included studies for complete references.

Selection criteria for studies

Animal studies

Inclusion criteria: animal models were in line with IA standard. Appropriate control groups were set, and uniform evaluation indexs were included.

Exclusion criteria: any study which was only related to pharmacokinetic study, combination of triazole or echinocandin with amphotericin B, not set with a blank or a placebo-control or repeatedly published data, was excluded.

Clinical studies

Inclusion criteria: any study in which IA was diagnosed according to the European Organization for Research and Treatment of Cancer and the Mycoses Study Group consensus criteria was included (10). We included studies in which patients were diagnosed with either proven or probable IA. We included cohort or RCT studies that assessed the efficacy of combination therapy of triazole and echinocandin with appropriate control groups.

Exclusion criteria: any study with only a case report or repeatedly published data, without control group or lacking uniform diagnostic criteria, was excluded.

Data extraction

Two reviewers independently applied selection criteria, performed quality assessment, and extracted data, including the sample size, antifungal dose, duration of treatment (days), the observed indicators and evaluation criteria. If we found that the information provided in a literature is not comprehensive, we contacted the author to get detailed information. Disagreement on whether some specific studies should be included into this study between the two reviewers was attempted to be reached a consensus in a subsequent discussion between the two reviewers, which otherwise was resolved by a third researcher.

Study quality assessment

A quality assessment of all selected full-text articles of animal studies was performed according to the ARRIVE guidelines (11,12). The Newcastle-Ottawa Quality Assessment Scales (13) for cohort clinical studies was applied to assess selection bias, comparability of exposed and unexposed groups of each cohort, outcome assessment, and attrition bias. The quality of the RCT was assessed according to modified Jadad score (14), including details of randomization, generation of random numbers, implementation of double-blinding, information on withdrawals, and allocation concealment. Two reviewers independently evaluated these components of the scale. Disagreements among reviewers were resolved by discussion until a consensus was reached.

Statistical methods and data analysis

The survival was reported as risk ratio (RR) with 95% confidence interval (95% CI). A heterogeneity test was performed to examine the homogeneity. If there was homogeneity, the fixed-effect model was used; if there was heterogeneity, the random-effect model was used. Z-statistic test for over effect was done, $P \leq 0.05$ was considered to

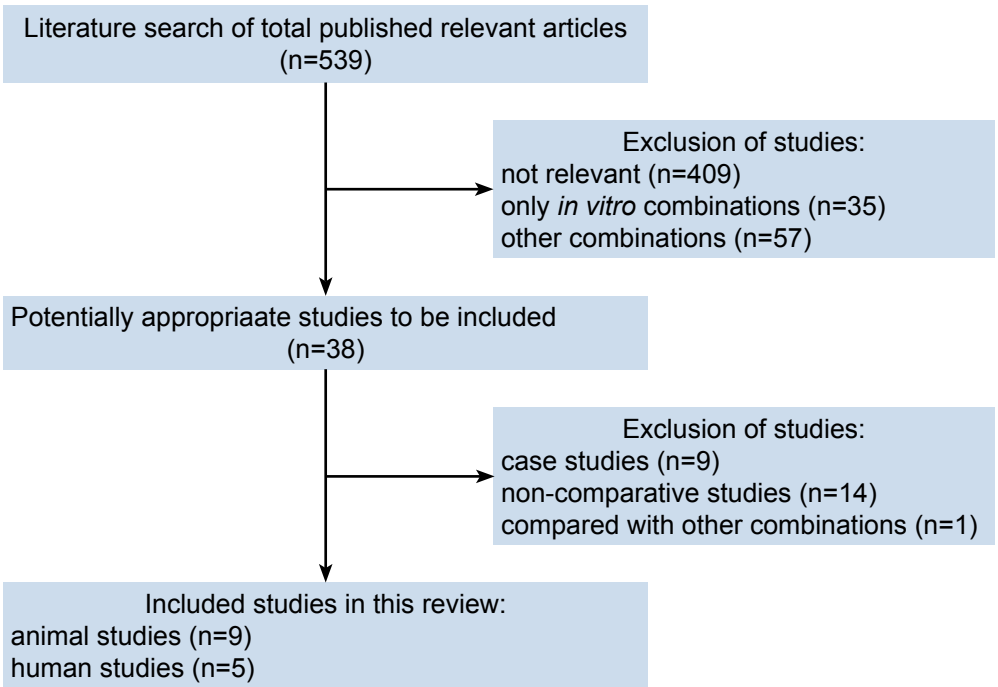

Figure 1 Flow chart of included and excluded studies.

Table 1 Quality assessment of combination therapy in animal models (Kilkenny *et al*. 2010a)

Studies	Items																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Kirkpatrick WR *et al.*, 2002 (7)	1	2	1	1	0	1	0	1	0	1	1	2	2	0	2	2	0	2	1	2
Luque JC *et al.*, 2003 (6)	1	1	1	1	0	1	1	1	0	1	1	2	2	0	2	2	0	1	0	2
Petraitis V *et al.*, 2003 (5)	1	2	2	1	0	1	1	1	0	1	1	2	2	0	2	2	0	1	1	2
MacCallum DM *et al.*, 2005 (15)	1	2	1	1	0	1	1	1	0	1	1	2	2	0	2	2	0	1	0	1
Clemons KV *et al.*, 2006 (9)	1	2	1	1	0	1	0	1	0	1	1	2	2	0	2	2	0	1	0	1
van de Sande WW *et al.*, 2009 (8)	1	2	1	1	0	1	0	1	0	1	1	2	2	0	2	2	0	1	0	1
Petraitis V *et al.*, 2009 (16)	1	2	1	1	0	1	1	1	0	1	1	2	2	0	2	2	0	1	0	2
Calvo E *et al.*, 2012 (17)	1	2	1	1	0	1	1	1	0	1	1	2	2	0	2	2	0	1	1	2
Seyedmousavi S *et al.*, 2013 (18)	1	2	1	1	0	1	0	1	0	1	1	2	2	0	2	2	0	1	0	2

be statistically significant. All statistical analyses were performed using Review Manager Version 5.1 (The Nordic Cochrane Centre, Cochrane Collaboration, 2011) software.

Results

Database searched results

The search process, the number of initially searched studies, and the number of excluded studies are illustrated in *Figure 1*. Nine animal studies (5-9,15-18) and five clinical studies, including one RCT and four cohort studies (19-24) were eligible for final review. *Tables 1* and *2* show that the included studies were of high quality. The Jadad scale score of the RCT was five.

Animal study characteristics

The main characteristics of the analyzed animal studies are summarized in *Table 3*. The survival of the included

Table 2 Newcastle-Ottawa quality assessment scale for cohort studies included in this review

Studies	Selection				Comparability	Outcome			Total score
	Representa-tiveness of the exposed cohort	Selection of the non-exposed cohort	Ascertainment of exposure	Outcome of Interest not present at start of study		Assessment of outcome	Adequacy of duration of follow-up	Adequacy of completeness of follow-up	
Marr KA *et al.*, 2004 (19)	A	A	A	A	A	B	A	A	7
Singh N *et al.*, 2006 (20)	A	A	A	A	A	B	A	A	7
Upton A *et al.*, 2007 (21)	A	A	A	A	A	B	A	A	7
Rieger CT *et al.*, 2008 (22)	A	A	A	A	A	B	A	A	7

animal studies with combination therapy was significantly prolonged compared with echinocandin alone [67.3% versus 28.9%; RR =2.26, (95% CI, 1.79-2.87; P<0.00001); *Figure 2*], but no statistical difference compared with triazole alone [67.2% versus 52.3%; RR =1.19, (95% CI, 0.98-1.44; P=0.08); *Figure 3*].

IA models infected by *A. fumigatus* (8,16,18) or *A. flavus* (17) were treated with combination therapy of voriconazole and anidulafungin or either of monotherapy of voriconazole or anidulafungin. The efficacy of the combination therapy was synergistic compared with either of the monotherapy (16-18) (survival, P<0.05). Meanwhile, Petraitis *et al*. (16) concluded that anidulafungin at a dosage of 10 mg/kg/day was antagonistic to voriconazole. Seyedmousavi *et al*. (18) showed that the combination therapy was additive in treatment of voriconazole-resistant IA. However, Van de Sande *et al*. (8) showed that the monotherapy of voriconazole was therapeutically effective and superior to the monotherapy of anidulafungin and that the combination therapy did not significantly improve the therapeutic outcome of either of the monotherapy.

Combination therapy of voriconazole and caspofungin in male Guinea pig IA model was demonstrated to be highly effective compared with caspofungin monotherapy, but no differences compared to voriconazole (7). However, another study showed highly effective (15) (survival, P=0.048). Combination therapy of itraconazole and micafungin in female mice IA model significantly improved the efficacy in prolonging survival compared with either of the monotherapy of micafungin (6), while traconazole and micafungin might be antagonistic to each other (9). Petraitis *et al*. (5) found that combination therapy of ravuconazole and micafungin might increase efficacy, sparing toxicity, or both (P<0.05).

Human study characteristics

A summary of the human study characteristics included in this review is presented in *Table 4*. The sample sizes of the reviewed human studies varied widely [47-405]. Five of the studies had treatment duration of 12 weeks or 90 days and used mortality as the endpoint.

Four studies (19-22) compared the combination therapy of voriconazole and caspofungin with voriconazole, caspofungin, or lipid formulation of amphotericin B. Marr *et al*. (19) found lower mortality in the combination therapy of voriconazole and caspofungin than monotherapy of voriconazole. Rieger *et al*. (22) showed that the mortality at the end of treatment of the combination of voriconazole and caspofungin and other treatment was 11% and 34% three months after initiation of combination therapy. Meanwhile, Singh *et al*. (20) considered that the combination therapy of voriconazole and caspofungin might be a preferable therapy. However, Upton *et al*. (21) did not observe any significant difference between this combination therapy and either of the monotherapy.

In the RCT (24), 277 patients enrolled from 93 sites in 24 countries were randomised to receive either voriconazole plus placebo (monotherapy) or voriconazole plus anidulafungin (combination therapy). Of the 135 patients who received this combination therapy, 26 (19.3%) died by week 6, compared to 39/142 (27.5%) recipients receiving either of the monotherapy (P=0.09; 95% CI, –18.99, 1.51); 39 (28.9%) died by week 12, compared to 55/142

Table 3 Characteristics of included animal studies

Studies	Types of animals	Sample sizes	Aspergillus	MIC (μg/mL)	Infective doses	Treatments		Duration of treatment (days)	Findings
						Combination therapy	Monotherapy		
Kirkpatrick WR *et al.*, 2002 (7)	Male guinea pigs	72	A. fumigatus	VRC 0.5, CAS 32	1×10^6 conidia	CAS 1 or 2.5 mg/kg/day IP + VRC 5 mg/kg/day PO	CAS 1 or 2.5 mg/kg/day IP or VRC 5 mg/kg/day PO	5	Mortality↓ (P<0.0025 compared to CAS); no differences compared to VRC
Luque JC *et al.*, 2003 (6)	Female mice	40	A. fumigatus	ITZ 1.56, MICA >16	8×10^6 conidia	MICA 3 mg/kg q12h + ICZ 100 mg/kg/day	MICA 3 mg/kg q12h or ICZ 100 mg/kg/day or no drug	12	Survival↑ (P<0.05 compared to MICA); no differences compared to ICZ
Petraitis V *et al.*, 2003 (5)	Female rabbits	36	A. fumigatus	RAV 1.0, MICA 0.25	1×10^8-1.25×10^8 conidia	MICA 1 mg/kg/day IV + RAV 2.5 mg/kg/day IV	MICA 1 mg/kg IV or RAV 2.5 mg/kg IV or no drug	12	Mortality↓ (P≤0.001); residual fungal burden↓ (P≤0.05); galactomannan indexes↓ (P≤0.01)
MacCallum DM *et al.*, 2005 (15)	Male Guinea pigs	90	A. fumigatus	VRC 0.032 to 0.5, CAS 0.125	10^4 or 10^3 conidia/g	CAS 1 mg/kg/day IP + VRC 1 mg/kg PO q12h	VRC 1 mg/kg PO q12h or CAS 1 mg/kg/day IP	7	Survival↑ (P=0.048 compared to CAS with 10^3 conidia/g)
Clemons KV *et al.*, 2006 (9)	Female mice	40	A. fumigatus	NR	3.96×10^4 conidia	MICA 1 mg/kg/day + ICZ 100 mg/kg/day	MICA 1 mg/kg/day or ICZ 100 mg/kg/day or no drug	10	Survival↓ (P>0.05)
van de Sande WW *et al.*, 2009 (8)	Female rats	58	A. fumigatus	NR	NR	AFG 20 mg/kg/day on day 1, followed 5 mg/kg/day + VRC 7.5, 10, 12.5, and 15 mg/kg on days 0, 1, 2, and 3 and 17.5 mg/kg on day 4 and beyond, IP q12h	AFG or VRC or no drug	5-10	Survival↓ (P=0.3290); galactomannan indexes (P=0.0238 and P=0.0357)

Table 3 (*continued*)

Table 3 (*continued*)

Studies	Types of animals	Sample sizes	Aspergillus	MIC (μg/mL)	Infective doses	Treatments		Duration of treatment (days)	Findings
						Combination therapy	Monotherapy		
Petraitis V *et al.*, 2009 (16)	Female rabbits	70	A. fumigatus	VRC 0.5 to 1.0, AFG 0.25	1.0×10^8-1.25×10^8 conidia	AFG 5 or 10 mg/kg/day IV + VRC 10 mg/kg q8h IV	AFG 5 or 10 mg/kg/day IV or VRC 10 mg/kg q8h IV or no drug	12	Survival↑ (P<0.001) (AFG 5 mg/kg/day); survival↓ (P>0.05) (AFG 10 mg/kg/day); residual fungal burden↓ (P<0.05); galactomannan indexes↓ (P<0.05)
Calvo E *et al.*, 2012 (17)	Male mice	240	A. flavus	VRC 0.5 to 1.0, AFG > 32	8×10^3 CFU	AFG 1 mg/kg/day IP + VRC 12.5 mg/kg PO q12h	AFG 1 mg/kg/day IP or VRC 12.5 mg/kg PO q12h or no drug	7	Survival↑ (P<0.05); residual fungal burden↓ (P<0.05); galactomannan indexes↓ (P<0.05)
Seyedmousavi S *et al.*, 2013 (18)	Female mice	882	VRC-S and VRC-R A. fumigatus	VRC 0.25 and 4, AFG 0.031	2.4×10^7 and 2.5×10^7 conidia	AFG 20 mg/kg/day + VRC 20 mg/kg	AFG 10 mg/kg/day or VRC 20 mg/kg	7	Synergistic in VRC-S; additive in VRC-R

AFG, anidulafungin; AMB, amphotericin B; CAS, caspofungin; ICZ, itraconazole; L-AMB, liposomal amphotericin B; MICA, micafungin; POC, posaconazole; RAV, ravuconazole; VRC, voriconazole; VRC-S, voriconazole-susceptible; VRC-R, voriconazole-resistant; IP, intraperitoneal; IV, intravenous; PO, peros (oral); q12h, every 12 h; NR, not reported; MIC, minimal inhibitory concentration.

(38.7%) recipients receiving either of the monotherapy (P=0.08; 95% CI, –21.4, 1.09). The combination therapy of voriconazole and anidulafungin results in a trend towards improved overall survivals compared with monotherapy of voriconazole in patients with proven or probable IA.

Discussion

In this review, to assess the efficacy of the combination therapy of triazole and echinocandin in treatment of IA, we systematically assessed publications on the combination therapy of triazole and echinocandin in treatment of IA, including the animal studies and clinical studies. We found that the survival in the combined therapy groups were significantly improved in the animal studies compared with monotherapy of echinocandin [RR =2.26, (95% CI, 1.79-2.78; P<0.00001)], but no statistical difference compared with monotherapy of triazole [RR =1.19, (95% CI, 0.98-1.44; P=0.08)]. It only suggests that the addition of triazole to echinocandin results in a trend towards improved overall survival in animals with IA. Meanwhile, we also found that the combination therapy of triazole and echinocandin in treating IA also results in a trend towards improved the survival in clinical studies.

To the best of our knowledge, this is the first study to assess the efficacy of the combination therapy of triazole and echinocandin in IA in both animal studies and clinical studies. However, there are some limitations in this review. First, the animal species, infective dosages of *Aspergillus*, route of infections, and antifungal drugs and doses are different

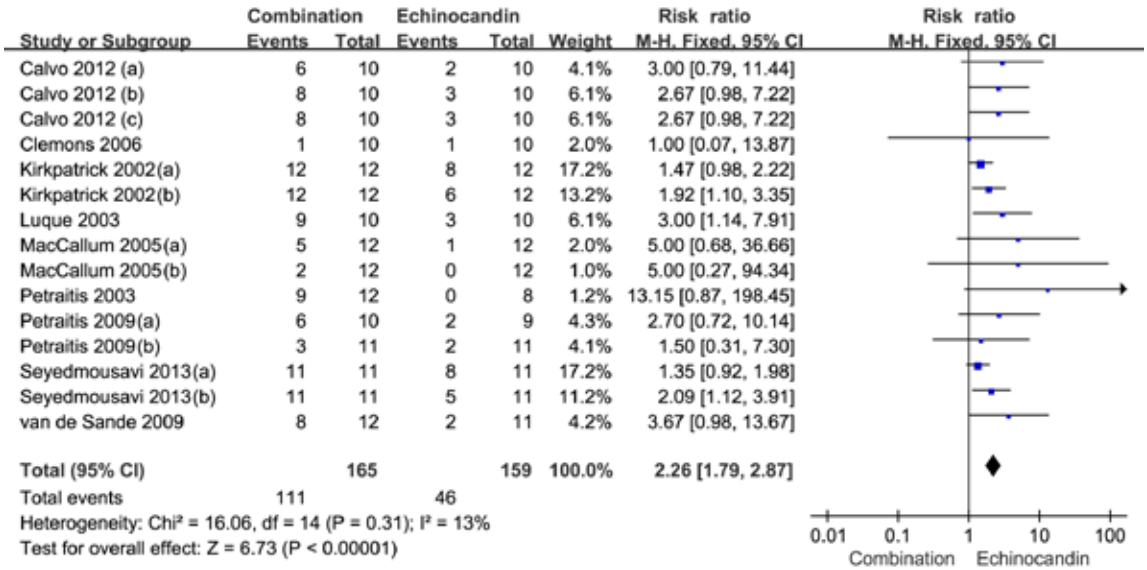

Figure 2 Forest plot showing the survival of the combination therapy of triazole and echinocandin compared with monotherapy of echinocandin in animal studies.

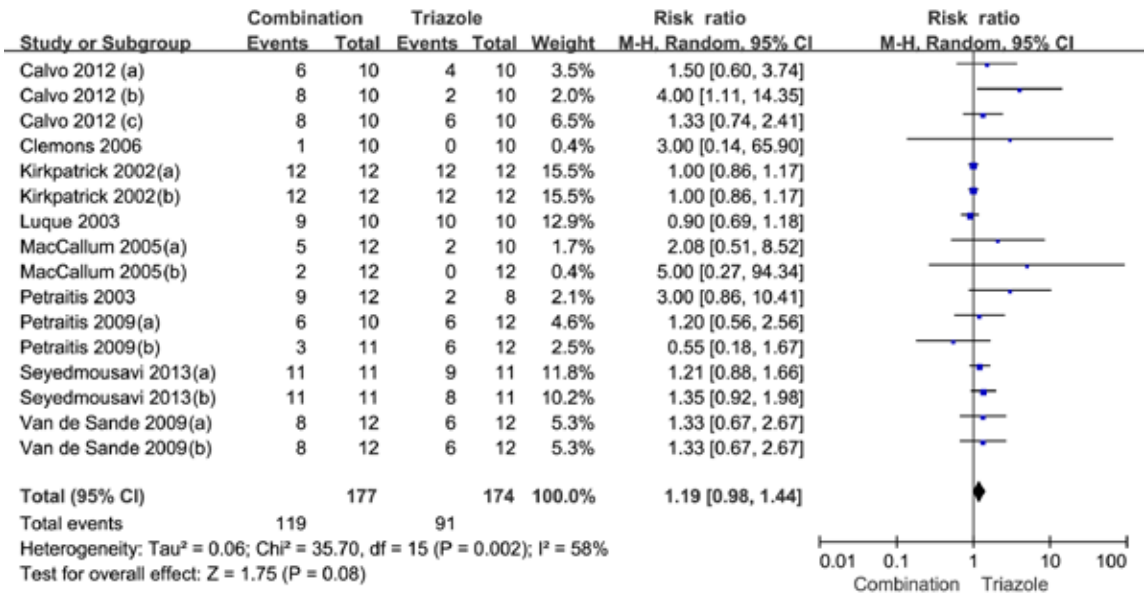

Figure 3 Forest plot showing the survival of the combination therapy of triazole and echinocandin compared with monotherapy of triazole in animal studies.

among some animal studies. Second, there may be difference between animals and humans in drug metabolism rate. For an example, the metabolic rate in rodents is faster than in humans. Third, the clinical studies contained only one RCT.

Due to the different targets of triazole and echinocandin, simultaneous inhibition of fungal cell-wall and cell-membrane biosynthesis may result in a synergistic or additive function against *Aspergillus*. However, we did not find this expected outcome in some animal studies and clinical studies. The possible causes may be ascribed to that the doses of triazole or echinocandin used in animal studies are different.

The area under the curve (AUC)/MIC ratio, a

Table 4 Characteristics of included human studies

Studies	Sample sizes	Age mean (years)	Study population	Types of studies	Treatments		Treatment duration (days)	End-point	Outcome measure
					Combination	Monotherapy			
Marr KA *et al.*, 2004 (19)	47	45	HSCT	Cohort	VRC 6 mg/kg q12h IV for 1 day and then 4 mg/kg q12h + CAS 70 mg IV for 1 day and then 50 mg/d	VRC 4 mg/kg q12h IV, AMB 1 mg/kg/day	90	Mortality	HR =0.28 (95% CI, 0.1-0.92); P=0.01
Singh N *et al.*, 2006 (20)	87	50	Organ transplant recipients	Cohort	VRC 6 mg/kg q12h IV for 1 day and then 4 mg/kg q12h + CAS 70 mg IV for 1 day and then 50 mg/d	L-AMB 5.2 mg/kg/d	90	Mortality	HR =0.58 (95% CI, 0.3-1.14); P=0.12
Upton A *et al.*, 2007 (21)	405	40.7	HSCT	Cohort	VRC + CAS	VRC (before 1996: AMB 0.5 mg/kg/day; after 1996: L-AMB 5 mg/kg/day)	90	Mortality	HR =2.3 (95% CI, 0.6-9.4); P=0.23
Rieger CT *et al.*, 2008 (22)	56	46	Haematological cancer	Cohort	VRC 6 mg/kg q12h IV for 1 day and then 4 mg/kg q12h + CAS 70 mg IV for 1 day and then 50 mg/d	L-AMB 3 mg/kg/d ± CAS 70 mg IV for 1 day and then 50 mg/d or VRC 6 mg/kg q12h IV for 1 day and then 4 mg/kg q12h	90	Efficacy; survival	No adjusted analysis
Marr KA *et al.*, 2012 (24)	277	51.9	HSCT and haematological malignancies	RCT	VRC 6 mg/kg q12h IV for 1 day and then 4 mg/kg q12h + AFG 200 mg IV for 1 day and then 100 mg/d	VRC 6 mg/kg q12h IV for 1 day and then 4 mg/kg q12h + placebo	42 or 84	Mortality	P=0.09; 95% CI, –19, 1.5 (42 days); P=0.08; 95% CI, –21.4, 1.09 (84 days)

HSCT, hematopoietic stem cell transplant; AFG, anidulafungin; AMB, amphotericin B; CAS, caspofungin; ICZ, itraconazole; L-AMB, liposomal amphotericin B; MICA, micafungin; POC, posaconazole; RAV, ravuconazole; VRC, voriconazole; IV, intravenous; IP, intraperitoneal; PO, peros (oral); q12h, every 12 h; HR, hazard ratio.

pharmacokinetic/pharmacodynamic (PK/PD) index, is used to predict triazole therapeutic efficacy (25,26) while both the AUC/MIC and the C_{max}/MIC are used to predict echinocandin therapeutic efficacy (27,28). However, according to Petraitis *et al*. (16), anidulafungin was synergistic at a dosage of 5 mg/kg/day but antagonistic at 10 mg/kg/day in the combination with voriconazole, suggesting that a higher dosage of echinocandin may be deleterious to the combination therapy. The reason for this phenomenon may be paradoxical echinocandin activity (29).

The resistance of *Aspergillus* to triazole may result in decrease of efficacy. According to a study by Seyedmousavi *et al*. (18), combination therapy of voriconazole and anidulafungin for IA was synergistic in voriconazole-susceptible *A. fumigatus*, but additive in voriconazole-resistant *A. fumigatus*.

In the RCT (24), the prolongation of survival, either six weeks (P=0.09) or 12 weeks (P=0.08), results in a trend towards improved in the combination therapy of voriconazole and anidulafungin compared with monotherapy of voriconazole. Of the four human cohort studies, two studies (19,22) observed that the combination therapy of triazole or echinocandin was associated with a significant reduction in mortality compared with other treatments and another study (20) might be a preferable therapy; However, one study (21) revealed that there was no significant difference between the combination therapy and either of the monotherapy. It suggested that the effectiveness of the combination therapy of triazole and echinocandin may be better than either of the monotherapy or other combination. Well-designed RCTs and further improved clinical trials are necessary to study the effectiveness of the combination therapy.

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Original Article

Transplantation of umbilical cord mesenchymal stem cells alleviates pneumonitis of MRL/lpr mice

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Objective: To investigate whether the umbilical cord mesenchymal stem cells (UC-MSCs) transplantation in the MRL/lpr mice has effect or not on their pneumonitis and the possible mechanisms underlying this treatment.

Methods: Twenty four 18-week-old MRL/lpr female mice were divided into three groups as following: the group 2 (UC-MSCT group) have been transplanted with 1×10^6 UC-MSCs through caudal vein, the group 3 (multi-UC-MSCT Group) have been transplanted with 1×10^6 UC-MSCs three times and the group 1 (control group) have been treated with 0.5 mL phosphate buffer saline (PBS) as control. The histopathology of the lung was observed. The pulmonary expression of high mobility group box protein-1 (HMGB-1) was measured by western blot and detected by quantitation real time polymerase chain reaction (PCR). Immunohistochemistry method was used to detect HMGB-1 expressions in pulmo.

Results: In comparision to control ground mice, UC-MSCs significantly reduced interstitial pneumonitis in the MRL/lpr mice. The lung peribronchiolar lesion index of UC-MSCT group (1.40±0.24) and multi-UC-MSCT group (1.02±0.29) were significantly decreased as compared to control group (1.95±0.35) ($P<0.01$). The perivascular lesion index of UC-MSCT group (1.20±0.18) and multi-UC-MSCT group (1.08±0.16) were also significantly reduced as compared to control group (1.56±0.32) ($P=0.018$, 0.002) and the lung alveolar areas lesion index of control group (1.72±0.34) was significantly increased as compared to UC-MSCT group (1.30±0.21) and multi-UC-MSCT group (1.05±0.15) ($P=0.011$, 0.000). The lung HMGB-1 protein in UC-MSCT group (0.32±0.04) and in multi-UC-MSCT group (0.28±0.06) were both significantly decreased as compared to that in control group (0.80±0.21) ($P<0.05$). The level of HMGB-1 mRNA in UC-MSCT group (4.68±0.37) and in multi-UC-MSCT group (4.35±0.10) lung were both significantly decreased as compared to those in control group (16.29±3.84) ($P<0.05$). In immunohistochemical staining lung sections, high expression of HMGB-1 was found mainly located in the cytoplasm and extracellular matrix of MRL/lpr mice pulmonary epithelial cells, the expression of HMGB-1 in UC-MSCT group and multi-UC-MSCT group was significantly decreased as compared to that in the control group.

Conclusions: These findings indicate that UC-MSCs have a therapeutic effect on systemic lupus erythematosus (SLE) pneumonitis, possibly by inhibiting HMGB-1 expression, which suggests a potential application of UC-MSCs in the treatment of human lupus.

Keywords: Umbilical cord mesenchymal stem cells (UC-MSCs); systemic lupus erythematosus (SLE); pneumonitis; high mobility group box protein-1 (HMGB-1)

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Introduction

Systemic lupus erythematosus (SLE) is a common and potentially fatal autoimmune disease characterized by multi-organ injuries including renal, pulmonary, cardiovascular, neural, musculoskeletal, and cutaneous involvements. SLE can affect any organ at any stage, during the course of the disease, but the lungs are relatively involved late (1). Lung involvement can be sometimes the presenting feature of SLE in the form of pleuritis, pleural effusion, lupus pneumonitis or interstitial lung disease (ILD). Once the lungs are involved, there are always some other organs involved, such as glomerulonephritis, which represent SLE highly active. Acute lupus pneumonitis (ALP) or chronic ILD without an accurate treatment may lead to hypoxic respiratory failure and cause death in final. In 1999, Wang *et al.* (2) found that high-mobility group box chromosomal protein 1 (HMGB-1) can be released into the extracellular and mediate inflammatory responses, which was considered to be an important inflammation mediator of endotoxemia and sepsis. Recent studies have demonstrated that HMGB-1, actively secreted by macrophage/monocytes under inflammatory stimuli (3), was found to act as a proinflammatory cytokine in SLE. The presence of anti-HMGB1 antibodies correlates with disease activity in SLE patients (4). The induction of this proinflammatory cytokines may play a pathogenic role in the development of pneumonitis in MRL/lpr mice. Despite improved supportive care, aggressive immunosuppressive medical therapies, and new therapeutic interventions, a subset of SLE patients continue to suffer significant morbidity and mortality from active disease. Therefore, it is urgent to develop more effective therapy for SLE, especially for those who are refractory to treatment.

Mesenchymal stem cells (MSCs) are multipotent stem cells which are able to differentiate into a variety of cell types, including osteoblasts, chondrocytes, adipocytes, and myoblasts (5-7). These cells have been shown to have immunosuppressive properties and to reduce inflammation (8-11). Human MSCs suppress lymphocyte alloreactivity *in vitro* in mixed lymphocyte cultures through human leukocyte antigen-independent mechanisms (8). Previous studies showed that MSCs could inhibit lymphocyte proliferation induced by a variety of mitogens (11-13). Transplantation of *ex vivo*-expanded bone marrow MSCs (BM-MSCs) proved effective in treating acute graft-versus-host-disease (GVHD) by inhibiting T-lymphocyte function (14-16). MSCs, which can produce important growth factors and cytokines, have a strong propensity to ameliorate tissue damage in response to injury and disease. Relevant to this investigation, Huang *et al.* demonstrated that BM-MSCs could be transplanted into lung tissues of rats, and transformed into type II alveolar cells and was shown to prevent the development of pulmonary fibrosis (17). Sun *et al.* have reported that MSCs in patients with SLE grew much slower and showed senescence behavior compared with those in normal control patients (18). Based on these findings, we hypothesized that transplantation of allogeneic MSCs may be a potential therapeutic approach for SLE. Currently, BM-MSCs represent the major source of MSCs for cell therapy. However, aspiration of BM-MSCs is invasive, and the population and differentiation potential of BM-MSCs decrease significantly with age (19). Compared to BM-MSCs, umbilical cord-MSCs (UC-MSCs) may be collected without causing pain to the donors, and these cells have greater proliferative potential. Therefore, for allogeneic transplantation, UC-MSCs should be more promising than BM-MSCs. We have also found that UC-MSCs transplantation is effective in preventing the development of lupus-like nephritis in MLR/lpr mice (20). Then, what about lupus pneumonitis? Weather UC-MSCs have effect on the other hazardous complication of SLE or not?

In this study, our results indicated that UC-MSCs can relieve the extent of pulmonary injury in MLR/lpr mice, which may provide a new feasible measure for the management of lupus pneumonitis in SLE patients.

Materials and methods

Mice

Twenty-four female MRL/lpr mice (six weeks old), weighing 20.4±0.5 g (mean ± standard deviation, SD), were purchased from Shanghai SLAC Laboratory Animal Institute Co. Ltd. The mice were maintained in a specific pathogen-free animal facility of the Affiliated Hospital of Nantong University. The MRL/lpr mice were randomly divided into the following three groups (eight mice in each group): group 1 mice receiving 0.5 mL phosphate buffer saline (PBS) at 18 weeks of age (control); group 2 mice receiving transplantation of 1×10^6 UC-MSCs (UC-MSCT) once at 18 weeks of age; group 3 mice receiving multi-transplantation of 1×10^6 UC-MSCs (multi-UC-MSCT) at three consecutive weeks (18, 19, and 20 weeks of age); At 29 weeks of age, the mice were sacrificed and the lung

tissue was collected. The experimental protocols conformed to the animal care guidelines of the China Physiologic Society and were approved by our Institutional Animal Research Committee.

MSCs culture

UC was obtained from the Gynecology Department at Affiliated Hospital of Nantong University. Tissue collection for this study was approved by The Affiliated Hospital Ethics Committee and informed consent was obtained from newborns' parents. The tissue was minced into 1-2 mm^3 pieces, and the minced tissue was incubated with 0.075% collagenase type II (Sigma, St Louis, MO, USA) for 30 min and then with 0.125% trypsin (Gibco, Grand Island, NY, USA) for 30 min with gentle agitation at 37 ℃. Cells from UC were plated at a density of 1×10^6 cells/cm^2 in non-coated T-25 cell culture flasks (Becton Dickinson, San Jose, CA, USA). Growth medium (GM) consisted of Dulbecco's modified Eagle's medium with low glucose (Gibco) and 5% fetal bovine serum (FBS, HyClone, Logan, UT, USA), supplemented with 10 ng/mL vascular endothelial growth factor (Sigma), 10 ng/mL epidermal growth factor (Sigma), 100 U/mL penicillin and 100 mg/mL streptomycin (Sigma), and 2 mmol/L glutamine (Gibco). Cultures were maintained in a humidified atmosphere with 5% CO_2 at 37 ℃. The medium was replaced after three days. The medium was changed twice weekly thereafter. A cell monolayer formed within two weeks, consisting of homogeneous bipolar spindle-like cells in a whirlpool-like array. Flow cytometric analysis showed that the UC-derived cells were positive for CD29, CD44, CD105, and CD166, but negative for CD14, CD34, CD38, CD45, and HLA-DR. Once 60-80% confluence had been reached, adherent cells were re-plated at a density of 1×10^4/cm^2 in UC-MSCs growth medium (UC-GM) for expansion. After passage 3, cells were used for transplantation. Flow cytometric analysis was performed on passage 2.

Histopathological analysis

To assess pathologic lung changes after MSCs transplantation, the left lungs were cut into small pieces and fixed in 10% formalin for 24 h at 4 ℃. Paraffin sections (4 mm) were stained with hematoxylin and eosin (HE) and Masson. The severity of airtube and vascellum injury was evaluated in a blinded manner by histologic examination of the sectioned lungs. Results were expressed according to the assay of Hasegawa *et al.* (21). The perivascular and peribronchiolar infiltrates were scored on the basis of histopathological findings: 0, normal; 1, less than three cell layers; 2, three to six cell layers; or 3, more than six layers. The index of perivascular lesion was indicated as the sum of all the scores per section divided by the number of all vessels per section. The index of peribronchiolar lesion was indicated as the sum of all the scores per section divided by the number of all bronchioli per section. The infiltrates in alveolar areas in high-power fields (×400 magnification) were scored as follows: 0, no infiltrating mononuclear cells; 1, less than 10 infiltrating cells; 2, less than 20 infiltrating cells; or 3, more than 20 infiltrating cells. The alveolar lesions index was indicated as the mean value of 20 random fields per section. The sections were evaluated by one of us, who was blinded to the treatment given.

RNA isolation and real-time quantitative PCR

To investigate the production of HMGB-1 in lung after the MSCs treatment, total RNA was extracted from pulmonary epithelial cells using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's recommendations. The production of HMGB-1 mRNA in lung was quantified by real-time quantitative polymerase chain reaction (PCR) using the TaqMan PCR MASTER MIX kit (Applied Biosystems, Foster City, CA, USA). The production of mRNA was determined and normalized to the expression of the internal housekeeping gene GAPDH. Primer and probe sequences are described as follows: HMGB-1 (359 bp): forward, 5'-ATGTTCTGCTCCTTACC-3', and reverse, 5'-AGTTTATCCGCTTTCC-3'.

Immunohistochemistry, western blot analysis

To detect HMGB-1 expression, lungs were snap-frozen in optional cutting temperature solution (OCT) compound (Sakura, Osuka, Japan) and cut into 5 μm pieces. Sections were analyzed by the avidin-biotin-peroxidase method, using biotin-labeled goat anti-murine HMGB-1 polyclonal antibody (Santa Cruz, CA, USA). Preimmune biotin-labeled goat serum served as a negative control. Analysis with monoclonal antibody (mAb) against human nuclei (MAB1281, Chemicon International) was performed following the manufacturer's instructions to detect UC-MSCs in kidneys of mice treated with UC-MSCs. For western blot analysis, lung homogenates were blotted with anti-mouse HMGB-1 antibodies (Santa Cruz, CA, USA).

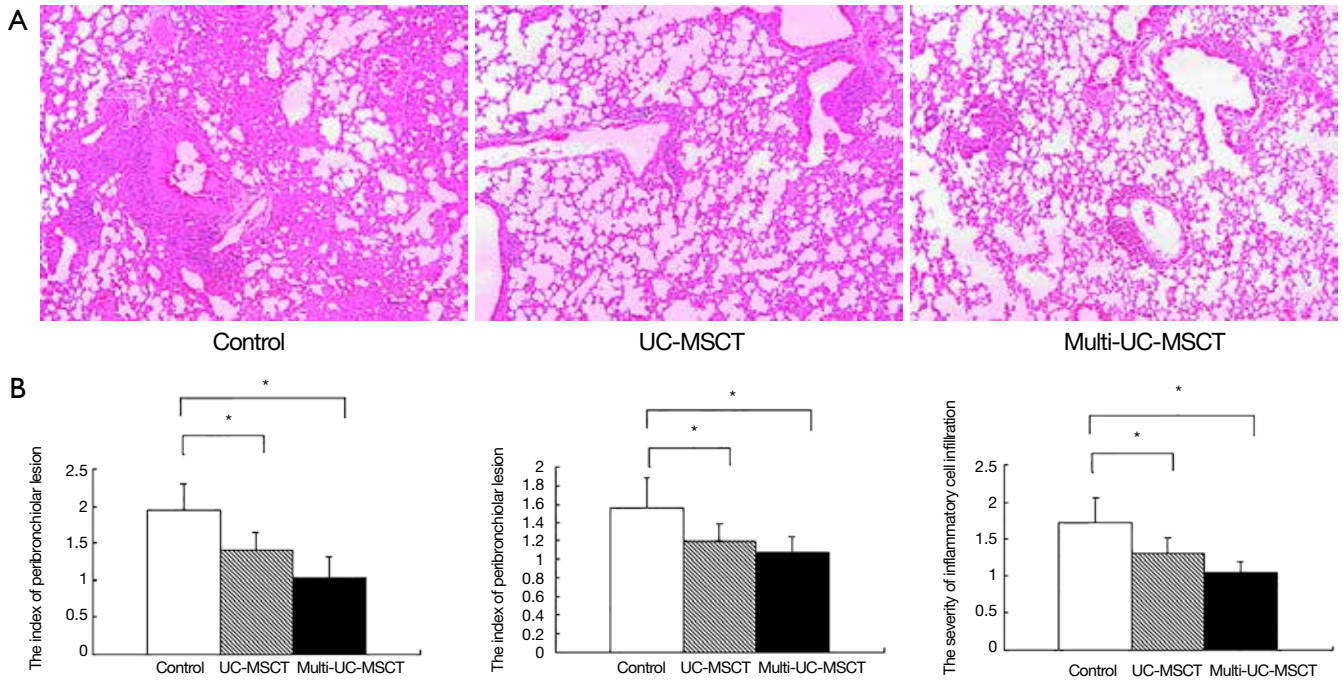

Figure 1 UC-MSCT improved pulmonary pathological injury in MRL/lpr mice. (A) UC-MSCT and multi-UC-MSCT reduced perivascular and peribronchiolar infiltration of inflammatory cell, improving vascular congestion and edema (H&E staining); (B) The degrees of pneumonitis of control and treated MRL/lpr mice were scored as described in Materials and methods. Values are presented as the mean and standard deviation (n=8 mice per group). All treatments (UC-MSCT and multi-UC-MSCT) exhibited a lower index of perivascular and peribronchiolar lesion (*P<0.05 *vs.* control). The severity of alveolar inflammatory cell infiltration was also much slighter (*P<0.05 *vs.* control).

Band detection was conducted using an enhanced chemiluminescence (ECL) detection system (Amersham Biosciences, Piscataway, USA).

Statistical analysis

Quantitative data were expressed as mean ± SD. SPSS 11.0 software was used for statistical analysis. The single-factor analysis of variance (ANOVA) was used for the comparison among multiple sample means. We considered P<0.05 as statistically significant.

Results

UC-MSCs transplantation alleviates pneumonitis of MRL/lpr mice

As mentioned above, there were three groups in the present study: control, UC-MSCT and multi-UC-MSCT. Two mice died respectively at 26 and 28 weeks of age in the control, which were also included in the following analysis. In control group, MRL/lpr mice showed typical interstitial lung disorders according to histopathology such as the perivascular and peribronchiolar focal aggregation of lymphocyte and mononuclear cell (*Figure 1A*). Besides the inflammatory cell infiltration, vascular congestion and edema were found in pulmonary interstitial. We found that all the treatment groups showed much less inflammatory cell infiltration in comparison with control mice. The index of perivascular and peribronchiolar lesion and the severity of inflammatory cell infiltration were much slighter (*Figure 1B*, *Table 1*). Further histological analysis demonstrated that the lungs from the treatment groups showed reduced deposition of collagen (*Figure 2*). In addition, the degree of lung injury in the multi-UC-MSCT group was significantly lower than that in the UC-MSCT. These findings suggest that UC-MSCT is a superior therapeutic approach for treating pneumonitis of MRL/lpr mice. Multi-infusion of UC-MSCs may enhance their effects.

UC-MSCs transplantation decreases the expression of HMGB-1 in lung of MRL/lpr mice

Recent studies have showed that HMGB-1 play an important

Table 1 The effect of UC-MSCT on pulmonary pathological injury in MRL/lpr mice

Group	Number	The index of peribronchiolar lesion	The index of perivascular lesion	The severity of inflammatory cell infiltration
Control	8	1.95±0.35	1.56±0.32	1.72±0.34
UC-MSCT	8	1.40±0.24*	1.20±0.18*	1.30±0.21*
Multi-UC-MSCT	8	1.02±0.29*	1.08±0.16*	1.05±0.15*

*, P<0.05 as compared to control.

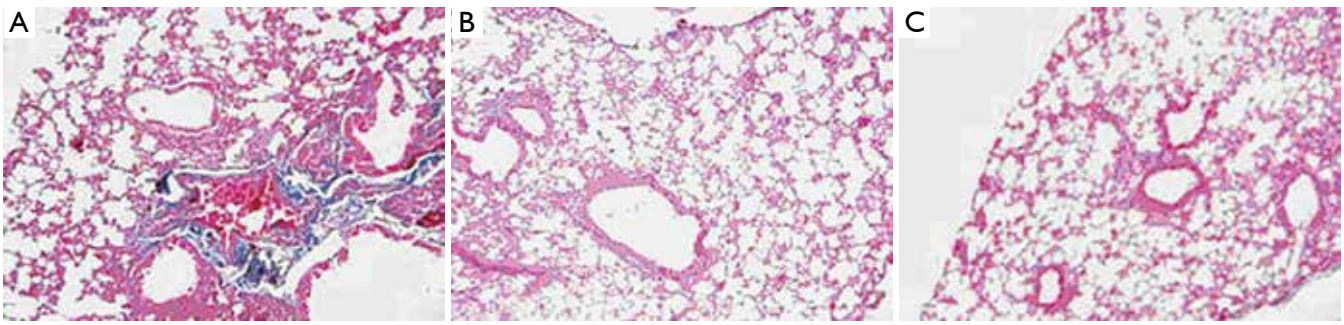

Figure 2 UC-MSCT and multi-UC-MSCT reduced deposition of collagen in the lung of MRL/lpr mice (Masson staining) (A-C).

role in the pathogenesis of SLE (4,22,23). We found that the expression of HMGB-1 protein in all the treatment mice was significantly lower than in control (*Figure 3A*,*B*; P<0.05). The differences in mRNA expression corresponded well with protein expression (*Figure 3C*, *Table 2*). Immunohistochemical staining for HMGB-1 showed marked intense staining in the control lungs. This positive staining was much weaker among all the treatment groups (*Figure 3D*). These results indicate that transplanting UC-MSCs is effective in the treatment of SLE pneumonitis, possibly by inhibiting HMGB-1 expression.

Discussion

SLE is sometimes a severe and thorny disease that often represents a therapeutic challenge because of its heterogeneous organ manifestations. Symptomatic pulmonary manifestations occur in 40% to 50% of the patients with SLE during the course of the disease (24). At autopsy, histological changes associated with SLE are found in almost all cases (25). Pneumonitis in SLE can be a severe and potentially life-threatening complication even despite current optimized therapy. The pulmonary manifestation of lupus is an important indicator of overall prognosis (26). Generally, pneumonitis in SLE patients will primarily be treated with glucocorticoids, cytotoxic and immunoregulatory agents (27). Alveolar haemorrhage is seen as an indication for additional plasma exchange (28-30). Rituximab has also been reported to provide benefit in these conditions (31-33) and infliximab has suggested effective to ILD which is refractory to cyclophosphamide (30). Though, the severe and life-threatening pulmonary manifestations in SLE, such as the acute course of lupus pneumonitis and the more smouldering course of ILD, represent a therapeutic challenge.

Previous studies about MSCs and ILD mainly concentrated in bleomycin (BLM) induced lung injury. MSCs can homing to and locate in the damaged lung tissue (34-36). After transplanting MSCs from male BALB/c rats to female C57BL/6 rats which have been caused lung damage by BLM, Ortiz *et al.* have found that donor derived MSCs can be settled at the site of injury of pulmonary receptors induced by BLM, and showed the epithelial like morphology, and can reduce the degree of inflammation and collagen deposition (35). Studies from Zhao *et al.* showed that MSCs in rat lung tissue damaged of BLM to differentiate into alveolar epithelial cells and bronchial epithelial cells (36). Rojas *et al.* (34) has also found that the protective mechanism of MSCs on lung injury induced by BLM is related to not only the MSCs differentiation to specific phenotype of lung cell, but also the increasing granulocyte colony stimulating factor (G-CSF) and granulocyte macrophage

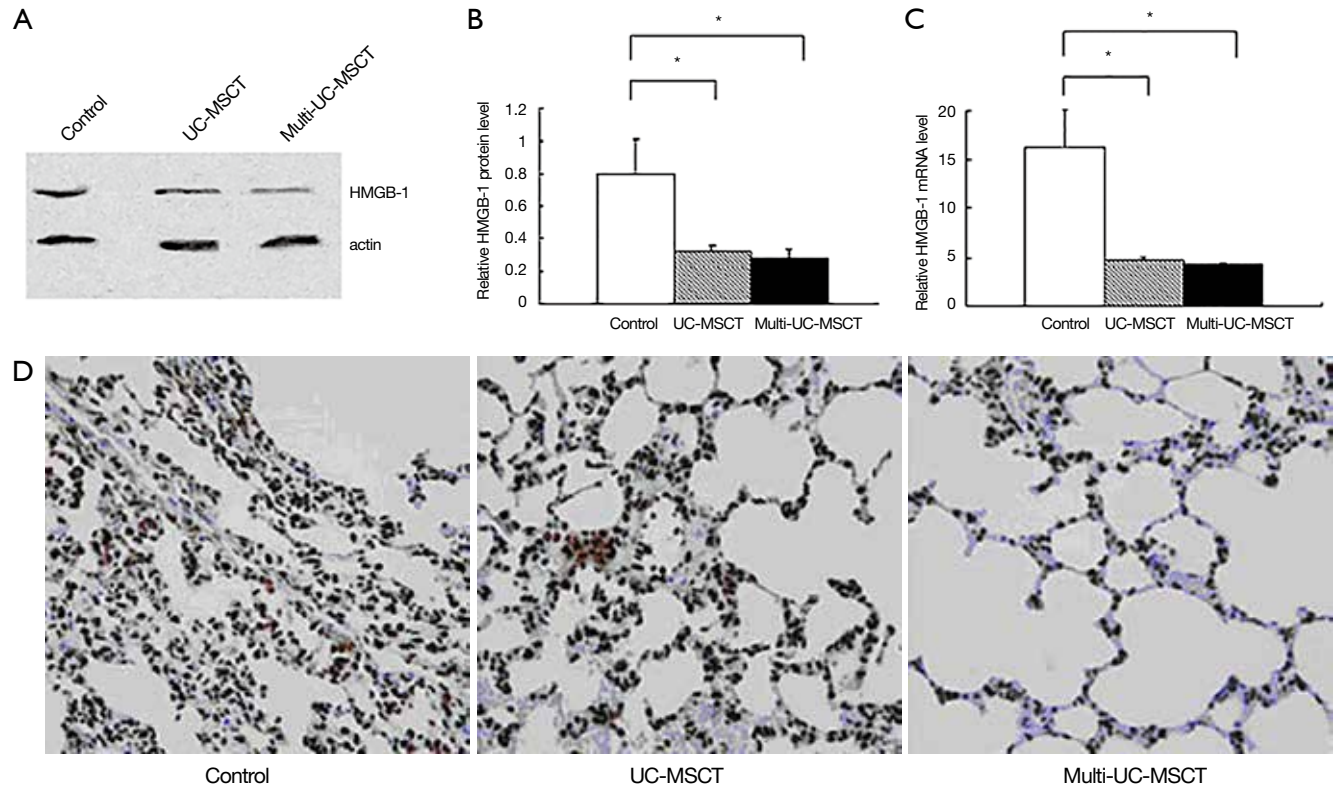

Figure 3 UC-MSCs treatment decreased the expression of HMGB-1. (A,B) HMGB-1 protein expression in control lungs was significantly higher than in all the treatment groups (*P<0.05 *vs.* control). β-actin was used as loading controls in western blot. Three repeated tests per group showed similar results; (C) HMGB-1 mRNA expression in control lungs was significantly higher than in all the treatment groups (*P<0.05 *vs.* control). GAPDH was used as internal control in RT-PCR. Three repeated tests per group showed similar results; (D) The expression of HMGB-1 in lung tissues was represented by immunohistochemical analysis. In the UC-MSCT and multi-UC-MSCT groups, lung HMGB-1 expression was significantly lower than that in the control group. Representative photomicrographs of the lung immunohistochemistry (×400) showed the increased redistribution of HMGB-1 from nucleus to cytoplasm and extracellular areas.

Table 2 Relative HMGB-1 mRNA level in MRL/lpr mice

Group	Number	Relative HMGB-1 mRNA level
Control	8	16.39±3.56
UC-MSCT	8	4.66±0.37*
Multi-UC-MSCT	8	4.45±0.10*

*, P<0.05 as compared to control.

colony stimulating factor (GM-CSF) which promote its own stem cell mobilization, and the decreasing release of inflammation factors involved in.

In the present study, we have demonstrated that transfusions of xenogeneic UC-MSCs significantly attenuate the severity of lung injury in MRL/lpr mice. There were significant differences in the airtube and vascellum injury levels between the treatment and control groups. Light microscopic examination of the lung tissues showed that the improvement of pulmonary pathology correlates well with reduced deposition of collagen and the infiltration of the interstitial inflammatory cell. It is of interest that three transfusions provided more significant reduction in the above-mentioned disease activity manifestations.

HMGB-1, originally characterized as a nuclear DNA-binding protein to be a regulator of transcription, has also been described to have an extracellular role when it is involved in cellular activation and proinflammatory responses (3,37-39). Monocytes and macrophages stimulated by LPS, TNF-α, or IL-1, secrete HMGB-1 (40,41). Addition of HMGB-1 to monocytes, macrophages, or neutrophils in culture induces the release of proinflammatory cytokines, including TNF-α, IL-1α, IL-1β, IFN-γ, MIP-2, and IL-8 (40-43). It can also activate the endothelial cells, increasing the expression of vascular cell adhesion molecule and cell adhesion molecules (44), which leads to the accumulation of inflammatory cells in the vascular wall to produce

vasculitis. HMGB-1 has been considered as a new pattern of inflammatory factor.

HMGB-1 has also been shown to act as an endogenous immune adjuvant by activating antigen-presenting cells (including dendritic cells and macrophages), through the receptor of advanced glycation end products (RAGE) and possibly toll-like receptor 2 and 4 mechanisms (45,46). Interestingly, it was recently shown that HMGB-1 and RAGE mediated TLR9-dependent activation of plasmacytoid dendrite cells by DNA-containing ICs (47). Thus, HMGB-1 plays an important role on the function of the immune system.

SLE is an autoimmune disease, whose pathological basis is vasculitis. Anti-HMGB-1 antibody was found in SLE patients (22). Popovic *et al.* colleagues have found high amounts of extracellular HMGB-1 in skin lesions of lupus (23). Deocharan and his colleagues found that immunization of non-autoimmune mice with a-actinin induced strong anti-nuclear antibody (ANA) response, particularly against chromatin. Furthermore, kidney glomerular IgG deposition and proteinuria were present in a-actinin-immunized mice (48). All above indicated HMGB-1 had an important effect on the genesis and development of SLE.

Al-Mutairi *et al.* reported that proinflammatory cytokines (TNF-α, IFN-γ, IL-8, IL-6) were more prevalent in the serum of SLE patients with pulmonary involvement compared with those without pulmonary manifestations (49). And HMGB-1 is deeply involved in inflammation and immunity. Studies from Maria *et al.* showed that inhibition of HMGB-1 protects against pseudomonas aeruginosa pneumonia in cystic Fibrosis (50). In the present study, we showed that the expression of HMGB-1 in lung was significantly reduced in all the treated mice in comparison with that in control animals. Therefore, downregulation of HMGB-1 expression may be one of the mechanisms involved in the treatment of MRL/lpr mice pneumonitis by UC-MSCs.

In summary, our study has shown that infusion of UC-MSCs exerts a therapeutic effect in treating pneumonitis in MRL/lpr mice without obvious major side effects. UC-MSCs were able to improved pulmonary pathological injury, reduced inflammatory cell infiltration, and reduced HMGB-1 expression in MRL/lpr mice. The results demonstrate that UC-MSCs could effectively prevent the development of pneumonitis of SLE. However, it remains to be determined whether UC-MSCs transfusions will reverse progression of established pneumonitis in SLE. Nevertheless, our findings provide an impetus for further investigations of the treatment of SLE with allogeneic MSCs readily available from umbilical cords.

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Original Article

Lentivirus vector-mediated Rho guanine nucleotide dissociation inhibitor 2 induces beta-2 adrenergic receptor desensitization in β_2AR desensitization mice model

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Background: It is well-known that chronic administration of β_2AR agonists can induce β_2AR desensitization. Our previous study showed that Rho guanine nucleotide dissociation inhibitor 2 (RhoGDI2) overexpression induced beta-2 adrenergic receptor (β_2AR) desensitization in airway smooth muscle cells. The purpose of this study was to further study the function of RhoGDI2 in β_2AR desensitization by β_2AR desensitization mouse model.

Methods: Studies were performed using a β_2AR desensitization mice model induced by salbutamol. The mice were randomly divided into five groups (n=45): RhoGDI2 overexpression group (n=10); RhoGDI2 siRNA group (n=10); empty viral vector group (n=10); experimental control group (n=10); blank control group—without any drug treatment (n=5). The first four groups were used the same methods and the same dose to establish β_2AR desensitization mice model by salbutamol. The first three groups that salbutamol-treated were used for intratracheal delivery of lentiviral vectors. Airway hyperreactivity was measured through a whole-body plethysmograph system. RhoGDI2, β_2AR, GRK2 mRNA and protein expression levels were then detected by RT-PCR and western blot analyses in fresh lung tissues. As well as the activity of GRK was assessed by light-dependent phosphorylation of rhodopsin.

Results: We successfully constructed β_2AR desensitization mouse model. As expected, airway responsiveness after inhaling acetylcholine chloride (Ach) was markedly increased in the RhoGDI2 overexpression group compared to experimental control group and blank control group when concentrations of Ach was 45 mg/mL (all $P<0.05$), while, it was markedly decreased in the RhoGDI2 siRNA group compared to experimental control group ($P<0.05$). RhoGDI2, GRK2 expressions and GRK enzymatic activity were significantly increased in RhoGDI2 overexpression group compared to experimental control group and blank control group (all $P<0.05$). RhoGDI2, GRK2 expressions and GRK enzymatic activity were significantly decreased in RhoGDI2 siRNA group compared to experimental control group and blank control group (all $P<0.05$). Conversely, β_2AR expression were significantly lower in RhoGDI2 overexpression group compared to experimental control group and blank control group (all $P<0.05$), exhibiting an inverse correlation with RhoGDI2 expression.

Conclusions: To sum up, our present studies found that RhoGDI2 might induce β_2AR desensitization and GRK2 might take part in RhoGDI2-mediated β_2AR desensitization.

Keywords: Rho guanine nucleotide dissociation inhibitor 2 (RhoGDI2); GRK2; β_2AR desensitization; lentivirus vector

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Introduction

Airway smooth muscle can be relaxed by β-adrenoceptor stimulation. This primarily involves β_2-adrenoceptors in human airways while airway β_2-adrenoceptors are sensitive to agonist-induced desensitization. The initial β-adrenoceptor response to salbutamol treatment is active; however, the treatment efficacy decreases with the duration and therapeutical cycles increasing. Desensitization is acquired rapidly, contributing to therapy failure. However, the mechanisms by which β-adrenoceptor develop desensitization to salbutamol are not fully understood. Previous study showed that Rho guanine nucleotide dissociation inhibitor 2 (RhoGDI2) level increased in asthmatic murine model of β_2-adrenoceptor desensitization by a proteomics approach (1). While the association and function of RhoGDI2 with β_2AR in β_2AR desensitization remains unclear.

RhoGDI2 has been identified as a regulator of RhoGTPase (2). It is mainly in hematopoietic, endothelial, and epithelial cells (3,4). RhoGDI2 has been linked to tumorigenesis and metastasis. Its precise role in cancer varies with tumor type (5). RhoGDI2 expression is downregulated in several cancer types, such as bladder, lung and lymphoma (6,7), but is upregulated in prostate and gastric cancer (8,9). Further, RhoGDI2 protein was significantly upregulated in high-grade compared with low-grade ovarian cancers, correlated with histological subtype, and did not correlate with stage of ovarian cancer (10). Thus, RhoGDI2 appear to carry out different functions within the same tumor type.

Previous study had shown that RhoGDI2 was overexpressed in chemotherapy-resistant paclitaxel-resistant ovarian cancers and fibrosarcoma cells, respectively (11). At the same time, it was reported that RhoGDI2 conferred resistance against cisplatin-induced apoptosis in gastric cancer cells (12). In previous study revealed that RhoGDI2 is a contributor to 5-FU resistance in colon cancer (13). The subsequent study also demonstrated that RhoGDI2 also confers resistance to 5-FU in gastric cancer cells (14). The results lead to the conclusion that high levels of RhoGDI2 expression are associated with chemotherapy resistance in certain types of cancers. Our previous study showed that lentivirus vector-mediated RhoGDI2 overexpression induces beta-2 adrenergic receptor (β_2AR) desensitization in airway smooth muscle cells. This study was to further study RhoGDI2 induces β_2AR desensitization in mice model.

To date, there has been no report on the significance of RhoGDI2 expression for β_2AR desensitization. Hence, in this study, we studied the effects of RhoGDI2 on β_2AR desensitization and the underlying mechanism *in vivo*.

Materials and methods

Reagents

Lentivirus vectors were created as previously described (15-17). The *RhoGDI2* gene (NCBI NM_001175.4) coding sequence was amplified by PCR and subcloned into a lentivirus expression plasmid pWPXL-eGFP vector (TronoLab, France) along with BamH I and Mlu I restriction sites to construct a lentivirus-based overexpression vector carrying the RhoGDI2 sequence (pWPXL-eGFP-RhoGDI2), confirmed by PCR and DNA sequencing. Additionally, siRNA interference sequences of RhoGDI2 were designed based on GenBank. Interference sequences were synthesized by Biomics Biotechnologies Co., Ltd (China). Lentivirus expression plasmids were cotransfected into 293T cells to construct pLenti-GDI viral stock and pRNAi-GDI viral stock. Lentivirus vector stocks with titers ranging from 0.1×10^9 to 2.0×10^9 particles/mL were used.

Mice and in vivo delivery of lentivirus supernatant

For experiments BALB/c mice were used at 6-8 weeks of age. All animal care and surgical procedures were carried out in accordance with the Guide for Care and Use of Laboratory Animals (National Research Council, 1996, USA) and were approved by the Chinese National Committee to Use of Experimental Animals for Medical Purposes, Jiangsu Branch. All efforts were made to minimize the number of animals used and their suffering. Lentiviral vectors were delivered intratracheally (i.t.) as previously described (18,19). In brief, the neck was extended and cleaned with a chlorhexidine solution. A small midline incision was made with a sterile scalpel to expose the trachea. About 0.5×10^8 to 1×10^8 vector particles in a final volume of 50 μL of phosphate-buffered saline (PBS) were instilled into the trachea with a 27-gauge needle. RhoGDI2 overexpression group (n=10)—intratracheal delivery of pLenti-GDI Viral Stock; RhoGDI2 siRNA group (n=10)—intratracheal delivery of pRNAi-GDI Viral Stock; empty viral vector group (n=10)—intratracheal delivery of the empty viral stock; experimental control group (n=10) and blank control group (n=5)—intratracheal delivery

of the same volume of PBS. Upon completion of the instillation, the skin and fascia were closed in one layer with interrupted sutures (4-0 Vicryl). RhoGDI2 overexpression group, RhoGDI2 siRNA group, empty viral vector group, experimental control group underwent the same procedure daily intraperitoneal injection of 60 μg salbutamol and inhaling an aerosol of 0.01% salbutamol 30 min/d the same day as intratracheal delivery of lentiviral vectors for 21 days. But blank control group was treated with PBS. Mice were allowed freely to get water and food and housed under a 12-hour light-dark cycle. Room temperature was kept at 22±0.5 ℃.

Measurement of airway hyperreactivity

At the 21 days, the mice were anesthetized by intraperitoneal injection of 1% pentobarbital sodium 0.15 mL. Hyperresponsiveness to increasing concentrations of acetylcholine chloride (Ach) was measured through a whole-body plethysmograph system (AniRes2005, Beijing SYNOL High-Tech Co. Ltd., China) and by determining airway expiration resistance (Re).

RNA extraction and reverse transcription-PCR

Mice were killed after measuring airway hyperreactivity. Fresh lung tissue samples were obtained and stored at –80 ℃ until extraction. Total RNA was extracted using Trizol® reagent (Invitrogen™ life technologies). After complete treatment of RNA with RNase-Free DNase (Promega) for 45 min at 37 ℃, a cDNA library was generated using M-MLV reverse transcriptase (Promega) and oligo (dT) primers. For PCR amplification, specific oligonucleotide primer pairs (10 pmol each) were incubated with 1 μL of cDNA template in a 20 μL PCR reaction mixture. Primer sequences were: 5'-GCCTGAGGAGTATGAGTTC-3'(F) and 5'-GAGGTGGTCTTGCTTGTC-3'(R) for RhoGDI2; 5'-AGATCAAGAAGTACGAGAAG-3' (F) and 5'-GATGTATGGCTGGAAGAG-3' (R) for GRK2; 5'-CCTGCTGACCAAGAATAAG-3' (F) and 5'-AAGGACACGATGGAAGAG-3' (R) for β_2AR; 5'-CCATTTGCAGTGGCAAAG-3' (F) and 5'-CACCCCATTTGATGTTAGTG-3' (R) for GAPDH. Dilutions of the cDNAs were amplified for 35 cycles at 94 ℃ for 30 sec, 60 ℃ for 30 sec, and 72 ℃ for 30 sec. The amplified PCR products were analyzed by 1% agarose gel electrophoresis and gel red staining.

Western blot

The fresh lung tissue samples were homogenized in a lysate buffer (5 mmol/L EDTA, 50 mmol/L Tris, 1% SDS, pH 7.5, 10 μg/mL aprotinin, 1% sodium deoxycholate, 1% NP-40, 1 mM PMSF, 1% Triton-X 100, and 10 μg/mL leupeptin) and then centrifuged in a microcentrifuge at 4 ℃ for 20 min to collect the supernatant. Protein concentrations were determined with the Bradford assay (Bio-Rad). After diluted with SDS loading buffer and boiled protein samples were subjected to SDS-polyacrylamide gel electrophoresis (PAGE) and transferred to polyvinylidine difluoride filter (PVDF) membranes (Millipore). The membranes were blocked with 5% dried skim milk in TBST (20 mM Tris, 150 mM NaCl, 0.05% Tween-20). After two hours at room temperature, the membranes were washed and incubated with primary antibody against RhoGDI2 (anti-rabbit, 1:500; Epitomics), GRK2 (anti-rabbit, 1:500; Epitomics), β_2AR (anti-rabbit, 1:100; Santa Cruz), Glyceraldehyde-3-phosphate Dehydrogenase (GAPDH, anti-rabbit, 1:1,000; Sigma) at 4 ℃ overnight. After incubating with horseradish peroxidase-conjugated secondary antibody, the protein was visualized using ECL (Pierce Company, USA).

Measurement of GRK activity

GRK enzymatic activity was assessed using light-dependent phosphorylation of rhodopsin (Benovic *et al*., 1987). Rod outer segment (ROS) membranes were prepared from dark-adapted bovine retinas via stepwise sucrose gradient centrifugation, and then treated with 5 M urea to inactivate endogenous kinase activity as substrate. GRK-dependent phosphorylation was determined by incubating 60 μg of lung tissue protein with 0.5 μM ROS in a buffer containing Tris-HCl (pH 7.4) 20 mM, EDTA 2 mM , $MgCl_2$ 5 mM, ~1 μCi [γ-^{32}P]ATP and 2 nmol ATP in a final reaction volume of 20 μL. The reactions were carried out at 30 ℃ for 30 min in the presence or absence of light. The incubations were terminated by the addition of 10 μL of 3× sodium dodecyl sulphate (SDS) sample buffer (8% SDS, pH 6.8, 20% glycerol, 50 mM Tris-HCl, 0.005% bromophenol blue and 5% β-mercaptoethanol). Samples were then electrophoresed on 10% PAGE. After electrophoresis, the gel was stained with Coomassie blue, dried, and phosphorylated rhodopsin was visualized by autoradiography. Bands corresponding to rhodopsin (~38 kDa) were cut from the gel and quantitated

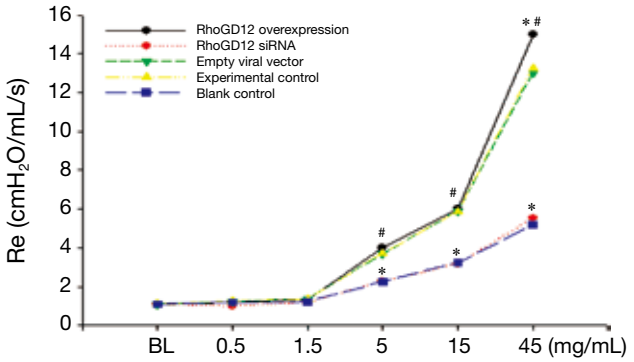

Figure 1 Measurement of airway hyperreactivity. Airway responsiveness was monitored by airway expiration resistance (Re) as described in methods. BL, baseline. Results are the mean ± SEM of three independent sets of analyses. *, Indicates significant difference compared with experimental control groups (P<0.05). #, Indicates significant difference compared with black control group (P<0.05).

by liquid scintillation counting.

Statistical analysis

Statistical analyses were performed using SPSS software version 16.0. Each experiment consisted of at least three replicates per condition. All values are expressed as mean ± SEM. Data with a normal distribution were analyzed using a *t*-test. A P-value of less than 0.05 was considered to be significant.

Results

Airway responsiveness after inhaling Ach

To assess β_2AR functional changes, we measured airway hyperreactivity (acetylcholine chloride-induced resistance). β_2AR agonists might decrease airway responsiveness, while Ach might trigger airway hyperreactivity. Airway responsiveness determined by airway resistance and described through an animal ventilator after inhaling acetylcholine chloride. Airway responsiveness after inhaling Ach was markedly increased in the RhoGDI2 overexpression group compared to experimental control group and blank control group when concentrations of Ach was 45 mg/mL (all P<0.05). Airway resistance was markedly decreased in the RhoGDI2 siRNA group compared to experimental control group (P<0.05) (*Figure 1*).

RT-PCR detection of RhoGDI2, β2AR, and GRK2 mRNA expressions

Total RNAs of fresh lung tissue were extracted and mRNA expression of RhoGDI2, GRK2, and β_2AR was measured by semi-quantitative RT-PCR. A representative gel of RT-PCR products from the recipient is shown in *Figure 2A*. The RhoGDI2 and GRK2 mRNA level were significantly increased in RhoGDI2 overexpression group compared to experimental control group and blank control group (all P<0.05) (*Figure 2B,C*). The RhoGDI2 and GRK2 mRNA level were significantly decreased in the RhoGDI2 siRNA group compared to experimental control group and blank control group (all P<0.05) (*Figure 2A-C*). The mRNA expressions GRK2 were significantly increased along with increasing RhoGDI2 expression, demonstrating a positive relationship. The mRNA expression of β_2AR was significantly lower in the RhoGDI2 overexpression group compared to experimental control group and blank control group (all P<0.05) (*Figure 2D*).

Protein expressions of RhoGDI2, β2AR and GRK2 were detected by western blot

Lung tissues protein from RhoGDI2 overexpression, RhoGDI2 siRNA group, empty viral vector group, experimental control group and blank control group were separated by SDS-PAGE and analyzed by western blotting. As expected, RhoGDI2 and GRK2 protein level were significantly increased in the RhoGDI2 overexpression group compared to experimental control group and blank control group (all P<0.05) (*Figure 3A-C*). The RhoGDI2 and GRK2 protein level were significantly decreased in the RhoGDI2 siRNA group compared to experimental control group and blank control group (all P<0.05) (*Figure 3A-C*). Conversely, β_2AR expression were significantly lower in the RhoGDI2 overexpression group compared to experimental control group and blank control group (all P<0.05) (*Figure 3A,D*), exhibiting an inverse correlation with RhoGDI2 expression.

GRK activity in lung

In lung tissue protein from each group, as expected, the GRK enzymatic activity was significantly increased in the RhoGDI2 overexpression group compared to experimental control group and blank control group (all P<0.05) (*Figure 4A,B*). The GRK enzymatic activity was significantly decreased in the RhoGDI2 siRNA group compared to experimental

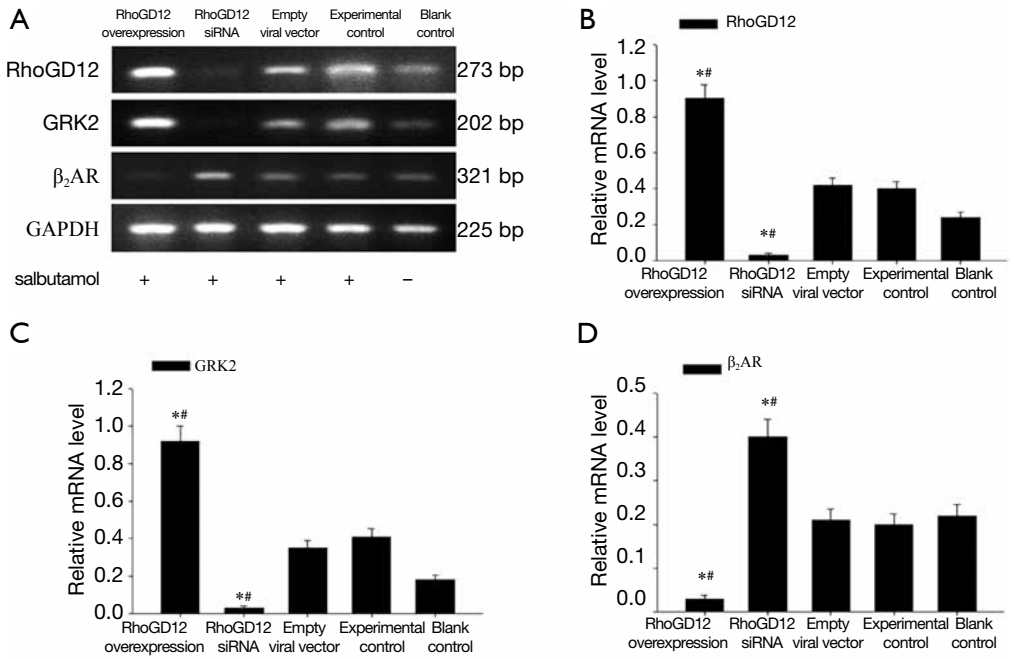

Figure 2 RhoGDI2, GRK2, and β_2AR mRNA expression level in fresh lung tissue. Twenty-one days after transfection, the mRNA expression of RhoGDI2, GRK2, and β_2AR in each group were assessed by RT-PCR analysis (A). Densitometric measurements of RhoGDI2, GRK2, and β_2AR mRNA expression were normalized to GAPDH mRNA expression (B-D). Results are the mean ± SEM of three independent sets of analyses. *, Indicates significant difference compared with experimental control groups (P<0.05). #, Indicates significant difference compared with black control group (P<0.05).

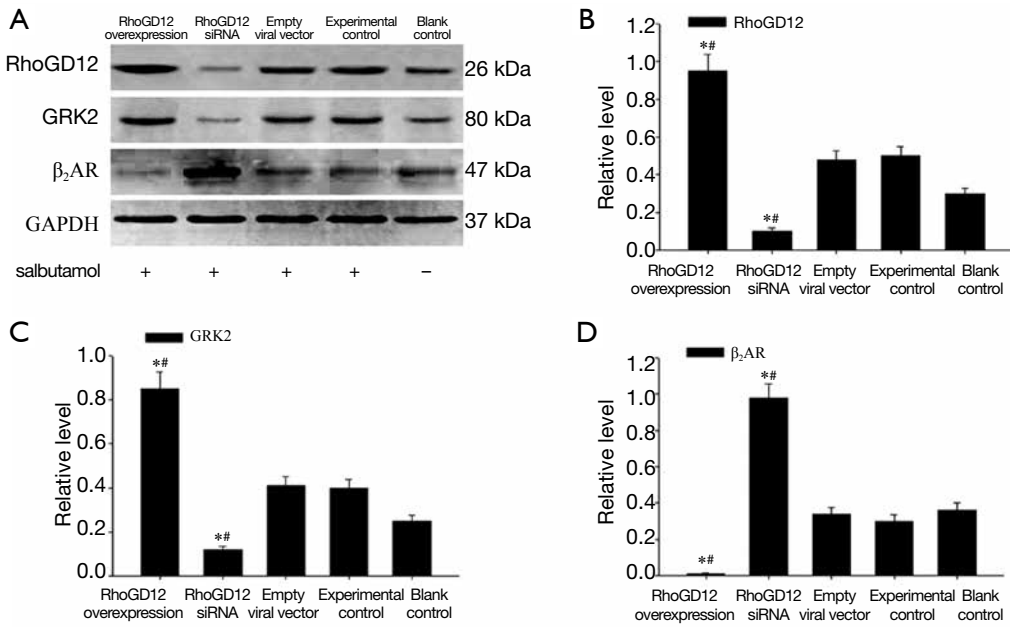

Figure 3 RhoGDI2, GRK2, and β_2AR protein expression level in fresh lung tissue. Twenty-one days after transfection, the protein expression of RhoGDI2, GRK2, and β_2AR in each group were detected by western blotting (A). Densitometric measurements of RhoGDI2, GRK2, and β_2AR immunoreactivity normalized to GAPDH (B-D). Results are the mean ± SEM of three independent sets of analyses. *, Indicates significant difference compared with experimental control groups (P<0.05). #, Indicates significant difference compared with black control group (P<0.05).

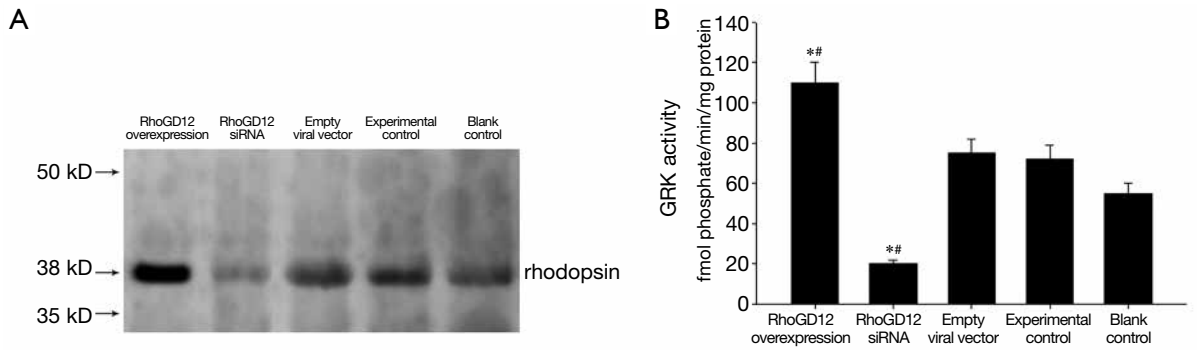

Figure 4 GRK enzymatic activity was assessed in lung tissue protein from different treatment groups. Autoradiographs depicting light-dependent phosphorylation of rhodopsin (~38 kD) (A). Quantification of GRK enzymatic activity was measured in each group (B). Results are the mean ± SEM of three independent sets of analyses. *, Indicates significant difference compared with experimental control groups (P<0.05). #, Indicates significant difference compared with black control group (P<0.05).

control group and blank control group (all P<0.05) (*Figure 4A,B*).

Discussion

β_2AR agonists are effective drugs for clinical treatment of respiratory diseases. However, their application is limited because of β_2AR desensitization (20). β_2AR downregulation is an important aspect of β_2AR desensitization (21). In addition, the functional changes of β_2AR desensitization were airway responsiveness to cholinergic stimulants.

To study the molecular mechanisms of β_2AR desensitization, models should adopt a stimulation method similar to the clinical setting during establishment (22). Therefore, construction of an animal model of β_2AR desensitization by stimulating the airway with salbutamol may reflect the molecular mechanisms of β_2AR desensitization. Salbutamol is one of the most commonly used short-acting β_2AR agonist because of a rapid effect of relieving acute dyspnea, minor systemic side effects, and low-cost (23). Our study adopted salbutamol as the stimulus to construct the animal model, so that it was more similar to the pathological status of clinical patients who are medicated with salbutamol. We constructed the animal model of β_2AR desensitization by adding salbutamol over a long-term. The time between drug administration and bronchodilation is various *in vivo*, it was significantly depended on the β_2-adrenoceptor agonist used (e.g., <2 minutes for salbutamol compared to ~30 minutes for salmeterol) (24).

Our study showed that overexpression of RhoGDI2 had a significant correlation with β_2AR desensitization as compared with RhoGDI2 silenced group. However, whether RhoGDI2 may be involved in the development of desensitization to β_2AR agonists is not known yet. In this study, we demonstrated that RhoGDI2 induced β_2AR desensitization in a murine model by measuring the activity of GRK, protein levels of GRK2, and the total amount of β_2AR receptor (western blotting analysis). In addition, the functional changes of airway responsiveness in desensitized mice were measured by airway hyperresponsiveness to Ach. To investigate the mechanisms involved, we used lentivirus-mediated overexpression and siRNA interference targeting RhoGDI2. Forced expression of RhoGDI2 increased tolerance of β_2AR to β_2AR agonists. Conversely, silence of RhoGDI2 sensitized β_2AR to β_2AR agonists. These results suggested that silence of RhoGDI2 confers a survival mechanism to β_2AR, which protects them from desensitizing these β_2AR agonists.

Our date showed that GRK2, an important G protein-coupled receptor kinase that phosphorylates β_2ARs specifically targets agonist-occupied receptors (25), expression is upregulated in RhoGDI2-over-expressing salbutamol treated animal model and downregulated in RhoGDI2-depleted salbutamol treated animal model. These results suggested that GRK2 might take part in β_2AR agonist tolerance in RhoGDI2-overexpressing salbutamol treated animal model. There are many reports demonstrated the importance of GRK2 in β_2AR agonist tolerance and the importance of GRK2 in β_2AR agonist tolerance makes GRK2 an important target for the prevention of the development of desensitization (26). Although, how RhoGDI2 upregulates GRK2 expression and whether upregulated GRK2 could mediate resistance to other therapeutic drugs-induced β_2AR desensitization in

RhoGDI2-expressing animal requires further analyses, all of our results suggested an important role of RhoGDI2 in regulating β_2AR desensitization by reinforcing the activity of GRK2 was accompanied with downregulation of β_2 adrenergic receptors.

In this study, we demonstrated that RhoGDI2 induced β_2AR desensitization. Investigations into the etiology of β_2AR desensitization have largely focused on the roles played by GPRKs in mediating phosphorylation of β_2AR. Such phosphorylation ultimately results in uncoupling of the agonist-occupied form of the receptor from the G proteins. Although changes in receptor responsiveness may serve as the primary means of desensitization, previous studies demonstrated that homologous desensitization was associated with, but not dependent on, a ~45% loss of cell surface β_2ARs (27). These data provide strong evidence that RhoGDI2 regulates β_2AR desensitization by indirectly affecting β_2AR number and function and may offer a distinct idea from the traditional mechanisms.

In conclusion, our present studies found that RhoGDI2 might induce β_2AR desensitization and GRK2 might take part in RhoGDI2-mediated β_2AR desensitization in β_2AR desensitization mice model. A better understanding the function of RhoGDI2 in β_2AR desensitization might provide a new perspective for the prevention and treatment of β_2AR desensitization.

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Disclosure: The authors declare no conflict of interest.

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Original Article

Reliability of intramyocardial electrogram for the noninvasive diagnosis of acute allograft rejection after heart transplantation in rats

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Objectives: To examine the reliability of the QRS amplitude of the autonomous intramyocardial electrogram (IMEG) and the maximum slope of the descending T wave (Tslew) of the ventricular evoked response (VER) for surveillance of acute allograft rejection (AR) after heart transplantation in rats.

Methods: Forty rats underwent heterotopic heart transplantation, including ten isograft (isograft group) and 30 allograft (allograft group) recipients. Autonomous IMEG and VER were recorded with epicardiac pacing leads. Isograft recipients were sacrificed on postoperative day 7 and allograft recipients on postoperative days 3, 5 and 7. Graft heart histopathological examinations were performed at the corresponding time points.

Results: Postoperative QRS amplitude and Tslew gradually decreased in the allograft group, but were unaltered in the isograft group. Decreases in the allograft group QRS amplitudes and Tslew values correlated with the histopathological results. At the optimal cutoff point of 90%, Tslew had 94.74% sensitivity, 81.82% specificity, 82.61% positive and 90% negative predictive values. QRS had 68.42% sensitivity, 90.91% specificity, 92.86% positive and 62.50% negative predictive values at its optimal cutoff point of 72.3%.

Conclusions: The QRS amplitude of the autonomous IMEG and Tslew of VER are reliable markers for monitoring AR after heart transplantation in rats.

Keywords: Heart transplantation; acute allograft rejection; intramyocardial electrogram (IMEG)

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Introduction

Heart transplantation is the treatment of choice for eligible patients with end-stage heart diseases, and acute rejection after transplantation can severely threaten patient survival (1,2). The development of new transplantation techniques and immunosuppressants has greatly improved heart transplantation efficacy. However, studies on the diagnosis of allograft rejection (AR) based on clinical manifestations, surface electrocardiograms, X-ray, cellular and humoral immune responses, and cardiac ultrasounds do not yield satisfactory results. According to an international multicenter study (Cardiac Transplant Research Database) (1), 15% of all deaths associated with heart transplantation result from allograft. AR occurs most frequently during the 1st or 2nd postoperative month (2) and can cause cardiac function deterioration and chronic rejection, significantly affecting the long-term survival of heart transplant recipients. Accordingly, a delay in AR detection can have severe consequences.

Although endomyocardial biopsy (EMB) displays good diagnostic accuracy and uniform diagnostic criteria (3), making it the current gold standard for AR diagnosis

after heart transplantation, its use retains several inherent disadvantages. AR can be focal, and local biopsy may fail to represent the overall cardiac condition. Humoral-mediated AR may be mild in manifestation but lead to severe consequences (2). EMB is expensive, difficult to perform on pediatric patients, can damage the cardiac conduction system, and can induce arrhythmia, tricuspid regurgitation (3), cardiac perforation, or even death (2). Dynamic surveillance is not possible with EMB, and diagnosis is not immediate. The interpretation of EMB results is subject to inter- and intra-observer variation, and thus the consistency and objectivity of the results are impaired. There is, therefore, a pressing need for a non-invasive, fast, sensitive, specific, and dynamic method to replace or supplement EMB.

Surface electrocardiogram has also been employed in monitoring immunological rejection. However, its method specificity is extremely low, due to influences from many external factors, and surface electrocardiograms are poorly correlated to histological results (4). As a relatively stable tool capable of accurately reflecting electrophysiological changes of focal cardiac muscles, intramyocardial electrogram (IMEG) has emerged as a promising method for AR diagnosis after heart transplantation. Most studies on the utility of IMEG have focused on the diagnostic value of the QRS amplitude of autonomous IMEG and the maximum slope of the descending T wave of the ventricular evoked response (VER or paced IMEG) (5-7). Such studies indicate that IMEG offers non-invasive, efficient, safe, and convenient AR surveillance (7,8), and give high credence to using the QRS amplitude and the maximum slope of the descending T wave as noninvasive markers. However, controversy exists as to their actual usefulness in AR diagnosis (9). In addition, no systematic or statistical evaluation has been performed with regard to the utility of IMEG for AR surveillance.

To address this issue, here we monitored changes in the QRS amplitude of the autonomous IMEG and the maximum slope of the descending T wave of VER in an abdominal heart transplantation model in rats. We analyzed and compared the reliability of both indices in AR diagnosis after heart transplantation, using interval estimation and receiver operating characteristic (ROC) curve analysis.

Methods

Animals

Eight-week-old male Lewis rats (n=10) and Sprague Dawley rats (n=30) with an average body weight of 280 g (250-300 g) were obtained from the experimental animal center of Nantong University, and were used as recipients and donors. Epicardial electrodes were implanted at the right ventricular outflow tract, left ventricular free wall, and left ventricular apex. All surgical interventions and postoperative animal care were performed in accordance with the National Institutes of Health Guide-lines for the Care and Use of Laboratory Animals (National Research Council, 1996, USA) and were approved by the Chinese National Committee to the Use of Experimental Animals for Medical Purposes, Jiangsu Branch. All procedures were performed on animals in an unconscious state. All efforts were made to minimize the number of animals used and their suffering.

Acquisition of autonomous and paced intramyocardial electrograms

Autonomous IMEG and VER were recorded at days 3, 5 and 7 after heart transplantation, at the same time each day. At least 50 autonomous and paced QRS complexes were recorded at each time point, and at least ten consecutive QRS complexes per minute of a 5-min period of continuous recording were selected for data analysis.

Observation indices

The QRS amplitude of IMEG was defined as the voltage (mV) of the QRS complex from trough to peak, and was calculated with a biomedical signal acquisition and processing system (PCLAB-UE, Beijing Microsignalstar, China). The maximum slope of the descending T wave of VER was obtained directly from PCLAB-UE (*Figure 1*). All data collected during each recording were averaged.

Experimental endpoints

Day 7 after operation was chosen as the experimental endpoint for the ten isograft group syngeneic recipients. The 30 allograft group allogeneic recipients were randomized into three groups (n=10) with experimental endpoints of days 3, 5 and 7 after operation, respectively. When animal subjects were eliminated before their experimental endpoints for various reasons, new matched rats were introduced to maintain a constant number of animals within each group.

Pathological examination

At the end of the experimental endpoints, laparotomy was

performed under general anesthesia and the transplanted heart was removed while still beating. The heart was fixed in 10% formaldehyde for 24 h. Subsequently, two myocardial sections of ~1 mm thick were cut, embedded in paraffin, and stained by hematoxylin and eosin stain. Diagnosis of rejection was established by the same group of pathologists in a blind fashion, according to the International Society of Heart and Lung Transplantation (ISHLT) system for rejection grading (10). Rats with a rejection grade of II or above were rejection positive, while those with grades of I or 0 were rejection negative.

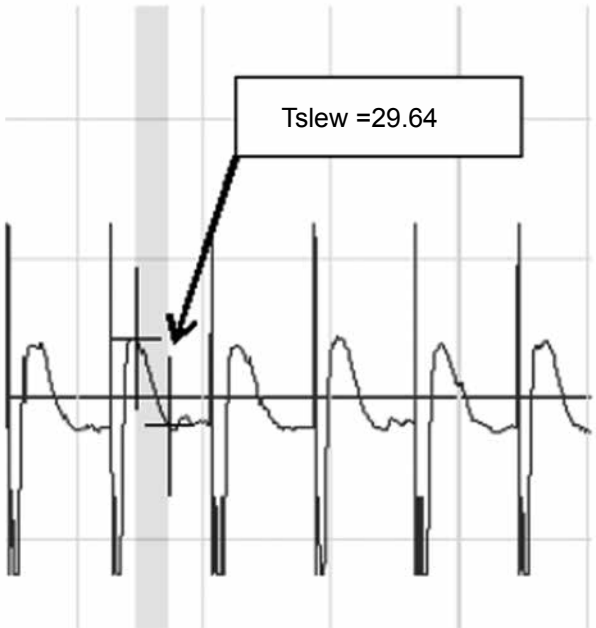

Figure 1 PCLAB-UE interface for the maximum slope of the descending T wave calculation: the maximum slope of the descending T wave of ventricular evoked response (VER) was 29.64.

Statistical analysis

Results are expressed as the means ± standard deviations. Data analysis was performed using Stata 10.0 with the significance level set at $\alpha=0.05$. P-values <0.05 were considered statistically significant.

Results

Values day 2 after operation were designated as baseline and set to 1. The relative value at each time point was calculated as the absolute value at each time point divided by the absolute value at day 2. The syngeneic hearts were visually similar in size to the autogenous hearts, while the allogeneic hearts were significantly increased in size (*Figure 2*). Pathological examination at the experimental endpoints revealed no cases of rejection in the isograft group, but 19 positive and 11 negative cases in the allograft group. In the allograft group, rejection began at day 3 with a rejection grade of 2 or 3, and all rejection episodes were of grade 3-4 at day 7 (*Figure 3*). Evaluation of the diagnostic cutoff values and diagnostic values was performed on the allograft group, and the pathological results were chosen as the gold standard.

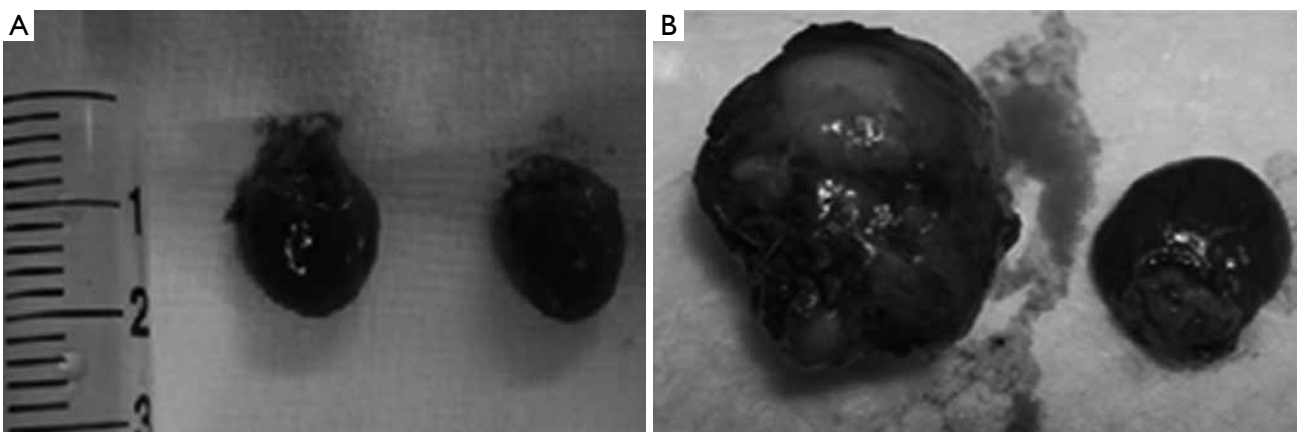

Figure 2 Syngeneic and allogeneic hearts were compared with autogenous hearts. (A) The syngeneic hearts (Lewis to Lewis, left) were visually similar in size to the autogenous hearts (right); (B) Allogeneic hearts (SD to Lewis, left) were significantly increased in size compared with autogenous hearts (right).

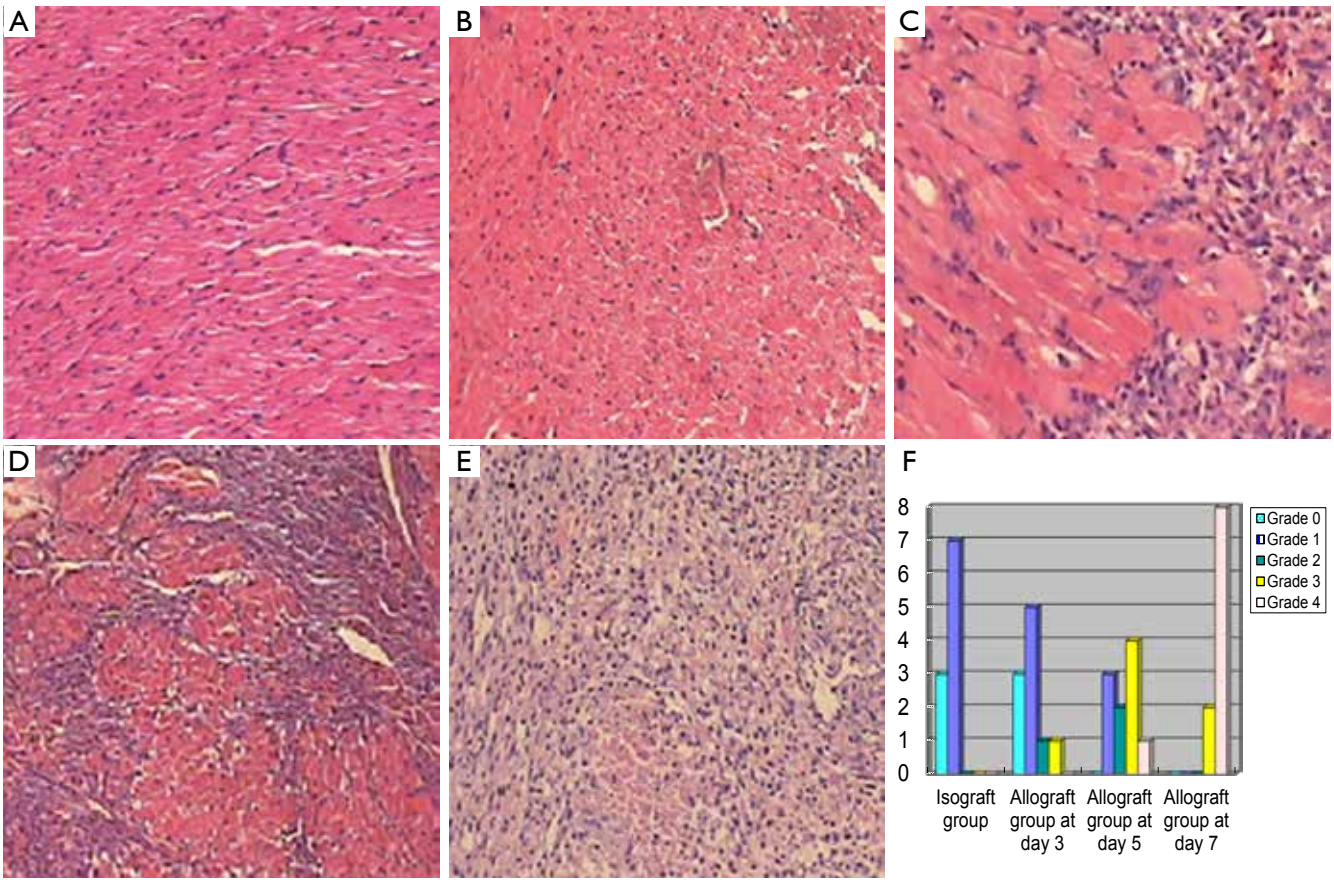

Figure 3 According to the International Society of Heart and Lung Transplantation (ISHLT) system for rejection grading, pathological sections of allograft rejection were shown. (A) grade 0, there is no lymphocytes inflammation and myocyte necrosis in allografts; (B) grade 1, there were no obvious inflammation and medium limited inflammatory infiltration and oedema in myocardial cell; (C) grade 2, there were local lymphocytic infiltration and faint myocardial cell necrosis in vascular and myocardial cell; (D) grade 3, it became moderate lymphocytic infiltration and faint myocardial cell necrosis; (E) grade 4, cardiomyocytes necrosis and multifocal aggressive interstitial lymphocytic infiltration in allografts; (F) Number of rats with different rejection grades in the pathological biopsy. [Original magnification (A,B,D,E) ×10, (C) ×40].

QRS amplitude of the autonomous intramyocardial electrogram and the maximum slope of the descending T wave of ventricular evoked response

The autonomous QRS amplitudes and the maximum slope of the descending T wave values of VER are shown in *Table 1*, respectively. No significant change in the QRS amplitude or the maximum slope of the descending T wave occurred in the isograft group during the study period. In contrast, these two indices were significantly decreased at the experimental endpoint in the allograft group compared with baseline values.

Comparisons of the QRS amplitudes between groups at various time points after surgery are listed in *Table 1*. A group *t*-test was performed on data collected at days 3 and 5, and a rank-sum test was performed on values obtained at day 7 due to heterogeneity of variance. We observed no marked difference between the QRS amplitudes on the postoperative day 3 of the two groups, but significant differences were noted in the subsequent two time points. Comparisons of the maximum slope of the descending T wave values between groups after surgery showed a similar pattern (*Table 1*). Comparisons of the allograft group QRS amplitudes and the maximum slope of the descending T wave values at different time points are summarized in *Table 2*. Except for those between days 5 and 7, comparisons between all time points showed significant differences in the QRS amplitudes and the maximum slope of the descending T wave values.

Table 1 QRS amplitudes and Tslew values

Index	POD 3	POD 5	POD 7
QRS			
Isograft group	0.91±0.07	0.89±0.08	0.88±0.07
Allograft group	0.88±0.10	0.71±0.13	0.61±0.13
P	0.3799	0.0006	0.0009
Tslew			
Isograft group	0.95±0.04	0.94±0.04	0.93±0.04
Allograft group	0.95±0.05	0.82±0.09	0.76±0.06
P	0.8127	0.0001	0.0000

POD, postoperative days; QRS, QRS amplitude; Tslew, the maximum slope of the descending T wave of ventricular evoked response (VER); the data are represented as the mean ± SD (n=10).

Table 2 Comparison of P values at different time points in the allograft group

Index	POD 3-5	POD 5-7	POD 3-7
QRS	0.000	0.093	0.000
Tslew	0.000	0.079	0.000

POD 3-5, postoperative days 5 compared with postoperative days 3; POD 5-7, postoperative days 7 compared with postoperative days 5; POD 3-7, postoperative days 7 compared with postoperative days 3; QRS, QRS amplitude; Tslew, the maximum slope of the descending T wave of ventricular evoked response (VER).

Correlation of the QRS amplitude and the maximum slope of the descending T wave

A linear correlation was observed between the two indices of the allograft group (r=0.5816, P=0.0007), but not of the isograft group (r=0.2639, P=0.1587) (*Figure 4*). The correlation of the QRS amplitude and the maximum slope of the descending T wave value with pathological results in the allograft group are shown in *Table 3*. Of note, allogeneic recipients that were rejection positive had remarkably lower QRS amplitudes and the maximum slope of the descending T wave values than those negative in the pathological biopsy.

Evaluation of diagnostic values of the QRS amplitude and the maximum slope of the descending T wave

A cutoff point separates positive from negative values. The specificity (Sp), sensitivity (Se), positive (PV+) and negative

Table 3 Correlation of the QRS amplitude and Tslew with pathological biopsy in the allograft group

Index	Rejection negative (n=11)	Rejection positive (n=19)	P value
QRS	0.85±0.11	0.68±0.15	0.0048
Tslew	0.95±0.07	0.77±0.09	0.000

QRS, QRS amplitude; Tslew, the maximum slope of the descending T wave of ventricular evoked response (VER).

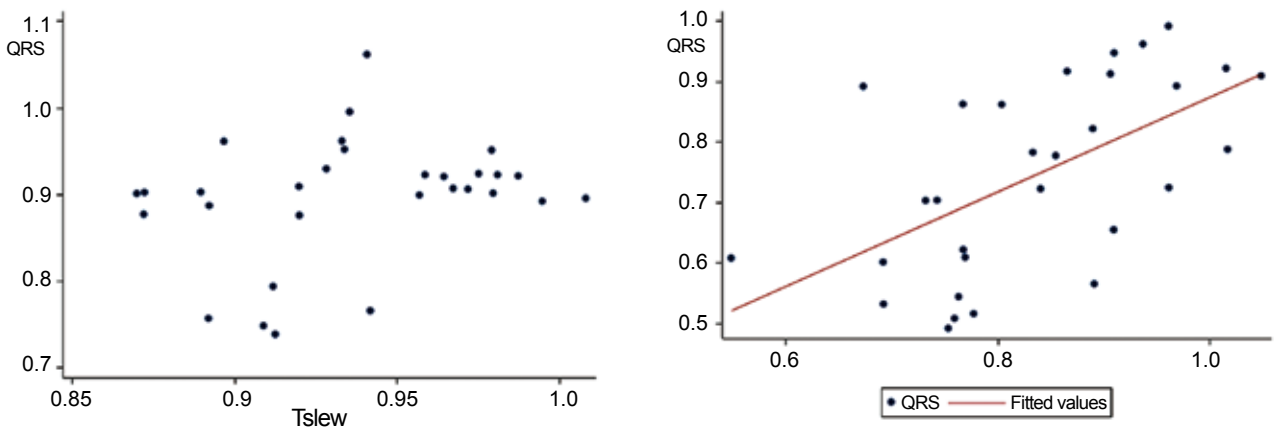

Figure 4 Correlation of the QRS amplitude and the maximum slope of the descending T wave. (A) No linear correlation was observed between the QRS amplitude and the maximum slope of the descending T wave in the isograft group (r=0.2639, P=0.1587); (B) Linear correlation between the QRS amplitude and the maximum slope of the descending T wave in the allograft group (r=0.5816, P=0.0007).

Table 4 Diagnosis at the optimal cutoff points of the QRS amplitude and Tslew

Index (%)	% (95% CI)				
	Se	Sp	PV+	PV–	Coincidence rate
QRS ≤72.3	68.42 (43.45, 87.42)	90.91 (58.72, 99.77)	92.86 (66.13, 99.82)	62.50 (35.43, 84.80)	76.67 (57.72, 90.07)
Tslew ≤90	94.74 (73.97, 99.87)	81.82 (48.22,97.72)	90.00 (68.30, 98.77)	90.00 (55.50, 99.75)	90.00 (73.47, 97.89)

QRS, QRS amplitude; Tslew, the maximum slope of the descending T wave of ventricular evoked response (VER); Se, sensitivity; Sp, specificity; PV+, positive predictive values; PV–, negative predictive values; CI, confidence interval.

Table 5 Diagnosis at given cutoff points of the QRS amplitude

Cutoff point (%)	% (95% CI)				
	Se	Sp	PV+	PV–	Coincidence rate
≤90	89.47 (66.86, 98.70)	45.45 (16.75, 76.62)	90 (68.30, 98.77)	71.43 (29.04, 96.33)	73.33 (54.11, 87.72
≤85	73.68 (48.80, 90.85)	54.55 (23.40, 83.25)	73.68 (48.80, 90.85)	54.55 (23.40, 83.25)	66.67 (47.19, 82.71)
≤80	68.42 (43.45, 87.42)	54.55 (23.40, 83.25)	72.22 (46.52, 90.31)	50 (21.09, 78.91)	1.33 (43.86, 80.07)

Se, sensitivity; Sp, specificity; PV+, positive predictive values; PV–, negative predictive values; CI, confidence interval.

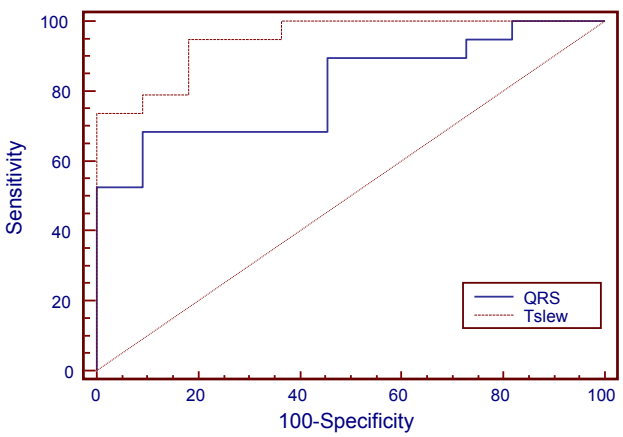

Figure 5 Areas under the ROC curves of the QRS amplitude and the maximum slope of the descending T wave: significantly differed (χ^2 =4.32, P=0.0377).

(PV–) predictive values and the coincidence rate of the QRS amplitude and the maximum slope of the descending T wave at their corresponding optimal cutoff points are shown in *Table 4*. Diagnoses at various cutoff points of the QRS amplitude and the maximum slope of the descending T wave are given in *Tables 5* and *6*, respectively.

At a cutoff point of 92% (≤92% considered positive), the maximum slope of the descending T wave had 100% sensitivity, 63.64% specificity, and 82.61% positive and 100% negative predictive values. At the optimal cutoff point of 90%, the maximum slope of the descending T wave had 94.74% sensitivity, 81.82% specificity, and 90% positive and 90% negative predictive values. At the optimal cutoff point of 72.3%, QRS had 68.42% sensitivity, 90.91% specificity, and 92.86% positive and 62.50% negative predictive values. The area under the ROC curve of the QRS amplitude (0.8086; 95% CI: 0.65319, 0.96404) significantly differed (χ^2=4.32, P=0.0377) from that of the maximum slope of the descending T wave (0.9474; 95% CI: 0.87528, 1.000) (*Figure 5*).

Discussion

Previous studies on IMEG have been limited to the empirical selection of one or several diagnostic criteria (or threshold values/cutoff points) to calculate the sensitivity, specificity, and other indices. Diagnostic value evaluation was reportedly based on point rather than interval estimation, overlooking the effects of sampling error or sample rate. Since these indices are related to the selected diagnostic criteria or threshold values, different diagnostic criteria may yield different results for the diagnostic value of IMEG. Therefore, it is insufficient to assess the diagnostic value of IMEG simply based on the empirical selection of diagnostic criteria.

In the present study, we used ROC analysis to assess the diagnostic value of the QRS amplitude and the maximum slope of the descending T wave. At the optimal cutoff point of the maximum slope of the descending T wave, we obtained a sensitivity and specificity of 94.74% and 81.82%, respectively, and a 90% positive and negative predictive value and coincidence rate. At the optimal cutoff point of the QRS amplitude, the sensitivity was 90.91% and the

Table 6 Diagnosis at given cutoff points of the Tslew

Cutoff point (%)	(%) (95% CI)				
	Se	Sp	PV+	PV–	Coincidence rate
≤92	100	63.64 (30.79, 89.07)	82.61 (61.22, 95.05)	100	86.67 (69.28, 96.24)
≤90	94.74 (73.97, 99.87)	81.82 (48.22, 97.72)	90 (68.30, 98.77)	90 (55.50, 99.75)	90 (73.47, 97.89)
≤85	78.95 (54.43, 93.95)	90.91 (58.72, 99.77)	93.75 (69.77, 99.84)	71.43 (41.90, 91.61)	83.33 (65.28, 94.36)

Se, sensitivity; Sp, specificity; PV+, positive predictive values; PV–, negative predictive values; CI, confidence interval.

specificity was only 68.42%, while the positive and negative predictive values and coincidence rate were 92.86%, 62.50%, and 76.67%, respectively. The area under the ROC curve of the QRS amplitude of autonomous IMEG was 0.8086, suggesting that the QRS amplitude is a moderately reliable diagnostic criterion. In contrast, the area under the ROC curve of the maximum slope of the descending T wave was 0.9474, suggesting that the maximum slope of the descending T wave is a highly reliable diagnostic criterion. The significant difference between these indices is attributable to the higher diagnostic coincidence rates of the maximum slope of the descending T wave at its various selected cutoff points. While both have the same sensitivity level, the maximum slope of the descending T wave usually has a higher specificity and lower false positive rate than the QRS amplitude.

The QRS amplitude and the maximum slope of the descending T wave of VER in both groups declined progressively after transplantation. In allograft recipients, these values were correlated to the pathological findings, and were markedly lower in patients positive in pathological examination, confirming that changes in the QRS amplitude and the maximum slope of the descending T wave were associated with AR. These results seem to suggest that the QRS amplitude and the maximum slope of the descending T wave followed similar patterns of change. However, correlation analysis revealed that the indices were linearly correlated in the allograft group, but not in the control. Interference from factors other than AR may produce different effects on the two indices. However, rejection seemed to synchronize the variation of both indices, which might contribute to the better performance of the maximum slope of the descending T wave versus the QRS amplitude in AR surveillance.

One possible reason for the false positive diagnosis by the QRS amplitude is poor electrode contact. We eliminated three rats (one syngeneic and two allograft recipients) from the study for this reason. The QRS amplitudes in these three rats were significantly reduced to 30-60% of the baseline values, suggesting that AR occurred according to the general criteria. However, the autopsy results showed the sliding of at least one cardiac electrode to the muscular layer of the abdominal wall, indicating that the marked decline in the autonomous IMEG in the three rats may have been derived from the cardiac electrical conduction of the transplanted heart to the body surface. For example, the QRS amplitude was reduced by >30% at day 3, while the maximum slope of the descending T wave was reduced by <8% in one of the three rats. At the experimental endpoint, the recording electrode fell off and one pacing electrode fell to the abdominal cavity, while the other was left *in situ*. This suggests both the possibility of false positive diagnosis by the QRS amplitude, and also that this misjudgment could be eliminated when the maximum slope of the descending T wave was used alone or in combination with the QRS amplitude.

When using ≤92% as the positive cutoff point, the maximum slope of the descending T wave yielded a high sensitivity of 100% and a specificity of 63.64%, suggesting that AR should be suspected and the maximum slope of the descending T wave could be used as a sensitive index for screening AR. When using ≤85% as the positive cutoff point, the maximum slope of the descending T wave gave a higher specificity of 90.91% and a slightly lower sensitivity of 78.95%, indicating a high probability of AR. When the optimal positive cutoff point (≤90%) was used, the maximum slope of the descending T wave provided a satisfactory sensitivity, specificity, and coincidence rate. Therefore, if EMB biopsy is not indicated clinically, AR could also be diagnosed when the optimal positive cutoff point of the maximum slope of the descending T wave is met.

Taken together, these results indicate that the QRS amplitude of IMEG and the maximum slope of the descending T wave of VER can be used as noninvasive tools for AR diagnosis after heart transplantation, and that the maximum slope of the descending T wave is more reliable in AR surveillance than the QRS amplitude. The use of such indices may minimize the need for EMB or serve as a

useful supplement to EMB. There are several limitations to our study. No correlation has been done between IMEG, gross pathology and microscopic pathology. We did not examine the physiological and ultrastructural mechanisms responsible for IMEG changes in response to AR, or the changes in the QRS amplitude of IMEG and the maximum slope of the descending T wave after AR disappearance following immunosuppressive treatment. Modified splint tube technique can be used to get heart transplantation models easily (11). Further studies addressing these issues are warranted.

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Original Article

Different microbiological and clinical aspects of lower respiratory tract infections between China and European/American countries

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Background: National treatment/diagnosis guidelines for lower respiratory tract infections (LRTIs) are generally based on local epidemiological data. Etiology and drug-resistance patterns could differ between China and European/American countries, and simply following their respective guidelines might cause problems in clinical practice. Therefore, we need to summarize the microbiology and clinical manifestations of LRTIs in China and develop our own guidelines.

Methods: Three major national multicenter epidemiology surveillance studies on LRTI were completed recently. The data were compared in detail with those from European/American studies.

Results: Clinical and microbiological differences were observed in community-acquired pneumonia (CAP), hospital-acquired pneumonia (HAP), and pulmonary mycosis between our country and European/American countries.

Conclusions: The microbiological and clinical characteristics of the major LRTIs in China differ in many respects from those in European/American countries. Patients should have personal treatment plans instead of simply following the guidelines from foreign countries

Keywords: Pathogens; epidemiology; antimicrobial resistance; lower respiratory tract infections (LRTIs)

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Introduction

Lower respiratory tract infection (LRTI) is one of the most common diseases in humans and a long-term global public health concern. Worldwide, it places considerably more strain on health budgets than do cancer, cardiovascular diseases, and malaria (1). In the United States, the incidence of LRTI and its mortality rates are higher than for any other infectious diseases. According to a 2002 WHO report, LRTIs accounted for 6.9% of all deaths in that year (2). High rates of LRTI incidence and the high medical cost involved are found worldwide, and the importance of their diagnosis and treatment is accordingly emphasized. Since the 1990s, European/American countries have developed guidelines for diagnosis and treatment of LRTI. These guidelines have been subject to regular evaluations and revisions on the basis of repeated evidence-based medical research and epidemiological investigations. The most comprehensive and influential guidelines are currently presented by the American Thoracic Society (ATS), the Infectious Diseases Society of America (IDSA), and the British Thoracic Society (BTS). Compared with European/American countries, China has diverging socioeconomic models, different medicare systems, and varying LRTI etiology and drug-resistance patterns. Therefore, simply copying the existing European/American guidelines is inappropriate and might cause serious problems in clinical practice. In recent years, large epidemiological investigations of community-acquired pneumonia (CAP), hospital-acquired pneumonia (HAP), and pulmonary mycosis were carried out in several large/middle-sized cities in China, and preliminary data on LRTI were obtained.

This paper summarized these results and discussed the differences in the microbiological and clinical features of

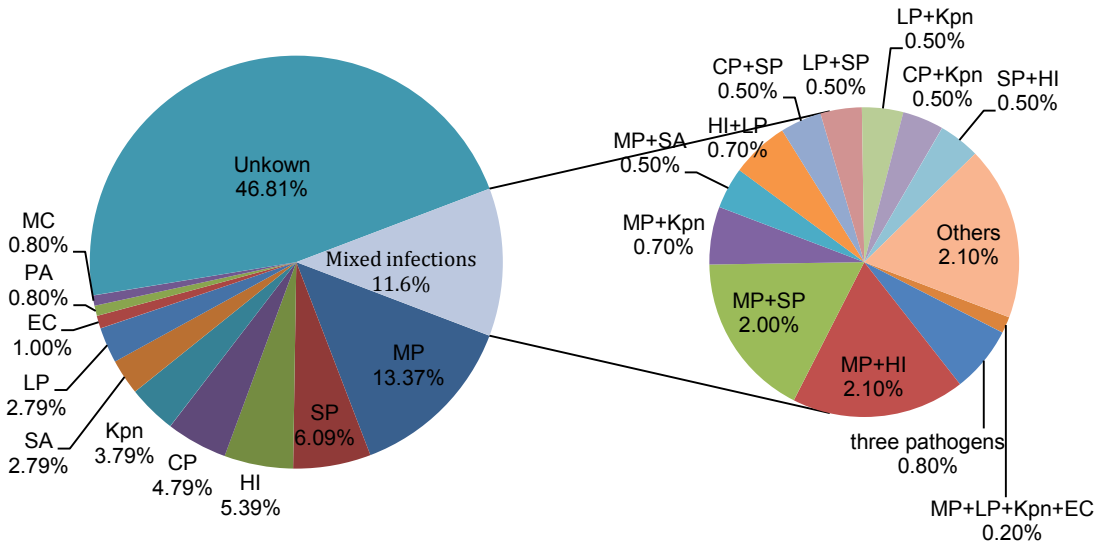

Figure 1 The pathogen distribution of 610 adult CAP cases in China. Unknown, No detectable pathogens; SP, Streptococcus pneumonia; HI, Haemophilus influenza; Kpn, Klebsiella pneumonia; MP, Mycoplasma pneumoniae; LP, Legionella pneumophila; CP, Chlamydia pneumonia; EC, Escherichia coli; SA, Staphylococcus aureus; PA, Pseudomonas aeruginosa; MC, Moraxelle catarrhalis.

LRTIs between China and European/American countries.

CAP

CAP is one of the most common LRTIs. The overall incidence rate is 5-11 per 1,000 people per year, accounting for 5-12% of all LRTIs (3). The pathogens causing CAP include viruses, bacteria, and other atypical pathogens. The pathogen composition is complicated and varies according to geographic area, population, and seasonal changes. The national pathogenic epidemiological investigation of CAP in 2006 indicated the following major characteristics in China.

(I) The infection rate of *Mycoplasma pneumoniae* surpassed that of *Streptococcus pneumoniae* and became the leading cause of adult CAP in our country. Whereas *S. pneumonia* and *Haemophilus influenzae* are still two kinds of the most common CAP pathogens (*Figure 1*). A high proportion of adult cases of CAP are derived from mixed infection with bacteria and atypical pathogens (4).

Regular sputum sampling was done with most (590/610) inpatients enrolled in the study, and blood samples were taken if patients had a fever of >38.5 ℃. Sputum was Gram-stained. Representative sputum originated from the lower respiratory tract was defined as that containing >25 granulocytes and <10 epithelial cells per low power field microscopic view. Validated sputum and blood samples were cultured.

(II) High resistance to macrolides among *S. pneumoniae* in China is another characteristic difference compared with European/American countries. The Alexander Project Group showed that in European/American countries, rates of erythromycin resistance (resistant + intermediate resistance) in *S. pneumoniae* are <30%, with 6.9% in Germany, 13.0% in the UK, and 28.8% in the USA (5). The resistance is mainly medicated by the *mef(A)* gene, and the common resistance phenotype is M-type (low level resistance to 14- or 15-membered macrolides and susceptibility to 16-membered macrolides and clindamycin) (6,7) (*Table 1*). Therefore, in those countries, macrolide antibiotics are recommended as the first-line empirical therapy in the clinic for CAP patients without risk factors (9). In contrast, in our country, the level of macrolide resistance in *S. pneumoniae* is higher. For example, the rate of azithromycin resistance is as high as 79.4%; mainly as constitutive resistance mediated by the *erm(B)* gene (cMLS$_B$, highly resistant to erythromycin) (8,10). Considering the high divergence in drug resistance to macrolides in different areas, the CAP guidelines from ATS/IDSA (2007 edition) still recommended macrolides as the drug of first choice for previously healthy patients. However, they also pointed out that in areas with high rates of macrolide resistance, alternative antibiotics should be selected (11).

(III) Many recent studies suggest that China has the

Table 1 Comparative prevalences of erythromycin resistance in S. pneumoniae between China and European/American countries

References	The main resistance gene type and phenotype	Country	n, isolates (intermediate + resistant)
Tiemei Z *et al*. (8)	erm(B), $cMLS_B$-phenotype	China	149/192*
Michael R *et al*. (5)	mef(A), M-phenotype	Germany	22/321
		UK	31/238
		USA	700/2,432

*, multiple comparisons of erythromycin resistance rates in S. pneumoniae isolates from different countries are based upon chi-square analog of Scheffé's theorem. The rate of erythromycin resistance in S. pneumoniae isolates between China and any of other West countries are all significantly different (P≤0.05).

Table 2 Comparative prevalences of macrolide resistance in M. pneumonia between China and European/American countries

Country	%, isolates (intermediate + resistant)	References
China	69.0*	Cao B, *et al*. (17)
Japan	30.6	Morozumi M, *et al*. (13)
France	9.8	Peuchant O, *et al*. (14)
USA	20.7	Dumke R, *et al*. (15)

*, multiple comparisons of macrolide resistance rates in M. pneumoniae isolates from different countries are based upon chi-square analog of Scheffé's theorem. The rate of macrolide resistance in M. pneumoniae isolates between China and any of the other three countries are of significances in statistics (P≤0.05).

highest rates of macrolide resistance of *M. pneumoniae*. Since the first macrolide-resistant *M. pneumoniae* strain was isolated from the lower respiratory tract of a Japanese child in 2001 (12), more macrolide-resistant strains have been obtained from other countries and the prevalence is increasing annually. In 2006, this resistance rate in Japan was 30.6% (13); from 2005 to 2007, it was 9.8% in France (14); it was 3% in Germany in 2009 (15) and from 2006 to 2007, it was 20.7% in USA (16). The surveillance data from our country indicated an even worse situation (17) (*Table 2*). Two clinical studies in Japan showed that the pneumonia derived from macrolide-resistant strains when treated with macrolides alone did not cause deaths from significant deterioration or treatment failure. However, the recovery from fever took longer. In such cases, alternative antibiotics had to be administered when clinical symptoms aggravated (13,18). These factors lead to new difficulties for treatment of *M. pneumoniae* infections. *In vitro* culture and susceptibility testing for *M. pneumoniae* take long, and alternative antibiotics should be used to treat *M. pneumoniae*-derived pneumonia before the laboratory results become available if macrolides are not effective. For children and young adults, there are no safe and effective antibiotics that can completely replace the macrolides. Therefore, tetracycline can be used for children aged >8 years, and respiratory quinolones *or tetracyclines can be used for adults. For adult patients with severe infection, respiratory quinolones alone or combined with other drugs can be used for initial treatment.*

(IV) CAP caused by methicillin-resistant *Staphylococcus aureus* (MRSA) is still rare in China. In 1993, the first community-acquired MRSA (19) was reported in Australia. Since then, a large number of cases have also been reported in the USA, and their severity and high mortality have attracted worldwide attention (20-22). It is generally accepted that community-acquired MRSA causes more infections of the skin and soft tissues than the respiratory tract. A study from Prof. Wang H in China suggests that MRSA-derived skin and soft tissue infections only accounted for 1% of cases (23). Therefore, except for patients with a high suspicion of MRSA infection, empiric anti-MRSA drugs are not necessary.

(V) We found that the probability of infection with *Escherichia coli* and *Klebsiellapneumoniae* were significantly higher in patients aged >50 years (10). In China, the drug-resistance rates in these two bacteria are as high as 50% (24), and the approximate rate of production of extended-spectrum β-lactamase is also high (~30%) (25). The drugs recommended by foreign guidelines, for instance, respiratory quinolones alone or third-generation

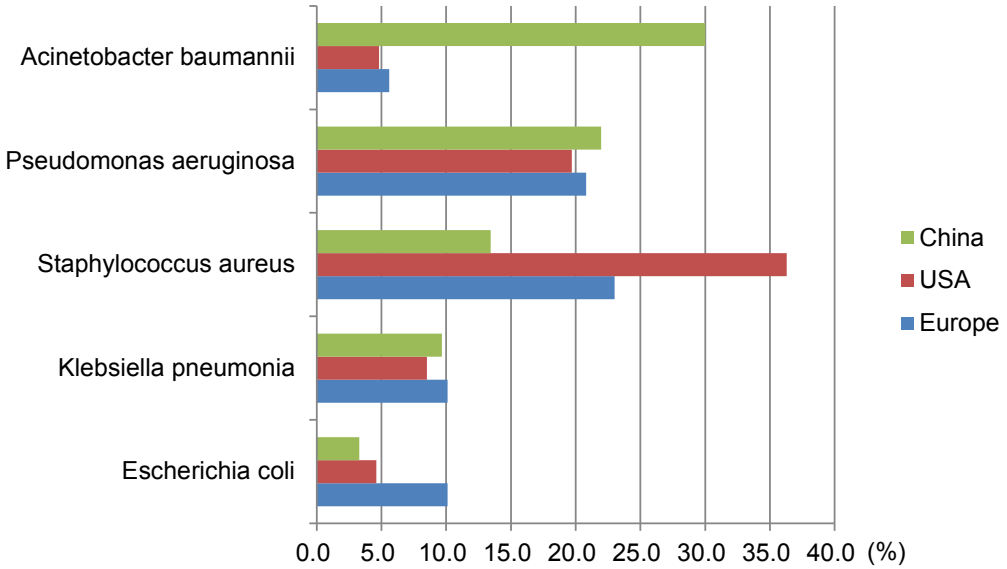

Figure 2 Regional incidence (%) of pathogens isolated from patients with hospital-acquired pneumonia (HAP).

cephalosporins combined with macrolides or respiratory fluoroquinolones, might result in treatment failure. For elderly patients with CAP caused by *E. coli*, β-lactam/β-lactamase inhibitors or carbapenems should be selected.

HAP

HAP, including ventilator-associated pneumonia, is the most common nosocomial infection worldwide, with a high incidence and mortality (26). In the USA, HAP is the second most common nosocomial infection with an incidence rate of 0.5-1%, which might lengthen hospital stay by 7-9 days and increase hospital cost by >$40,000. The HAP incidence rate in patients who receive mechanical ventilation can reach 6-20% (27,28). In 2012, we carried out a nationwide multicenter, prospective epidemiological survey of HAP.

In that study, we isolated the three most common pathogenic bacteria for HAP: *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Staph. aureus* (MRSA accounted for 87.8% of *Staph. aureus*) (29). In European/American countries, the most common HAP bacterium is *Staph. Aureus* (30) (*Figure 2*).

In addition to the differences in pathogen distribution, the drug resistance in non-fermentative bacteria is also more severe in China than that in European/American countries. The rates of non-susceptibility to carbapenems are nearly 80% for *A. baumannii* (*Figure 3*) and >70% for *P. aeruginosa* (*Figure 4*), indicating the reduced value of these drugs for treatment of HAP in our country. This might be associated with the uncontrolled usage of these drugs in our clinical practice. *P. aeruginosa* still has relatively high susceptibility to some carbapenems such as β-lactams, aminoglycosides, and quinolones. However, for *A. baumannii*, the drug options are relatively few and only polymyxin, sulbactam/β-lactam agents and tetracycline can be considered. The means to obtain polymyxin in China are still limited.

The most commonly isolated HAP-associated bacterium in European/American countries, *Staph. aureus*, is only the third most common in China, and MRSA accounts for most cases. MRSA in China still has ideal susceptibility to several anti-MRSA drugs, such as vancomycin, teicoplanin, linezolid, and tigecycline (*Figure 5*). The ineffectiveness of the treatments with commonly used glycopeptides in clinical practice might result from low dosage or no loading dosage. Vancomycin-resistant MRSA has not yet been found in China.

It is generally believed that the distribution of pathogenic bacteria differs significantly between early- and late-onset HAP, with the former mainly caused by susceptible bacteria and the latter by drug-resistant bacteria (31,32). In the present study, we applied a variety of tests including *S. pneumoniae* urinary antigen test for diagnosis. However, no significant differences were revealed between pathogens associated with early- and late-onset HAP (29). The possible explanation is that >90% of patients with either

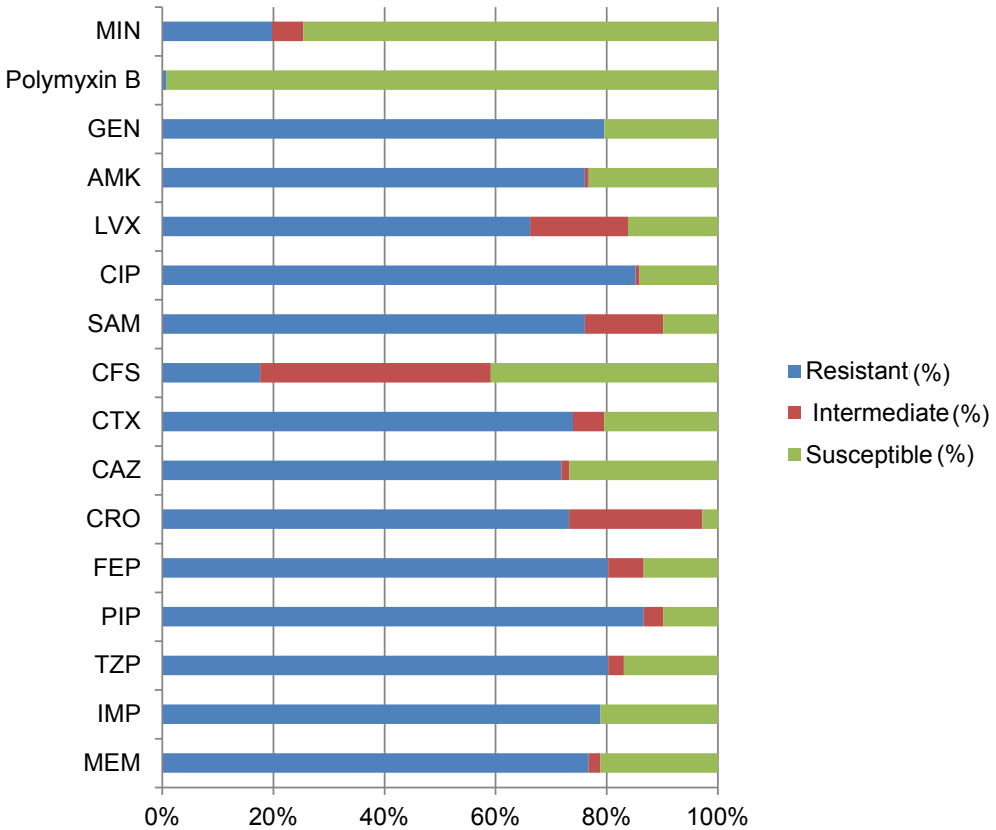

Figure 3 The antibiotics susceptibility of Acineto bacterbaumannii isolates.

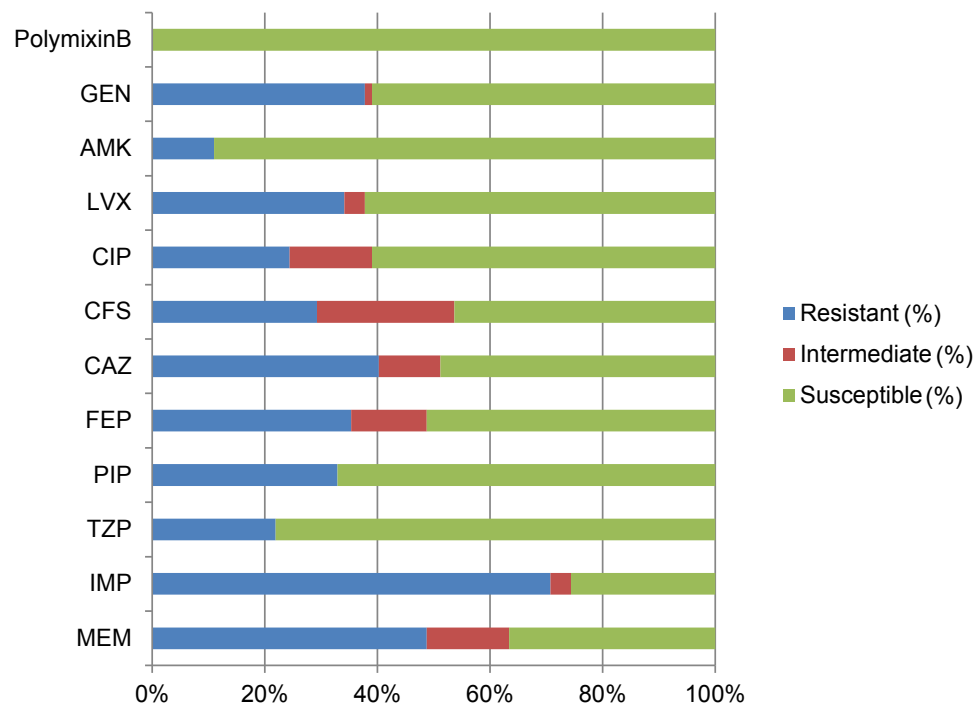

Figure 4 The antibiotics susceptibility of Pseudomonas aeruginosa isolates.

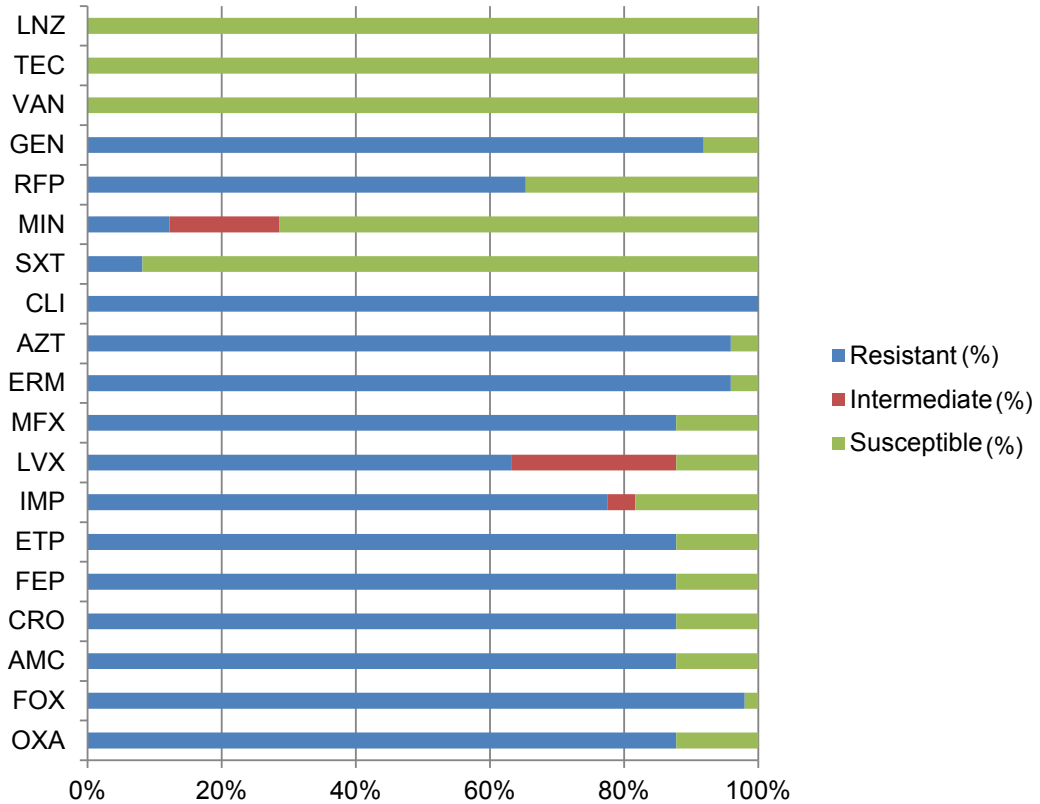

Figure 5 The antibiotics susceptibility of methicillin-resistant Staphylococcus aureus (MRSA) isolates.

early- or late-onset HAP have been exposed to antibiotics within 90 days of the first symptom. Compared with the timing of HAP onset, the application of the antibiotics may be more relevant to the type of the pathogenic bacteria and drug resistance of pathogens. This is another difference between China and European/American countries.

Pulmonary mycosis

The number of cases of pulmonary mycosis is growing. This results from the increased number of immunocompromised hosts, and widespread application of broad-spectrum antibiotics, immunosuppressive agents, and invasive diagnostic/therapeutic technologies. Compared with bacterial LRTI, pulmonary mycosis is more difficult to treat and has poorer prognosis. Most recent foreign pulmonary mycosis guidelines are evidence-based. They are valuable for correct clinical diagnosis and proper treatment, although they might not be applicable in our country. Therefore, a national multicenter 10-year retrospective study provides more useful information for our clinicians.

In the survey, we found in the past ten years in China that the three most common types of pulmonary mycoses were pulmonary aspergillosis, pulmonary candidiasis and pulmonary cryptococcosis (*Figure 6*). IDSA guidelines suggest that invasive pulmonary candidiasis is rare and lung histopathology evidence must be provided for its diagnosis. Positive sputum/bronchoalveolar lavage fluid culture cannot be used as a diagnostic criterion for pulmonary candidiasis and patients do not receive antifungal therapy in such cases (33). We included comparison of blood/pleural fluid culture with sputum culture as a criterion and found that the incidence of candidiasis was almost the same as that of aspergillosis. There were no less than 54 cases of candidiasis confirmed only by lung biopsy (34). This suggests that it is not as rare as indicated by the IDSA guidelines, which is consistent with studies in other countries. Kontoyiannis *et al.* performed autopsies on 676 cancer patients and found that 38% of them (254/676) had mixed pneumonia. Histopathology results showed that 14% (36/254) of cases were caused by candidiasis (35). Therefore, the description about the fungal distribution in the European/American guidelines is not applicable in China.

Cryptococcosis is the third leading form of pulmonary

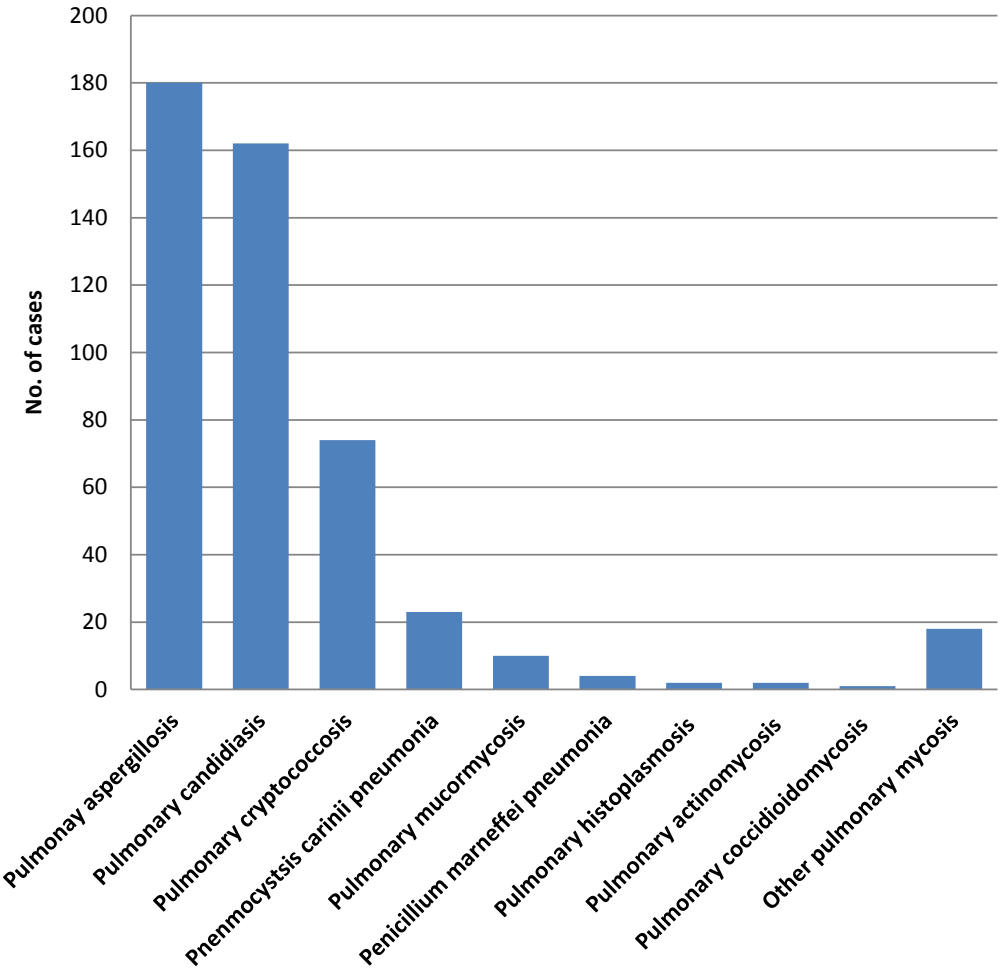

Figure 6 Type distribution of pulmonary mycoses diagnosed.

Table 3 Characteristics of patients with cryptococcosis or other pulmonary mycoses

Characteristics	Pulmonary cryptococcosis	Other pulmonary mycoses	P-value
Age ≤44	44/74 (58.7)	129/400 (31.8)	<0.01
Acquired in the community	71/74 (94.7)	243/400 (59.9)	<0.01
Without underlying diseases	53/74 (70.7)	110/400 (27.1)	<0.01
Without immunodeficiency	66/74 (88.0)	259/400 (63.8)	<0.01
All-cause mortality	3/74 (4.0)	98/400 (21.0)	<0.01

mycosis in China. Compared with other pulmonary mycoses, pulmonary cryptococcosis is characterized by high community incidence, less combined immunodeficiency or underlying diseases, and good prognosis (*Table 3*). Compared with foreign countries, its cure rate is higher in China (36,37), which might be related to the presence of different *Cryptococcus* subtypes (38).

In summary, the nationwide multicenter epidemiological studies on LRTIs revealed differences in microbiology and clinical practice between China and European/American countries. This suggests that the diagnosis/treatment guidelines should be developed based on local research results. China is a vast country and information from remote areas or medium-sized/small hospitals is still

missing because of resource limitations. Further studies are needed to improve the present findings. In clinical practice, when following empirical guidelines, it is important to apply individualized treatment plans while considering the patterns of endemic pathogens, differences in hospitals.

Acknowledgements

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Surgical Techniques

Bilateral pulmonary metastectomy through a unilateral single-port thoracoscopic approach

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Abstract: A 58-year-old woman underwent radical proctectomy 19 months prior to admission. The initial diagnosis was rectal adenocarcinoma of pathological stage T2N0M0. She was discharged five days after the operation. She was followed by abdominal computed tomographic (CT) scan at 3, 9 and 18 months after the operation. Eighteen months after the operation, follow-up abdominal CT scan revealed tiny nodules in the bilateral lower lobes. Subsequent CT scan of the chest showed two tiny nodules in the right lower lobe and a single tiny nodule in left lower lobe. She then underwent single port thoracoscopic surgery through the right side for resection of the nodules. Using a single port wound, we excised the two tiny nodules on the right side and the one tiny nodule in the left lower lobe across the mediastinum. She was discharged four days later. The final pathology report showed those three nodules were metastases from an adenocarcinoma in the colon.

Keywords: Empyema; single-incision thoracoscopic surgery (SITS); uniportal; thoracoscopy/video-assisted thoracic surgery (VATS); metastectomy

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Introduction

The current trend of management of metastatic colon cancer is to a more aggressive approach. Along with the advancement of endoscopic techniques, metastectomy of the lung is performed using an endoscopic approach. Conventional thoracoscopic surgery typically utilizes three or more incisions for an operation. An endoscopic approach has many advantages, but one potential problem of pulmonary metastectomy is the difficulty of identifying very tiny nodules during the course of the operation. Because direct palpation of the lung is not amenable, instrument palpation has become an alternative method to confirm the location of such nodules. At present, thoracoscopic surgery can be performed with a certain single port techniques (1-3). In the past, the single-port techniques were limited to the management of unilateral pleural space. In this case, we have extended the range to the bilateral pleural space. We used a unilateral single-port approach to excise bilateral lung lesions, a procedure which has hardly ever been performed, even with conventional multi-port techniques.

Case

A 58-year-old woman was found to have rectal cancer on the performance of an annual health exam two years ago. At that time, she underwent radical proctectomy after complete cancer survey. The final pathology report showed adenocarcinoma with a depth of invasion to the level of the submucosa and muscularis propria. Lymph nodes were metastasis free. The pathology stage was T2N0M0. Her postoperative recovery was adequate and she was discharged five days after the operation. Because of the stage I status, she did not receive any chemotherapy. She was followed in

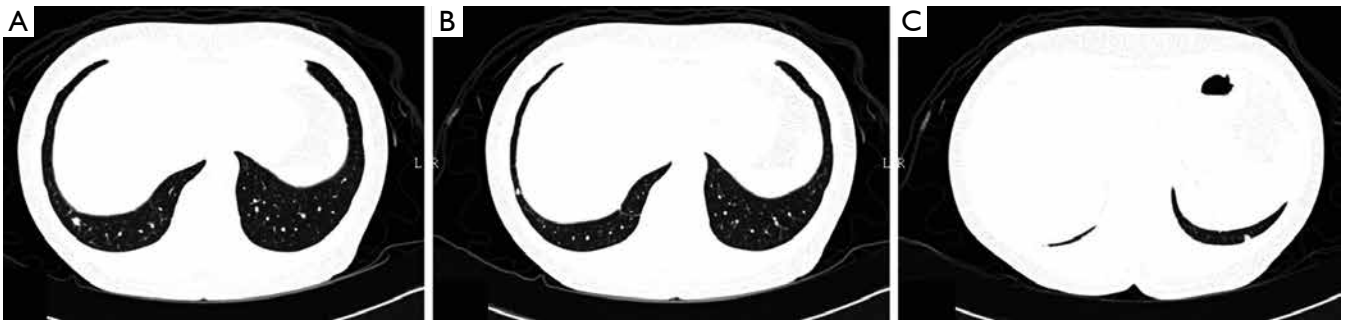

Figure 1 CT scan of the chest showed that there was one 5 mm nodule (A) and one 2-3 mm nodule (B) in the periphery of the right lower lobe. In the contralateral left lower lobe, there was a 3 mm nodule near the pleural surface (C).

the outpatient department.

In the later period of follow-up, she regularly underwent chest radiograph (CXR), whole abdominal computed tomographic (CT) scan, and various blood tests, including an evaluation of the serum level of carcinoembryonic antigen (CEA) at 3, 9 and 18 months after the operation. The CEA level was normal. CXR also displayed a normal pattern. The whole abdominal CT scan at 18 months revealed suspicious tiny lesions in the bilateral lower lung fields. One month later, she was examined with a CT scan of the chest, which showed two tiny nodules in the right lower lobe (*Figure 1A*,*B*) and another tiny lesion in the left lower lobe (*Figure 1C*). The size of the two peripheral lesions in the right lower lobe was 0.5 cm × 0.3 cm and 0.3 cm × 0.2 cm, respectively. The lesion in the left lower lobe was 0.3 cm × 0.3 cm. Because of the presence of newly developed neoplasms, lung metastasis was considered. After discussing with the patient about the choices of either follow-up three months later or thoracoscopic biopsy, she preferred immediate surgical biopsy.

The preoperative evaluation revealed the forced expiratory volume in the span of one second to be 2.21 liters, and the blood tests were all normal. The CEA level was 2.1. CT scan of the abdomen showed no local recurrence and there was no evidence of liver metastasis. After excluding the possibility of extra-pulmonary metastasis, she was prepared for surgical biopsy.

She received general anesthesia in a supine position and was intubated with a Fr. 35 double-lumen endotracheal tube. Then she was changed to a left lateral position and the operative field on the right side was disinfected and draped. We used the single-port approach reported by our team previously (1,2). The basic tools used included a plastic wound protector, a 5-mm endoscope with a 30-degree field-of-view, an endoscopic grasp and a stapler. An L-hook electrical cautery was used for dissection of the mediastinum. In consideration of an effective contralateral dissection, the selection of incision is critically important. We made the 2.5 cm incision in the 6th intercostal space and the incision was slightly medial to the anterior axillary line. We initially approached the right lower lobe lesion from an anterior approach, that is, the surgeon stood on the ventral site of the patient, from which position it was easier to carry out single port surgery. A 5 mm right lower lobe lesion (of 5-mm size) in the periphery was identified (the green arrow in *Figure 2A*), but the 3-mm nodule could not be seen on an endoscopic view. Because the two nodules were very close to each other, we used a stapler to excise the lung tissues that were thought to contain the two tiny lesions (*Figure 2B*). After the specimen extracted, the 5- and 3-mm nodules were palpated and then sent for frozen sectioning, which indicated metastatic adenocarcinoma. Before approaching the contralateral site, we changed the operator's position to the patient's back and then the operative table was slightly tilted backwards by approximately 30 degrees for a better angle of approach. Then we dissected the retrosternal soft tissues at the junction of heart, diaphragm and sternum (*Figure 2C*). With assistance of a ring forceps to push down the soft tissues and pericardial fat (*Figure 2D*), an L-hook electrical cautery was used to dissect the mediastinal pleura that is just beneath the sternum (*Figure 2E*). Using both electrical cauterization and blunt dissection, we created a safe entry route to the contralateral pleura (*Figure 2F*). Then the pleura was opened and we entered the left side pleural space (*Figure 2G*). The 3-mm nodule in the periphery of the left lower lobe was not identified endoscopically. Based on the CT scan of the lung, we excised the part of left lower lobe that was considered to contain the lesion (*Figure 2H*). After pull-out of the specimen, careful palpation showed a very tiny nodule. Frozen sectioning of the surface of the nodule displayed metastatic adenocarcinoma. After confirming lung metastasis, distilled water was used to

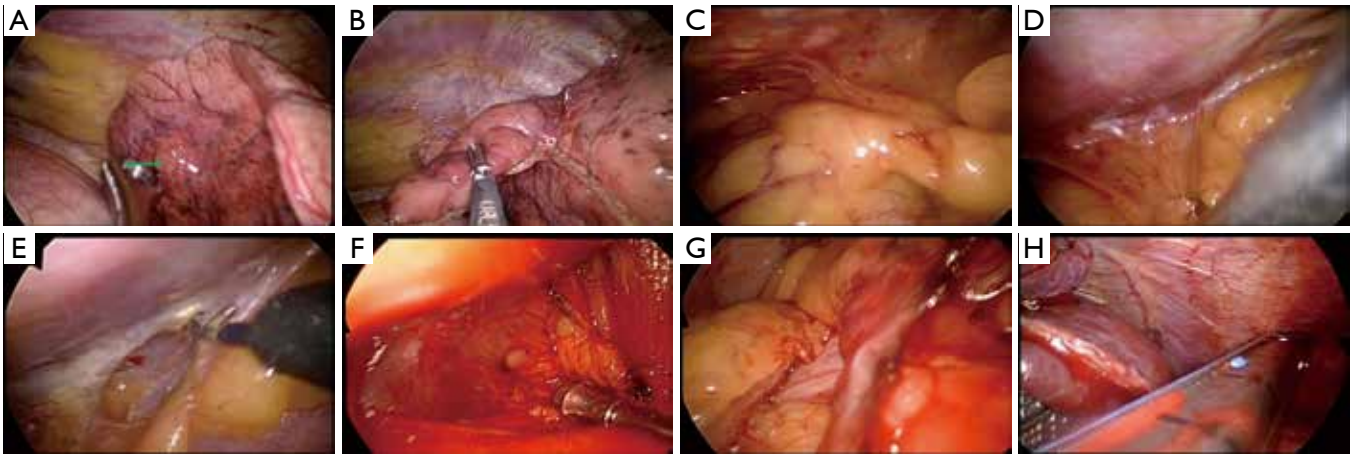

Figure 2 A peripheral nodule was palpated with an instrument in the right lower lobe (the green arrow in A). However, a second tiny nodule could not be palpated. We excised the related part of the lung because we knew the smaller nodule was adjacent to the larger nodule (B). After the completion of a wedge resection of the right lower lobe, we then sought a safe location at the junction of the heart, sternum and diaphragm (C). A ring-forceps was used to hold the mediastinal pleura downwards (D), and then an L-hook electrical cautery was used to open the mediastinal pleura (E). Blunt dissection was performed with either a ring-forceps or suction tube (F) until the contralateral pleura was opened (G). We then identified the suspicious lung tissues for resection with a stapler (H).

irrigate the pleural space. Then a Fr. 24 tube was placed in the left side pleural space through the mediastinum, after which a Fr. 28 chest tube was place in the right side pleural space (*Figure 3A*).

Immediate postoperative CXR indicated adequate lung expansion that was effusion free (*Figure 3B*). The postoperative course was uneventful. The Fr. 24 chest tube was removed 48 hours after the operation and the Fr. 28 chest tube was removed 72 hours after the operation. She was then discharged for subsequent chemotherapy.

Discussion

The management of colorectal cancer with distal metastasis has come to be aggressive, because the overall outcome has improved as a result. The cases reviewed by Kawano *et al.* showed that metastectomy may be of benefit for selected cases with better long-term survival (4). In some patients with very early metastasis to the lung, the lesions may be extremely small and not easy to identify by imaging alone (5). Therefore, surgical biopsy has a role in deciding the management plan of colorectal cancer. Follow-up at a certain interval may be an option, but may also delay treatment planning.

Conventional thoracoscopic surgery utilizes three or more incisions. In this case, we used the single-port approach proposed by us previously (1,6). This technique is comprised of four parts or concepts. The first is the use of a minimal incision to carry out procedures whenever possible. The theoretically smallest incision is just small enough to allow the specimen to be pulled out or to allow the instruments to be placed into the pleural space if there is no specimen to be extracted. The second guiding concept is to use an anterior incision. An incision in the 5th to 6th intercostal space (ICS) on the anterior axillary line is an advantageous site for a single-port approach because of the wider ICS (2,3). However, in considering the approach to the contralateral site, the surgeon must change positions to the patient's back. We tilted the operating table in order to create a more comfortable approaching angle for the surgeon (*Figure 4*). The third concept is to avoid the use of a rigid trocar, so we used a plastic wound protector instead (*Figure 4*). A rigid trocar limits the freedom of instrument handling. A wound protector may afford the maximal degree of freedom for instrument manipulation. The fourth concept or principle is to always try a single port approach at first. If necessary, there is always the option of converting to a conventional multi-port approach or even an open method. Using these same guiding principles, we extended the application of single-port techniques to the treatment bilateral disease.

There are multiple technical considerations which must be borne in mind. The first one is the entry route through the mediastinum must be kept safe. In the case described here, we created a retrosternal route slightly above the level of diaphragm while avoiding compression of the

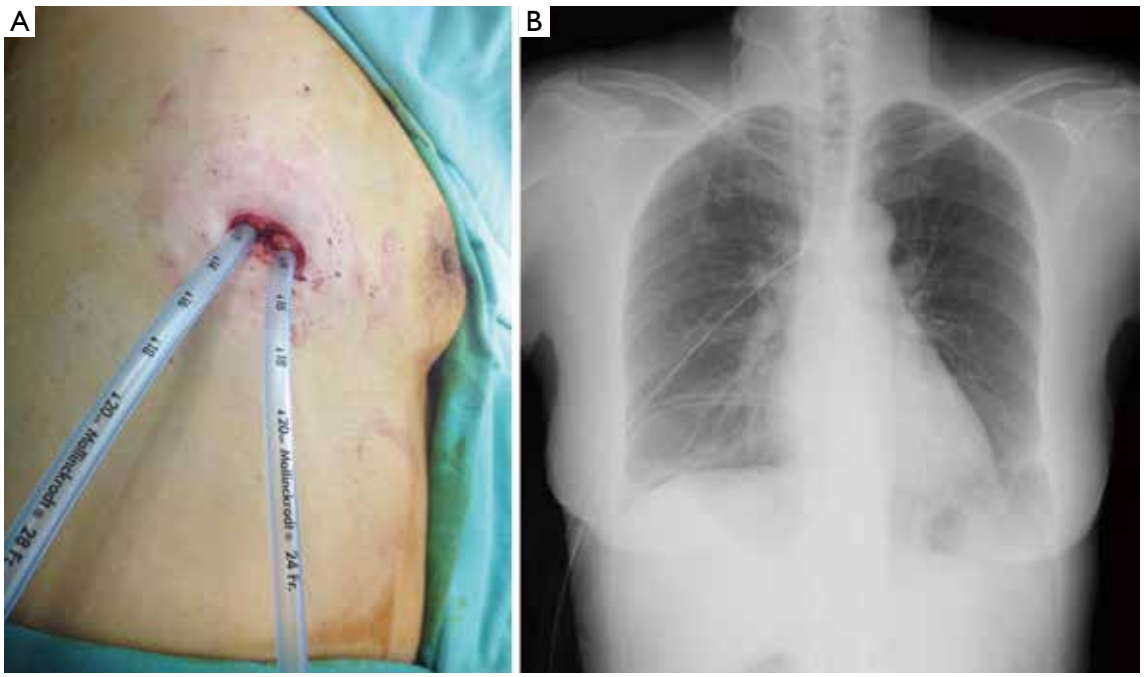

Figure 3 After the procedure, we placed two chest tubes in the respective bilateral pleural spaces. The two tubes were fixed in the same single wound (A). A chest radiograph was taken to confirm their location and function (B).

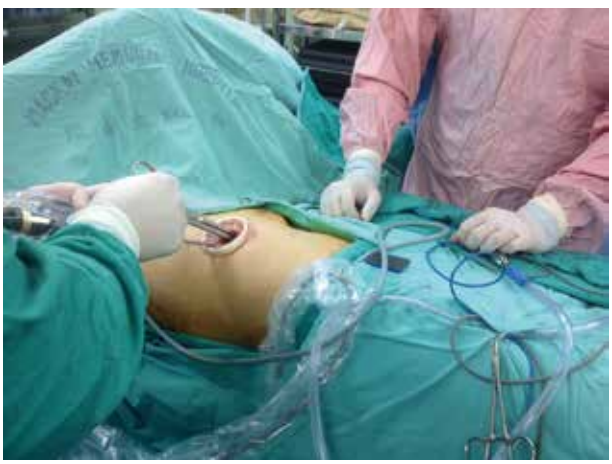

Figure 4 In contrast to the single-port techniques proposed by us that using a ventral location for the operation, the position was changed to the patient's back in order to provide an adequate approach to the contralateral lesion.

heart. If the entry route is higher in the level of heart, direct compression of the cardiac chambers may carry an additional risk of arrhythmia and/or hypotension during the procedure. The bilateral internal mammary arteries should not be transected. If the plane is correct, blunt dissection with minimal electrical cauterization is sufficient to create the space within a matter of minutes. The potential space across the mediastinum created in this way is prone to close if the endoscopic instruments are not in place. We still lack an effective tool for keeping the space open without any additional instruments to push the soft tissues downwards. This makes the procedures more complex and difficult in the single-port approach than is optimal. For the lesion in the contralateral lower lobe, the space we created in this case was sufficient. However, if the lesion is in another location that is more challenging, a different entry site should be considered.

A prospective study performed in 1996 showed that thoracoscopic identification metastatic lung nodules may be difficult and had higher failure rate (7). However, the techniques of thoracoscopic surgery and resolution of CT improved a lot in recent 15 years. At present time, high-resolution CT scan can detect pulmonary lesion around 2 to 3 mm in diameter. Endoscopic techniques also allowed us to detect superficial lesion more than 3 mm in diameter. However, for deep parenchymal lesions, detection may be very difficult. Even in ipsilateral side, CT-guided needle localization may be required.

The identification of tiny nodules in the contralateral lung using our method may be usefully assisted by CT-guided needle localization. In the absence of a

needle localization method, the identification of a tumor less than 3 mm is very difficult. Endoscopic inspection and instrument palpation are the only two means of confirming the location. Based on our experience, contralateral small nodules should be localized under CT-guidance.

The field-of-view is limited in this procedure. With some amount of improvement in the design of the endoscope, the field-of-view may be greatly improved (8). The manipulation is also limited by the current straight endoscopic instruments as well as the stapler. Therefore, the approach should be considered best suited to highly selected cases. Not all patients with bilateral lung tumors can be treated by this method. Another consideration is that when malignant effusion is encountered, the opening of the mediastinum may make the possibility of mediastinal metastasis a serious concern. Moreover, in a particularly complicated condition, such as adhesion, the approach may not be feasible and additional incisions or a contralateral incision may be required.

Conclusions

This report demonstrates the value of single-port thoracoscopic surgery in treating bilateral pleural and lung disease. It should be considered as an alternative time-saving method for certain conditions in which the simultaneous treatment of bilateral disease is required.

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Thoracoscopic purse string technique for minimally invasive Ivor Lewis esophagectomy

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Objective: Thoracolaparoscopic esophagectomy with chest anastomosis (TLE-chest) is increasingly performed for middle and lower esophageal cancer; however, gastroesophageal anastomosis for this surgery remains both challenging and inefficient. To address this issue, we previously reported our MIE technique with Ivor-Lewis anastomosis. Here we present the video to introduce our TLE-chest operation procedures.

Methods: TLE-chest with a combined thoracoscopic and laparoscopic technique was performed by one group of surgeons. From October 2011 to September 2013, 80 esophageal cancer patients were treated with TLE-chest using this improved anastomotic technique.

Results: The surgery was successful for all patients, although the anastomosis in one patient required intraoperative manual repair. No patients required open conversion. In this video, dissociation of stomach, and dissection of lymph nodes, creation of gastric tube and staple line embedding, jejunostomy were carried out by laparoscopic surgery. Dissection of esophageal cancer and mediastinal lymph nodes were done through rib 3 or 4 by a 3-4 cm video-assisted right anterior minithoracotomy, then esophago-gastric anastomosis was performed in right thoracic cavity This video shows the R0 resection of T3N0M0 esophageal cancer. Totally, 36 lymph nodes were dissected, including 21 mediastinal lymph nodes and 15 abdominal lymph nodes. The patient recovered well and was discharged on day 8 after the surgery, with good short term outcomes.

Conclusions: A safe, cost effective purse string stapled anastomotic technique has been presented for TLE-chest in our video. It is consistent with the oncology principles.

Keywords: Esophageal cancer; esophageal surgery; thoracolaparoscopic esophagectomy; anastomosis (gastroesophageal)

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Introduction

Surgery is the main treatment for resectable esophageal cancer; however, the mortality of open esophagectomy is high and consequently minimally invasive esophagectomy (MIE) was developed (1). MIE was initially developed by Cuschieri *et al.* (2) and DePaula *et al.* (3). Various technical modifications has been made to this technique, including laparoscopic assisted and thoracoscopic assisted techniques. Combined thoracoscopic laparoscopic esophagectomy with cervical anastomosis (TLE-neck) and intrathoracic anastomosis (TLE-chest) have gradually become the mainstream MIE techniques. The first randomized controlled study in the world showed significant advantages of MIE over traditional open surgery (4). TLE-chest is increasingly used for the treatment of mid and lower esophageal cancers. Studies (5-7) have shown that this technique is safe and effective, particularly in reducing perioperative complications such as recurrent laryngeal nerve injury, lung infection, and anastomosis fistula (6). Previously we described our MIE technique with Ivor-Lewis anastomosis. Here we present in the video our TLE-chest operation procedures (*Video 1*).

Video 1 Thoracoscopic purse string technique for minimally invasive Ivor Lewis esophagectomy.

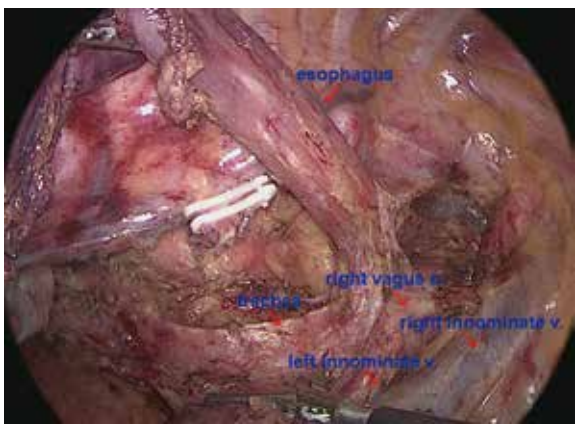

Figure 1 The border of 2R/4R and right laryngeal recurrent nerve lymph nodes.

Operative techniques

Abdominal part of the operation

Five ports are made. A forcep is placed through the 5 mm trocar below the xyphoid process to grasp the gastrohepatic ligament for liver retraction.

Stomach mobilization and lymph nodes dissection

After laparoscopic abdominal exploration, the gastric body is mobilized using a Harmonic scalpel. The mobilization is started at the lesser curvature. The three branches of the celiac trunk are "skeletonized" and surrounding lymph nodes are dissected (*Figure 1*). The gastrosplenic ligament is carefully transected. The dissection at the greater curvature side shall be made downwards to sufficiently separate the adhesions at the pylorus. The dissection is made upwards to expose both crus. Left and right cardia lymph nodes are dissected. Definitive attention shall be made to avoid gastric serosa injury, and to protect the integrity of right gastroepiploic vascular arch.

Gastric conduit preparation and staple line embedding

Vessels at the lesser curvature and 3-5 cm proximal to the pylorus are managed. Most of the gastric conduit is made using 3-4 firings of Echelon 60 stapler (Ethicon Endo-Surgery, Cincinnati, Ohio, USA). The staple line of the gastric conduit is embedded with gastric muscular and serosa layers using absorbable suture (4-0 Braided Absorbable Suture, Covidien, USA).

Jejunostomy

Jejunostomy is made laparoscopically at 20 cm distal to the Treitz ligament, and a feeding tube (Flocare CH08 jejunostomy feeding tube, Nutracia) is placed.

Thoracic part of the procedure

The patient is turned to left semi-prone position. Single lung ventilation is established. The thoracic procedure is operated using four ports.

Esophagus mobilization

Following thoracic exploration, the esophageal bed and mediastinal pleura are opened, and the esophagus is lifted. The azygos vein is divided after Hem-O-Lok double clipping at the proximal and distal sides. The esophagus is mobilized upwards until 2 cm above the azygos vein arch. Attention should be given to preserve the mediastinal pleura above the azygos vein arch.

Lymph nodes dissection

The lymph nodes of inferior pulmonary ligament and of the starting point of the left recurrent laryngeal nerve, and paraesophageal and subcarinal lymph nodes are dissected (*Figure 2*). Exploration is made along the anatomical location of the right recurrent laryngeal nerve chain and the lymph nodes are dissected. Neither recurrent laryngeal nerve is skeletonized during their lymph nodes dissection.

Thoracoscopic purse string

The 5 mm trocar in the 3/4 intercostal space on the posterior axillary line is extended to 3-4 cm, and a wound protector is placed. The gastric conduit is lifted to the thoracic cavity. A purse string is made using 3-0

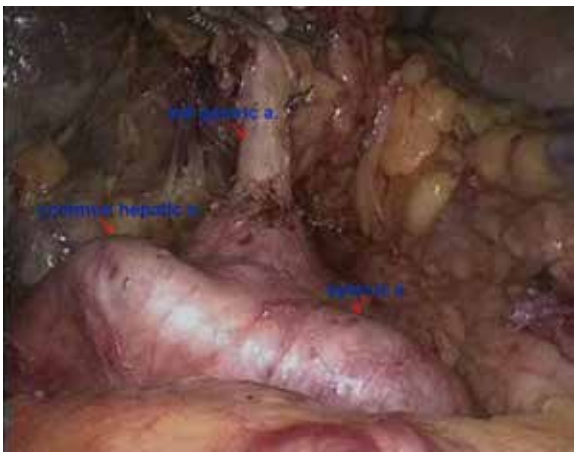

Figure 2 The left gastric artery, hepatic artery and splenic artery were skeletonized for adequate celiac lymphadenectomy.

Prolene 5-8 cm proximal to the tumor. An incision is made 2-3 cm distal to the purse string. An anvil is placed into the esophagus and the purse string is tightened and tied.

En bloc tumor resection

The esophagus is transected layer by layer. The esophagus and the stomach are pulled out of the chest cavity. The mid and lower esophagus with the tumor, cardia and lesser curvature lymph nodes are resected en bloc.

Gastroesophageal anastomosis

The incision of the gastric conduit is lifted using the triangle-shaped sutures and the body of the circular stapler (CDH stapler, Ethicon Endo-Surgery, USA). The anvil is connected to the stapler and an end-to-side gastroesophageal anastomosis is completed in the right thoracic cavity. The remaining gastric conduit is completed using Echelon 60, and the staple line is embedded.

Embedding of the anastomosis

The anastomosis is embedded with mediastinal pleura that has been preserved for this purpose.

Discussion

TLE-chest provides an effective technique for the treatment of mid and lower esophageal cancers; however, thoracoscopic gastrointestinal tract reconstruction remains challenging. Although devices such as OrVil (8,9) and Endo-Stich (6,10) are available, these devices are quite expensive. We previously reported a novel purse string technique for the first time in the world (11). We have been applying this technique to the TLE-chest of 80 consecutive cases since October 2011, and the perioperative results have been satisfactory.

This technique presents the following features: (I) the purse string is made thoracoscopically using an atraumatic suture and the process is convenient, fast and cost effective; (II) the transection of the esophagus is made layer by layer; the muscular layer is divided first, followed by the division of the mucosal layer. More importantly, the mucosal layer is retained 5 mm longer than the muscular layer to prevent mucosa retraction; (III) the tumor is resected en bloc, which is consistent with the oncology principle and it facilitates intrathoracic anastomosis; (IV) the anastomosis is made with a regular circular stapler, which is cost effective for wider application; (V) mediastinal pleura is preserved for the embedding of the anastomosis to enhance the safety of the anastomosis.

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Combined thoracoscopic and laparoscopic minimally invasive esophagectomy

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Abstract: With the improvement in thoracoscopic and laparoscopic surgery, thoracoscopic and laparoscopic esophagectomy (TLE), a minimally invasive approach, has attracted increasing attention as an alternative to open three-field esophagectomy. From June 2012 to October 2013, 90 patients underwent laparoscopic and thoracoscopic resection of esophageal carcinoma in our department. The VATS esophagectomy technique described here is the approach currently employed in the department of thoracic surgery at Sichuan Provincial People's Hospital of China.

Keywords: Thoracoscopic and laparoscopic; minimally invasive; esophagectomy

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Introduction

Esophagectomy remains effective for patients with localized esophageal carcinoma (1). However, invasive esophageal cancer surgery carries a high incidence of complications, and serious complications still occur although improvements have been made in surgical maneuvers and perioperative care. Regardless of the approach, open esophagectomy has been associated with considerable morbidity and mortality, the incidence of major or minor complications is still 70-80%, and the operative mortality is 4-7% in experienced surgery centers (2). Great progress has been made in video-assisted thoracoscopic technique during the past 20 years. Since thoracoscopic surgery involves minimal intercostal incisions, respiratory function can be retained, so the pulmonary complications such as pneumonia and atelectasis decrease (3-5). Many esophageal cancer surgeries take a long time, bringing stress to the operating staffs as well as the patients, so it's urgent to improve surgical procedures and reduce the operation duration (6,7). As the experience and skills for thoracoscopic and laparoscopic surgery improve, thoracoscopic and laparoscopic esophagectomy (TLE) has attracted increasing attention and becomes an alternative to open three-field esophagectomy with fewer complications.

Video 1 Combined thoracoscopic and laparoscopic minimally invasive esophagectomy.

The technique of VATS esophagectomy described here is currently employed in the department of thoracic surgery at Sichuan Provincial People's Hospital of China (*Video 1*).

Clinical summary

A 59-year-old lady presented with progressive dysphagia

Figure 1 The surgery position and four VATS ports in right chest wall to facilitate optimal views of the posterior hilum and placement of instruments.

for three months. Gastroscopy revealed a nodular neoplasm in the esophagus 25-30 cm away from the incisor teeth. Pathological biopsy demonstrated esophageal squamous cell carcinoma. CT scan revealed the middle of the esophageal wall was thickening and mediastinal lymph nodes were enlarged.

Formal spirometry showed a FEV1 of 3.02 (109.3% predicted), a FVC of 3.80 (105% predicted) and an FEV1/FVC ratio of 82.49%.

Pre-operative assessment

Esophageal cancer surgery is associated with a high incidence of complications, especially the anastomotic leakage and pulmonary complication. Therefore pre-operative selection and assessment of patient may be crucial. Moreover, the location and size of the tumor, foreign invasion, lymph node metastasis, heart and lung function are to be evaluated before operation. Operator is also a key factor and individuals unfamiliar with VATS techniques should not attempt VATS esophagectomy.

Anaesthesia and positioning

General anesthesia was performed with double lumen intubation, which allowed independent ventilation of either lung for better exposure. Meanwhile, artificial pneumothorax was employed and the patient was kept in left lateral position leaning forward with upper limb suspended, in which way, the posterior mediastinal tissues were better exposed.

Technique

Four VATS ports were made in right chest wall for optimal views of the posterior hilum and instrument insertion: one incision about 5 mm in length in the third intercostal axillary line and fourth intercostal anterior axillary as the operation port, and one incision 12 mm in length in the seventh intercostal axillary midline and eighth intercostal axillary line as the operating hole and the observation hole; cervical and abdominal incision include: incision over the front of left sternocleidomastoid muscle and 5-12 mm incision over subxiphoid, navel, left and right midclavicular line subcostal (*Figure 1*).

The first step in the procedure is to confirm resectability and to identify invasion of the chest wall, pleurae and hilar structures including the aorta, pulmonary artery and bronchus. The second step is thoracic operation: performing anatomical esophageal resection completely, the principle and the extent of resection of which is similar to conventional open thoractomy. Electric coagulation hook and ultrasonic knife were used in esophageal isolation from top to bottom, clamp the two ends of arch of azygos vein and cut them off with Hemolok. All lymph nodes in the operative field were dissected, including lymph nodes of paraesophageal, carina, left and right recurrent laryngeal nerve chains (*Figure 2*). Intraoperative care was required to avoid inadvertent injury to the vagus, phrenic, recurrent laryngeal nerves and membranous part of trachea. Then, a 32 F chest drain was placed through the anterior port before closure. The third step is gastric dissociation: assisted by laparoscopy, ultrasonic scalpel dissected gastric greater and lesser curvature, fully separated the left gastric artery

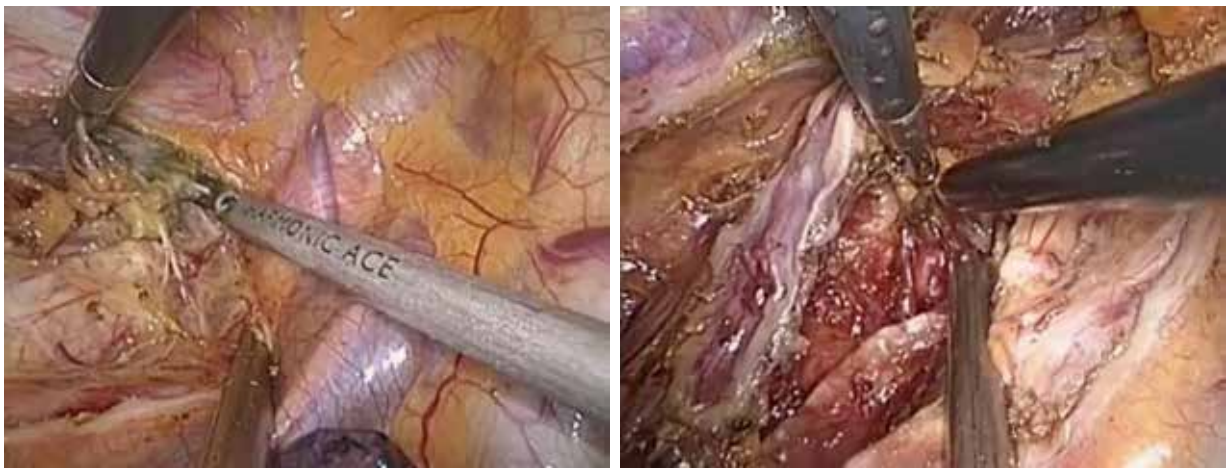

Figure 2 All lymph nodes in the operative field were dissected, including left and right recurrent laryngeal nerve chain.

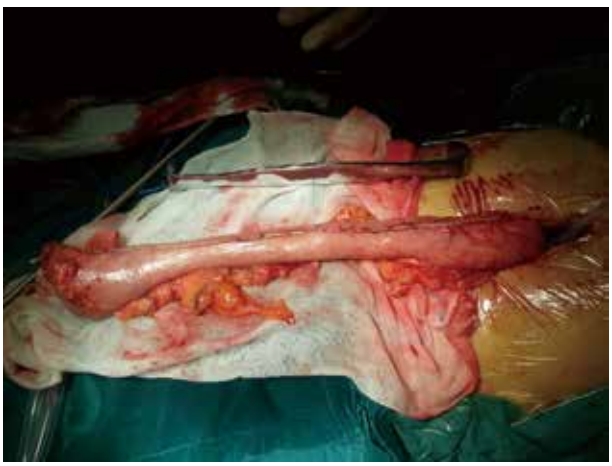

Figure 3 Making pipe type gastric.

and vein, and then clamped the two ends of left gastric artery and vein to cut them off with Hemolok. Stomach was fully separated with attentions paid to avoid injury of adjacent vessels, spleen and liver. The last step we dissected cervical esophagus, made a pipe type gastric (*Figure 3*), a circular stapler was used to complete cervical esophagogastrostomy. Operation time: 210 min, intraoperative blood loss: 200 mL.

Final pathology revealed a T3N1M0 highly differentiated squamous cell carcinoma involving the vagus nerve.

Post-operative management

All patients fasted 7-9 days after operation, and underwent enteral feeding by the duodenal feeding tube from the next day after the operation. On the ninth day after operation, patient took liquid diets without exception, and chest X-ray was normal, and the chest tube was extubated.

Comments

Combined thoracoscopic and laparoscopic minimally invasive esophagectomy is a safe and reliable option for esophageal carcinoma with smaller trauma, shorter hospital stay, less blood loss, less pain and better appearance, especially for the elderly and those with poor lung function. As reported by Richards, this approach also improves the view of the mediastinal node packets which facilitates lymphadenectomy (8). TLE is technically complex, so a steep learning curve is required to reach optimal surgical outcomes, however, thoracic surgeons will have to treat more cases to climb the learning curve because most of them are not familiar with laparoscopic skills (9).

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All authors have the same contribution to this article.
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Thoracoscopic and laparoscopic radical esophagectomy with lateral-prone position

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Abstract: With 20 years of development, minimally-invasive treatment for esophageal cancer has been widely spread. However, surgeons have not reached consensus about the optimal minimally-invasive operation method, or whether the effect of radical lymph nodes dissection is comparable to the traditional open procedure. Thoracoscopic esophagectomy with lateral-prone position combines the advantages of both lateral position (allowing quick conversion to open procedure) and prone position (good visual area and complete lymphadenectomy). Together with laparoscopic abdominal lymphadenectomy, gastric tube formation and jejunostomy, this approach provides an easier way for minimally-invasive radical esophagectomy. In this article, approaches for thoracoscopic esophagectomy with lateral-prone position and total mediastinal lymphadenectomy, combined with totally laparoscopic gastric mobilization, abdominal lymphadenectomy, gastric tube formation and jejunostomy, will be presented by video instructions. All the procedures were under the rule of radical lymphadenectomy. Cervical lymph nodes dissection and esophago-gastrostomy were the same as those in open procedure, which will not be discussed here.

Keywords: Minimally-invasive surgery; video-assisted thoracoscopic surgery (VATS); esophageal cancer; lateral-prone position

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Introduction

Esophageal cancer is one of the most malignant tumors threatening people's lives, and the 6th leading cause of cancer-related death worldwide. China is the high incidence area for esophageal cancer, with more than half of the patients with esophageal cancer in the world (1). Squamous cell carcinoma is the most common type for esophageal cancer, with main causes of death of post-operative lymph nodes recurrence and metastasis. Therefore, three-field lymphadenectomy involving neck, thorax and abdomen is highly suggested for its improved long-term survival rates, but it is also limited for its high rate of morbidity and mortality (2). Minimally-invasive surgeries employing thoracoscopic and laparoscopic techniques are widely performed over the past 20 years. According to the reports of Luketich *et al.* (3) in 2003 and Palanivelu *et al.* (4) in 2006, minimally-invasive esophagectomy (MIE) has the same long-term survival rate but less post-operative complications and higher survival quality of life compared with open procedures. These advantages have made MIE widely-spread and adopted in more and more cases (5,6) over the last decade.

MIE is an approach which combines thoracoscopy, mediastinoscopy and laparoscopy, however, which combination is optimal is still under discussion. Even more, whether radical lymph nodes dissection is fully completed as in open procedure is still under doubt (7).

In this article, main steps of thoracoscopic esophagectomy with lateral-prone position and total mediastinal lymphadenectomy, combined with totally laparoscopic gastric mobilization, abdominal lymphadenectomy, gastric tube formation and jejunostomy will be introduced, in order to reach the target of minimally-invasive radical esophagectomy,

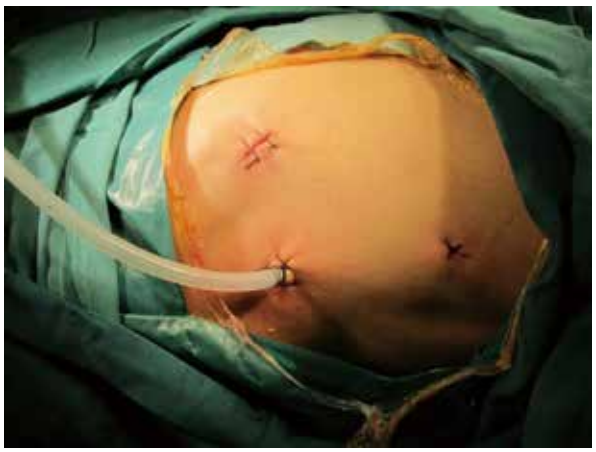

Figure 1 Thoracoscopic access at the end of the surgical procedure.

Video 1 Denudation of arch of azygos vein.

Video 2 Lymphadenectomy of right para-recurrent laryngeal nerve for intraoperative frozen section.

both thoracoscopically and laparoscopically.

Thoracoscopic esophagectomy with lateral-prone position and total mediastinal lymphadenectomy

Patient was intubated with single-lumen endotracheal tube, with lateral-prone position of 45 degree leaning to the left. Thoracoscopic observation port (12 mm) was placed at middle axillary line at the sixth intercostal space, inflated with CO_2 at 6-8 mmHg, collapsing right lung lobe. During operation, tidal volume can be properly lowered by anestheologist in order to better expose posterior mediastinum. Main operative port (5 mm) was placed at the right side of middle axillary line at the fourth intercostal space. Assisting port (12 mm) was place 2 cm behind the right side of the posterior axillary line at the eighth intercostal space (*Figure 1*). Esophagus mobilization and mediastinal lymph nodes dissection were successively done.

(I) Chest exploration, adhesion separation and azygos vein ligation. Mediastinal pleura in the posterior side of esophagus is incised to ensure the resectability of tumor (*Video 1*);

(II) Lymph nodes at the right side of para-recurrent laryngeal nerve were dissected and sent for intraoperative fast frozen pathological diagnosis, in order to decide whether three-field lymphadenectomy would be employed in patient's cervical area (8) (*Video 2*);

(III) Supra-diaphragmatic lymph nodes and fat tissue were then dissected and thoracic duct was ligated. Lower esophagus was mobilized and tied with tractor, in order to better reveal the left side of mediastinum (*Video 3*);

(IV) Esophagus, para-esophageal lymph nodes and subcarinal nodes were resected en bloc from distal to proximal end until thoracic inlet. Lymph nodes at the left side of para-recurrent laryngeal nerve and aortopulmonary window lymph nodes were further dissected (*Video 4*).

The lateral and prone positions are commonly employed in the thoracospic esophagectomy. The former goes in line with the visual habit of both open and video-assisted thoracoscopic surgery (VATS), facilitating multiple procedures, which needs veteran assistants to help reveal detailed structures, and affects lymph nodes dissection by restricting the exposure of the left side of mediastinum. The latter provides better exposure for the left side of mediastinum, thus complete lymphadenectomy

Video 3 Dissect the anterior diaphragmatic lymph nodes, mobilize lower esophagus and tie with tractor.

Video 5 Mobilization of greater gastric curvature.

Video 4 Mobilization of the thoracic esophagus.

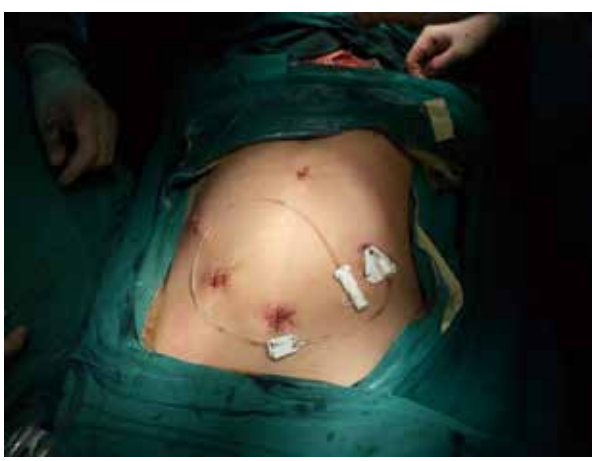

Figure 2 Laparoscopic access at the end of the surgical procedure.

is ensured. However, the visual habit differs from that of the traditional open procedure, which requires operator's accommodation. Another weak point for prone position is that patient's position should be shifted if conversion to open surgery is needed. Considering pros and cons of both positions, the author suggests esophagectomy be performed with lateral-prone position and total mediastinal lymphadenectomy, which can be an approach for thoroughly resecting thoracic esophagus and dissecting total mediastinal lymph nodes, as proved in 130 cases of study. Apart from that, advantages such as single-man operation, better exposure of the left side of mediastinum and more complete lymph nodes dissection are also significant. Moreover, immediate conversion to open procedure could be possible, especially when intraoperative bleeding occurs.

Totally laparoscopic gastric mobilization, abdominal lymphadenectomy, gastric tube formation and jejunostomy

Patient was reset to supine position, with head higher above feet and 30 degree leaning to the right. Incisions were made traditionally with five ports (see *Figure 2*). Gastric mobilization, abdominal lymphadenectomy, gastric tube formation and jejunostomy were performed in sequence.

(I) Great curve was separated up to gastro-splenic ligament and down to pyloric (*Video 5*);
(II) Lymph nodes located in common hepatic artery, splenic artery, celiac trunk and para left gastric artery were dissected (*Video 6*);
(III) Gastric tube formation (*Video 7*);
(IV) Jejunostomy (*Video 8*);

Video 6 Lymphadenectomy at lesser gastric curvature.

Video 9 Tubular stomach pulled-up.

Video 7 Tubular gastroplasty.

Video 10 Suture of diaphragmatic hiatus.

Video 8 Jejunostomy.

(V) Esophageal hiatus was mobilized, and the gastric tube was lifted to the neck (*Video 9*);

(VI) Diaphragm hiatus was sutured to prevent post-operative diaphragm hernia (*Video 10*).

All the above six steps were performed laparoscopically.

Laparoscopy was employed in esophagectomy as total laparoscopy and hybrid laparoscopy. The latter was less difficult during operation, with higher safety and shorter operation time, thus more widely adopted clinically. Total laparoscopy requires higher techniques such as suturing for formation of gastric tube and jejunostomy, but provides smaller trauma. By experience of 130 cases, the author believes total laparoscopy, compared with open procedure, can provide the same therapeutic effect of gastric mobilization and lymph nodes dissection, and safe gastric

tube formation and jejunostomy.

Conclusions

Thoracoscopic and laparoscopic radical esophagectomy and esophago-gastrostomy with lateral-prone position is a reliable approach to treat esophageal cancer. Comparing with open procedure, this approach provides the same lymphadenectomy but less surgical trauma and post-operative complications, as well as improved post-operative quality of life.

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ICC COLUMN: The Voice of the Patient

The global battle to improve patients' health outcomes: COPD awareness, activities, and progress

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Awareness and activities

The International COPD Coalition (ICC) has been working to improve COPD awareness and action since it was organized in 2001. Shortly after the launch of ICC in 2001 we did an omnibus phone and in-person interview study of COPD awareness in Canada, Brazil, China, and Germany and found that global public awareness of COPD was very limited. It ranged from 4% of the public in Brazil to about 10% in Germany. Increasing public awareness was a high priority to help COPD patients. This was important because these data showed us early on how few people knew about the disease and how important it was to increase awareness globally. By now—in 2014—many countries' COPD leaders are tracking public awareness of COPD every year. The data from a survey (*Figure 1*) that ICC has just completed of estimated COPD awareness in 41 different countries (26 developed and 15 developing countries) as provided by the ICC COPD patient organization leaders from each of the countries shows clearly that there has been an overall global increase in awareness since 2001, with almost 37% of the countries reporting public awareness figures of 20% or greater. *Figure 2* shows the differences in the estimates of awareness from developed and developing countries. 31% of developed countries have public awareness greater than 40%, while 0% of developing countries have such high awareness. However, even though developing countries have lower awareness of COPD, *Figure 3* indicates that awareness has been increasing in almost 60% of all countries, while no countries report decreasing awareness.

Mass communication by television and the internet have made a big difference in expanding COPD awareness. In the US, direct-to-consumer advertising of COPD products has played a key role, with its broad reach and frequent reminders. It is important to realize that overall COPD awareness in a country can be rapidly increased by well-planned communications, especially if the government, the medical and commercial leaders, and the patient organizations play active roles with the mass media.

Respiratory medical professional groups in Norway performed phone surveys from 2002 to 2005 in conjunction with a national COPD awareness initiative and asked a random sample of adults in Norway "Have you ever heard about COPD?" During the four years of this campaign the positive responses to this question went from 27% to 78% (*Figure 4*) (1). The success of the Norwegian awareness campaign has greatly improved our understanding of how to increase COPD awareness. The yearly World COPD Day campaigns, which ICC initiated in 2002 in collaboration with the GOLD initiative, have helped many countries build COPD awareness and action.

The activities of national respiratory patient organizations are crucial in proposing and implementing improvements in respiratory disease prevention and treatment. ICC has surveyed these activities globally as well. In wealthy countries, like the US, a great deal of COPD education and promotion is being done. Particularly successful have been the activities organized by the US COPD Foundation. *Figure 5* shows the algorithm that summarizes the comprehensive programs that the US COPD Foundation conducts for COPD education and awareness promotion in the US. Their work includes public policy liaison, COPD advocacy, and COPD research!

Figure 6 presents top line information from an

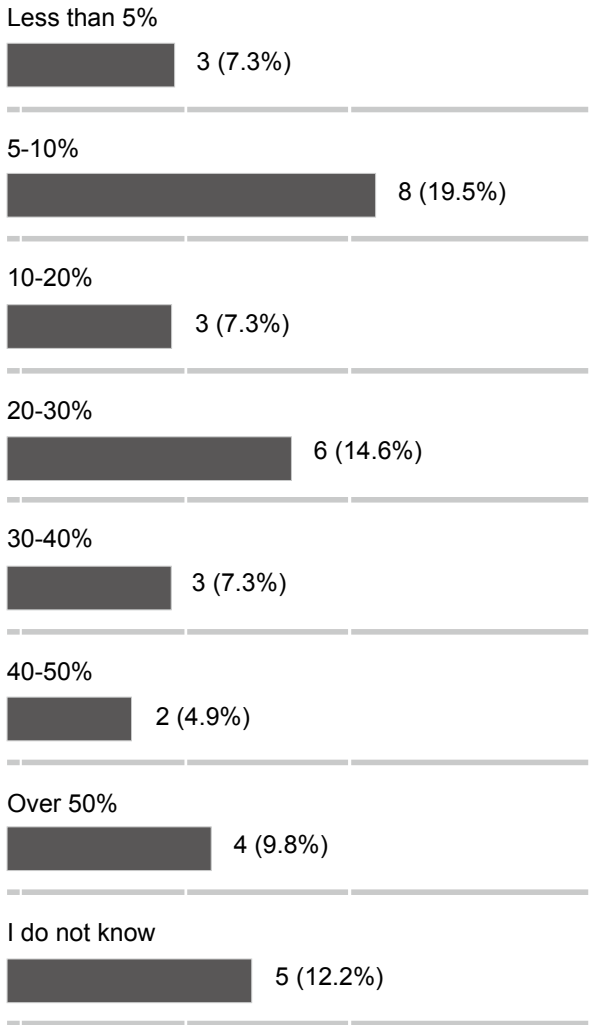

Figure 1 COPD awareness. What percentages of the public in your country are aware of COPD as a disease?

What percentages of the public in your country are aware of COPD as a disease?

Q2 Awareness	% Respondents	
COPD Awareness	developed	developing
<5%	8%	13%
05-10%	15%	27%
10-20%	8%	13%
20-30%	15%	13%
30-40%	8%	7%
40-50%	8%	0%
50%+	23%	0%
unknown	15%	27%
Total	**100%**	**100%**

Figure 2 ICC survey 3-COPD awareness.

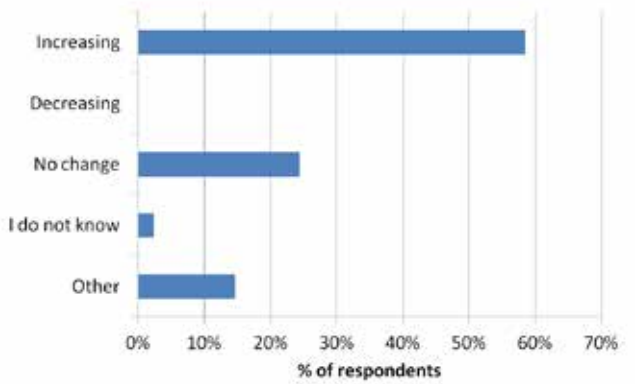

Figure 3 Have there been any changes in the past several years in COPD awareness among the public in your country?

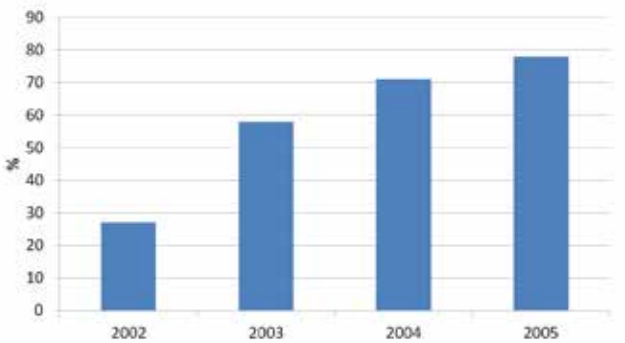

Figure 4 Have you ever heard about COPD? (Adapted from the *Clinical Respiratory Journal*).

ICC survey of the major activities globally of COPD organizations in their countries. There are many creative and effective educational programs of different types that have been carried out globally to serve the many different countries throughout the world. Some resemble those in the US, but most countries have their own COPD priorities and their own means of communication. In the developing world there are limited budgets and fewer COPD activities are possible. *Appendix 1* contains verbatim remarks from national ICC leaders concerning country-specific COPD patient organization activities. They include different approaches to COPD education, prevention, and management.

Budgetary limitations on respiratory patient organizations, particularly in the developing world, limit their ability to carry out educational programs. *Figure 7* presents data from an ICC survey in press in the *Journal of Thoracic Disease*. It tabulates the presence of various respiratory patient organizations globally. These organizations focus their

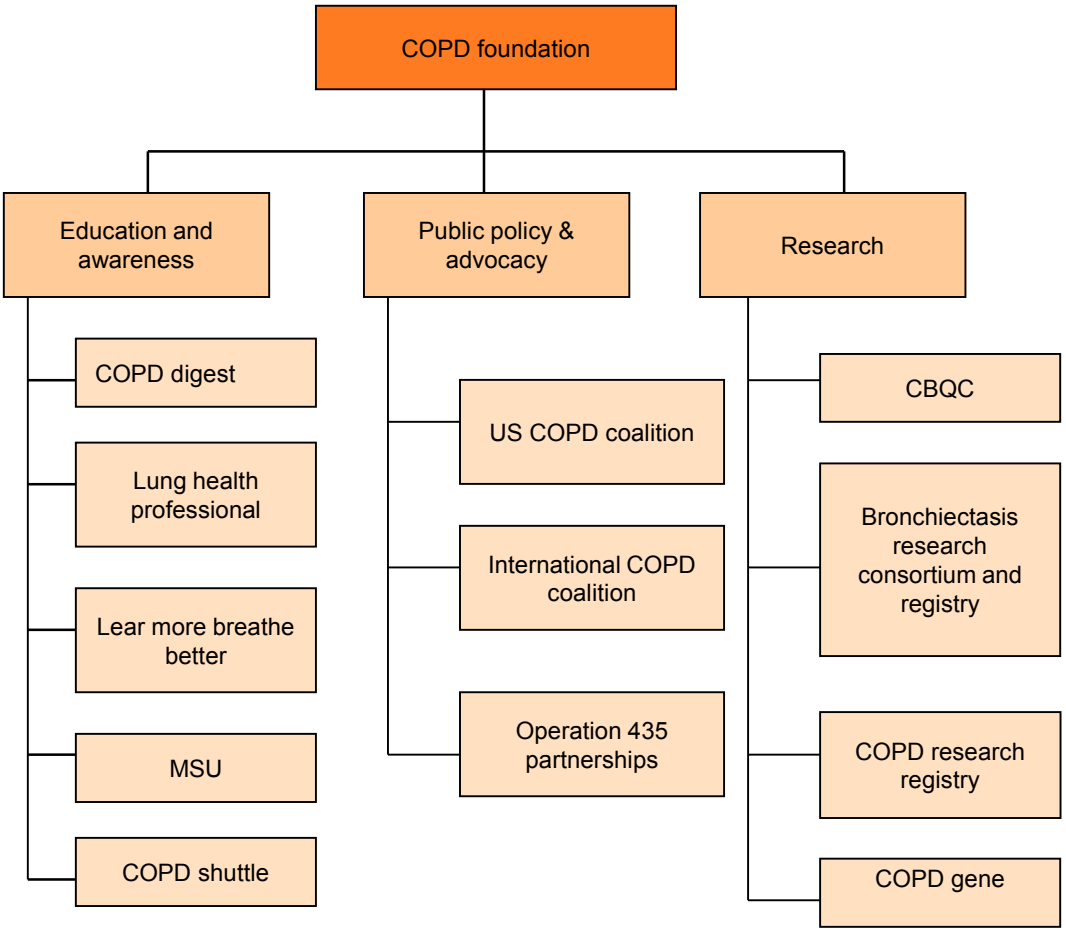

Figure 5 US COPD Foundation algorithm of services (2).

Examples of replies

- In Australia, LFA has been building a COPD Online series of tools for patients and carers, as well as for nurses, doctors, pharmacists and rehabilitation providers.
- In Japan, COPD being adopted in 2012 to the 4th targeted health problems due to mal-habit related life style in Japan after cancer, CVD and metabolic syndrome (DM).
- In China, COPD is the key disease of research supported by government funding; COPD patients receive additional medical care compensation in Guangzhou as the result of pulmonologist's effort to convince the government. Some researches on early diagnosis and early intervention in the community are going on.

Categories of replies

Categories of replies	Count
Advocacy, education and awareness campaigns	8
Reimbursement coverage/Affordable medication	7
Smoking cessation	7
None	5
Various	3
Improve PCP diagnosis/ acces to spirometry	2
Health policy priority	2
Guidelines	2
Research	2
International organizations	1
Rehab referrals	1
Oxygen	1

Figure 6 What is the most important action to benefit COPD patients that is going on in your country?

Appendix 1 This appendix provides the verbatim responses from all the ICC leaders who replied to the full questionnaire. It includes the detailed descriptions of the COPD patient activities in each of the countries represented

Q4	Q5	Q6
What percetage (%) of COPD patients cannot afford state-of-the-art medications in your country?	How do the patients deal in your country with the expense of COPD care if they do not have adequate financial resources?	What is your country?
30-40	It varies: some go without, some find help for paying for meds, some of the pharma companies have programs for low income folks	USA
10-20	Limited resourses at Govt Hospitals and voluntary agencies. I have established COPD Club for patients organising meetings and issue of medicines to the poor	India
Less than 5	Consult social workers' office	South Korea
Over 50	Public nor we gain health insurance	Norway
30-40	Liquid oxygen cost 4,300 per month In my case half of this cost comes out of my pocket, as in my case as with my case as with others, it may require substantial loans and re-mortgage of my home and others, there are grey areas in provincial coverage	Canada
Over 50	Patients only seek medical care when they are in acute exacerbation stage. Patients use only the cheap, affordable medications that are less effective	China
Less than 5	The need is sometimes for caregiver support, for respite and for home services	Canada
20-30	Deprived of care	USA
I do not know	Most of the ones I deal with here simply do not use their meds the way they are ordered if they can't afford them. They will either not get the scripts filled or they will only use the expensive meds when they are having a hard time	USA
30-40	They are covered "in this moment" put its too expensive	Castellon-Valencian comunity-Spain
20-30	Some access government funded or charity care, some do without	US
Less than 5	They just suffer and eventually die if they have no access to even oxygen	Philippines
I do not know	They do not they go without a lot of the time	Bermuda
30-40	They look for the lowest priced products, get family help or Civil society help. They quit smoking	Syrian Arab Republic
5-10	Medicare in the U.S.	United States of America
20-30	Rx assistance programs	USA
Over 50	They can get some of the medication for free	Brazil
I do not know	Many qualify for subsidy schemes. Our patient groups have not indicated concerns in this area with the exception of oxygen access	Australia
Over 50	They use aminophylline tbl. It is the cheapest drug. Most COPD patients believe they have asthma. COPD is not recognized as a distinct chronic disease. More than 50% of population smoke	Serbia
5-10	The patients either do not see the doctor or they do not pick up the prescribed medication	Czech Republic

Appendix 1 (*continued*)

Appendix 1 (*continued*)

Q4	Q5	Q6
Over 50	They remain under-treated. Sometimes get financial supports from NGOs	Bangladesh
5-10	There is "universal health insurance" (medicare) for everyone for medical and allied health care plus medications, PLUS those who can afford it buy extra private insurance	Australia
Guessed estimate, it's also about access	COPD care is generally well state funded access and personal prioritising can be financial strain	New Zealand
I do not know	If the compensation of the Social Insurance Service do not cover expences of the medicine, patients are useing only low cost medicine such as the inhaled opening medice instead of corticosteroid or others	Finland
0	Care is paid by national health insurance	Austria
Over 50	We are using Theophyllin and Triotopium as these two drugs are less costly than others	Bangladesh
Less than 5	They have free care	Italy
Less than 5	All COPD patients get their medicines on blue prescription, which in reality means free after a low amount early in the year	Norway
I do not know	They do not comply with treatment	Pakistan
30-40	They ask help from social workers and at the hospital	Portugal
I do not know	Buy meds from India I do not believe anyone in this country is denied health care	America
Are payed from the state we have only a little ticket	The poor people do not pay the ticket	Italy
I do not know	They were not treated or discontinued therapy	BULGARIA, Association of Bulgarian with Bronchial Asthma, Alergi and COPD/ABBA/Diana Hadzhiangelova, Chairman of ABBA www.asthma-bg.com asthma@mail.bg
Drug treatment is cheapest in the world. But poverty is also amongst biggest in the world despite economic growth which has not been entirely non-inclusive	Under diagnosis under monitoring and under-treatment is rampant. Non drug components such as smoking cessation, doctor patient motivational sessions, action plans, rehabilitation services, care giver training, home hospital care, end of life issues-all these are practically unheard of in India	Incredible India
Because they do not visit their primary care doctor	Government provide free medications for all registered in primary care COPD patients	Kazakhstan
Less than 5	In my country there is a public health service for everybody	Italy
Less than 5	90-70% of all medical cost are covered by public insurance system in Japan. The coverage extends to virtually all people	Japan
Over 50	If patients can not cover the expenses they use aminophylline tablets	Serbia
5-10	If elderly they are OK. If not they are on their own	USA
Over 50	They do not unfortunately	Philippines
Less than 5	Not applicable	UK

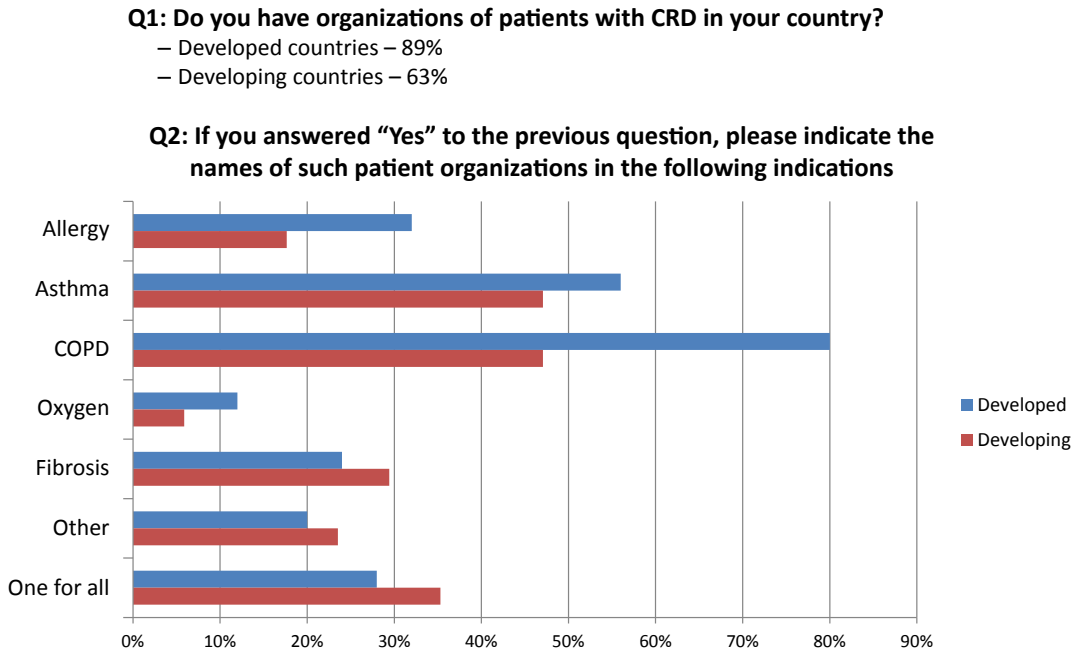

Figure 7 Availability of patient organizations.

education and advocacy on different respiratory diseases (asthma, COPD, pulmonary fibrosis, airway allergy) and treatments (oxygen). Only about one third of the countries have patient advocacy organizations for most chronic respiratory diseases. However, 80% of the developed countries have COPD patient organizations. Only about 45% of developing countries have them.

Figure 8 shows a global picture of the major categories of activities of respiratory patient organizations. The kinds of activities are the same in both developed and developing countries; however, because of financial limitations in developing countries, their activities are only about half of those in developed countries.

Finally, we see which groups of educators provide respiratory patient education in developed and developing countries (*Figure 9*). Clearly, respiratory specialists are the major providers of education for respiratory patients worldwide. However, the ICC survey shows some deficiencies in education. First, little education is provided by COPD patient groups in developing countries. This probably results both from the shortage of COPD patient organizations in developing countries as well as their limited resources. Another concern that is raised from these data is that primary care physicians, who do most of the care for respiratory patients, apparently do little patient education.

These data suggest that developing countries need assistance to develop their COPD patient organizations. They need this support so they can provide needed education, improve care, advocate for COPD patients rights, and improve COPD prevention and awareness in their countries.

The need for more efforts and resources in developing countries is highlighted by Prof. Yousser Mohammad, ICC Co-Chair, and her colleagues in their article (in press) in the *Journal of Thoracic Disease*. They observe that 63% of global deaths result from non-communicable diseases. A percentage of 80 of global deaths occur in low and middle income countries and 90% of global deaths from COPD occur in developing countries (3). To deal with respiratory diseases globally, the efforts must increase in developing countries.

Patient Micro-Organizations

Prof. Muhammad and her colleagues in the WHO Global Alliance against Chronic Respiratory Disease have a concept to improve respiratory patient education in developing countries through what they call Patient Micro-Organizations (4). These COPD patient groups are built locally and from the ground up rather than being mandated from top down by health ministries. They are organized around local institutions like hospitals and schools and they

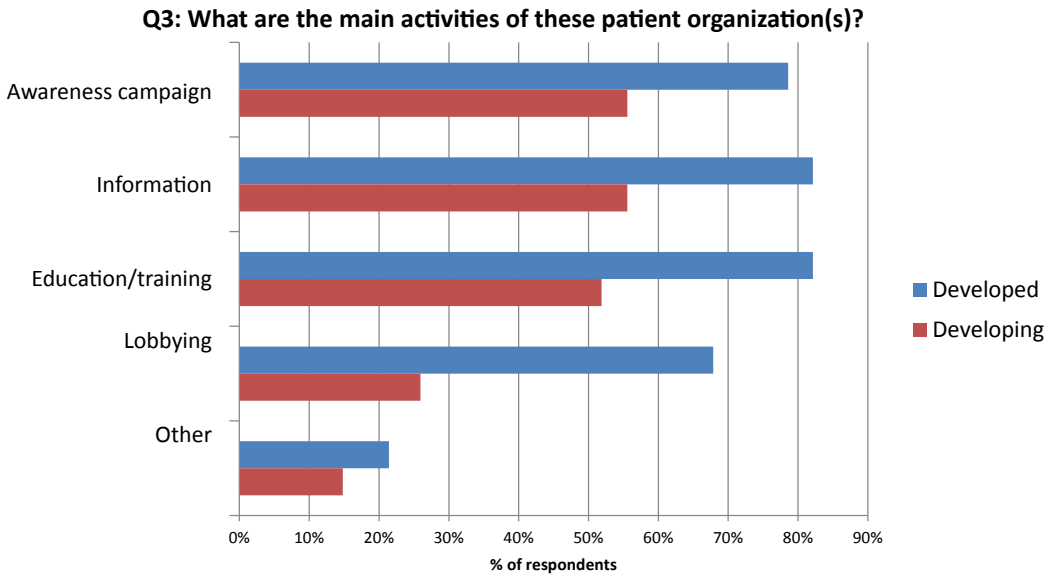

Figure 8 Activities of patient organizations.

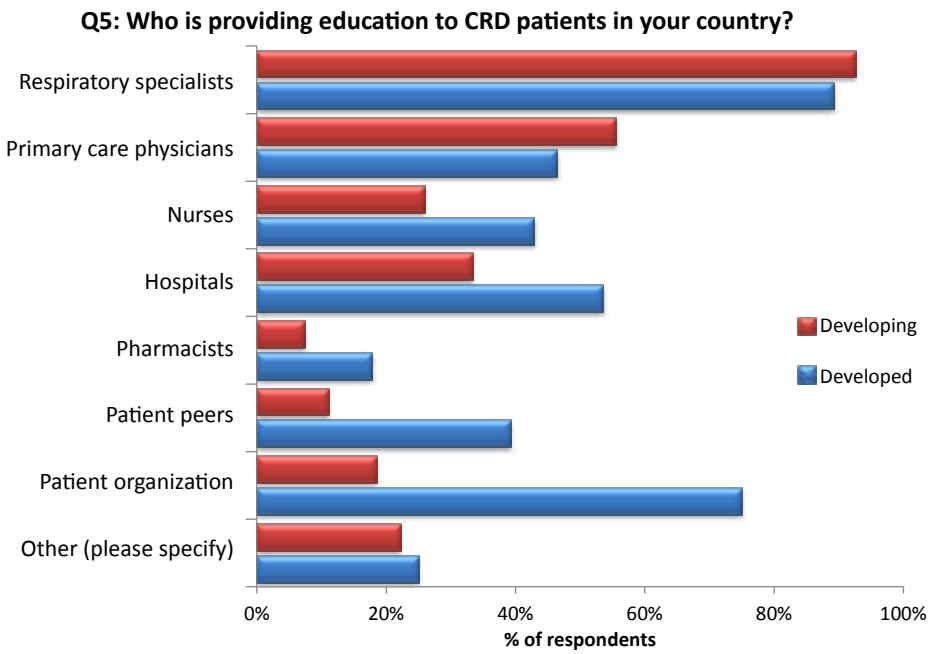

Figure 9 Providers of CRD patient education.

develop and provide educational materials about COPD for health care professionals and patients that are appropriate for their country and region. A number of these patient micro-organizations have been successfully established in developing countries. ICC believes that such local COPD organizations can make a big difference in COPD awareness and action. Patient Micro-Organizations follow the ICC philosophy, which is to think globally, but act locally.

Lack of progress

The final question is whether or not the global medical community is making progress in preventing and managing COPD. Data from the World Health Organization suggests

that the progress is very limited. COPD continues to increase as a global non-communicable disease epidemic. Stroke and coronary heart disease, the other leading non-communicable diseases, have had their mortality rates decrease. COPD has increased in its effects on years lived with disability (referred to as YLDs) (5). The World Health Organization Burden of Disease report recently compared data from 1990 and 2010 and showed a 46% rise in YLDs globally. The global deaths caused by COPD have made it the 3rd major cause of death in 2010, up from 4th in 1990 (6).

In many countries, such as the US, life expectancy has shown a small increase from 1990 to 2010; however healthy life expectancy, which is years lived without disability has actually decreased. That is, people are living longer but with more disability and less good health. This is especially true of COPD patients. Years lived with disability from COPD in the US increased from 1.3 million in 1990 to 1.8 million in 2010 (5). As respiratory health care providers, we want to improve life expectancy with COPD, but we want it to be healthy life expectancy.

In years lost prematurely (YLLs) from COPD, the global regions most severely affected are the Caribbean, Oceania, north and sub-Saharan Africa, the Middle East, and Latin America (5).

Although COPD awareness and activities have increased globally, COPD continues to increase as a deadly and costly health menace. In the second part of this report we will examine the efforts of the four key global medical partners in combating COPD and other respiratory diseases to try to understand why the progress has been so limited.

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My dreams

I have seen patients suffering from chronic obstructive pulmonary disease (COPD) for many years. Once the disease develops into its late stage, generally there is no effective treatment. Too many patients only come to doctors when they cannot bear the tortures of this devastating disease any more, but that's too late. Some others with early stage COPD don't pay enough attention to it. Once the pulmonary function decreases and no action is taken, severe consequences result.

This conference* has made me think about the COPD patients I have known and treated. I have had a dream about a global effort to help COPD patients everywhere. It would be an organized person to person effort to prevent COPD where we can. For those who are already developing COPD we would conduct a far-reaching initiative to diagnose patients in the very early phase of the disease. We would do our best to help these people avoid the dangerous progression of the disease before they suffer from the debilitating symptoms and the consequent decline of health. This would take place in rich countries as well as poor countries and would locate these patients in the big cities and in the small rural villages.

My second dream is that lung doctors like me and my colleagues will work closely with GPs and primary care doctors as well as other health care professionals and patients from all the communities of the world in this quest to help COPD patients and prevent others from developing COPD. Only by working together can we accomplish the great task of monitoring patients at risk for COPD and detecting the onset of the disease. Then, working with all our colleagues and our patients, we can use the best techniques to diagnose COPD and provide the medicines needed to benefit the patients. I believe that we can all work together to make these dreams come true!

Acknowledgements

*At the end of the World Conference of COPD Patient Organizations held in Shanghai, China, in November of 2011, Monica Fletcher, RN, the Chair of the European Lung Foundation, chaired a panel discussion of COPD experts from throughout the world. She challenged each of the participants to talk about what the meeting had meant to them and how they hoped that it would influence what they would work for upon their return home. Prof. Nanshan Zhong's remarks expressed the sentiments that most deeply affected the attendees from all walks of health care and from all parts of the world. Dr. Alfred Loh, the CEO of the World Organization of Family Doctors applauded Prof. Zhong's dreams. His wish was that he could mobilize his 128 member organization of GPs to take up the challenge that Prof. Zhong described. Dr. Lawrence Grouse adapted this essay from Prof. Zhong's remarks at the World Conference.

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Prof. Walter McNicholas: sleep apnea—a disease calling for attention

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Professor Walter McNicholas,MD, FRCPI, FRCPC, FCCP is Newman Clinical Research Professor at University College Dublin (UCD), Director of the Pulmonary and Sleep Disorders Unit and Consultant Respiratory Physician at St. Vincent's University Hospital, Dublin, Ireland. He is a medical graduate of UCD [1974] and has a long established track record in high level research and leadership (Figure 1). He is a leading international authority in translational research on the mechanisms and consequences of sleep-related breathing disorders particularly sleep apnoea, and has held competitive grants from agencies such as the Health Research Board (Ireland) on a continuous basis for close to 30 years. His research interests include the pathophysiology, treatment and outcomes of sleep apnoea syndrome, the cardiovascular and metabolic consequences of the disorder, basic cell and molecular mechanisms and consequences of intermittent hypoxia, in addition to sleep disturbances in COPD and other chronic respiratory disorders. He is also closely involved in the evaluation of novel ambulatory monitoring devices for sleep disorders. He is a past Associate Editor of the European Respiratory Journal, and has published over 170 papers in Pub-Med listed Journals (h-index in 2013 of 36), in addition to more than 30 book chapters, and has edited three textbooks on breathing disorders during sleep.

Prof. McNicholas has held many Leadership positions in National and International organisations, particularly the Presidency of the European Respiratory Society [2002-2005] and is current Vice President of the European Sleep Research Society and President of the European Board of Accreditation in Pneumology. He Chaired a COST Action (B26) on Obstructive Sleep Apnoea [2005-2010], and recently chaired a Working Group established by the European Commission on Sleep Apnoea and Driving [2012-2013], which is expected to lead to an official EU Directive on this topic.

In the Third International Conference on Respiratory Disease on October 11, 2013, we are honored to have an interview with Professor Walter Mcnicholas to provide further advice for management of sleep apnea.

Figure 1 Professor Walter McNicholas.

JTD: What is the common cause of sleep apnea?

Prof. McNicholas: Sleep apnea is a condition that occurs because of the narrowing of the upper airway and in many cases narrowing is congenital. In addition to the congenital factors, other acquired factors may develop which particularly relates to issues like weight gain. So the combination of congenital factors with acquired factors, most notably the development of obesity, can cause the development of upper airway obstructions sufficient to lead to sleep apnea.

JTD: Does the hereditary factor play a role as cause for sleep apnea?

Prof. McNicholas: Yes, absolutely. As I have already indicated that hereditary factors are important in sleep apnea. Population studies looking at the contribution of genetic factors suggest that between 40 to 50 percent of the varied morbidity of sleep apnea can be accounted for genetic factors.

JTD: What would be the consequences of sleep apnea?

Prof. McNicholas: Untreated sleep apnea results in a number of consequences with the most obvious one as the disturbance and unrefreshing nature of sleep and also daytime sleepiness. There are other less obvious

complications particularly relating to comorbidities. The major comorbidity of sleep apnea relates to the cardiovascular system, principally systematic hypertension and moreover it can lead to some cardiac consequences, such as cardiac arrhythmia, myocardial infarction, and congestive heart failure.

JTD: How to evaluate the progress of the disease? Is there any staging grade in classification?

Prof. McNicholas: Sleep apnea could be categorized as mild, moderate and severe, which indeed could be the case for most medical disorders, and we have arbitrary definitions of the characteristics of mild and severe models based on the frequency of breathing disturbance, the so-called Apnea Hypopnea Index which is commonly abbreviated as AHI. There is evidence to show that the increasing severity of sleep apnea based on the AHI classification is associated with increasing risk of downstream comorbidities (particularly cardiovascular comorbidities). These are more prevalent in patients with severe sleep apnea compared to the patients with mild disease.

JTD: People may be confused with snoring and sleep apnea as snoring is a common symptom for sleep apnea. How in your perspective does snoring relate to sleep apnea?

Prof. McNicholas: It needs to be remembered that snoring is highly prevalent but snoring itself is not a medical disorder. Therefore, it is understandable that the physician's approach towards patients simply with snoring may be relaxed as there is no evident health consequence of simply snoring. But problems arise if the physicians' concern dismisses everybody because a proportion of patients who snore will also have sleep apnea. If you take a person who snores in isolation, he would have no complaint but the partner will be affected. Because the snoring person will sleep well and wake up fine in the morning without any complaint during the daytime and they are not sleepy or fatigued, and in addition, they have no significant risks of developing comorbidities such as hypertension or heart attack. The person with sleep apnea also snores, but with lower quality of sleep and waking up feeling not refreshed, sleepy and tired in the daytime and he is quite likely to have systemic hypertension. Therefore it is actually straightforward to distinguish the simple snorer and the snorer suspected of having associated sleep apnea.

JTD: How do you see that relation between obesity and sleep apnea?

Prof. McNicholas: Obesity is an important factor in the development of the sleep apnea. But it is not the only factor. In reality what leads to the development of the overall picture of sleep apnea is a combination of genetic factors together with acquired factors, the most important of which, is the development of obesity. The relationship between obesity and sleep apnea could be considered as the analogue to relationship of the obesity and the development of type II Diabetes. If one looks at type II Diabetes, obesity is an important factor but not the only one. In sleep apnea, obesity is just one of the factors, although clearly important. Besides, when we look at the population cohorts with sleep apnea attending our own sleep apnea clinics, we found that the average Body Mass Index (BMI) of them is in the region between 30 to 31, which means they are obese but only in mild degree. We should take the cut-off between obesity and overweight as a BMI of 30. This is particularly evident in the European network study that I referred to in my presentation in this conference which is the European sleep apnea data base, and where the average BMI among the 15,000 patients in the data base is actually under 30.

JTD: Is COPD or asthma highly associated with sleep apnea?

Prof. McNicholas: The relationship of sleep apnea with COPD and asthma is not clear cut. There is evidence that COPD is a little more prevalent in the patients with sleep apnea than in the general population and the other side of the equation also holds true. However, if we look at for example the prevalence of systemic hypertension in sleep apnea, we would find systematic hypertension is very common in patients with sleep apnea. The excessive prevalence of COPD in sleep apnea is nothing like that of hypertension. It is common but not that much.

JTD: You have introduced the risk of drivers with sleep apnea. To help these patients, we have come to a very common question: how to identify the early stage of sleep apnea?

Prof. McNicholas: The importance of sleep apnea related to the driving risk is an increasing concern for

national regulative authorities, which brings into focus the recognition and diagnosis of these patients. If we look at the private drivers, we will see a huge burden identifying them. It is likely to be difficult to screen every driver, and thus for private drivers, we have to rely on the recognition of patients' complaints presented to Sleep clinics and those who we identify as having sleep apnea should do the appropriate test and treatment. In terms of screening of the general population for potential sleep apnea, it would be more important for commercial drivers, particularly drivers that operate trucks on the highways. It is likely to be a development towards screening procedures for the so-called group II drivers (drivers operating trucks). I expect that in the coming years there will be a move towards active screening of that type of drivers for possible sleep apnea.

JTD: We understand that you have recently chaired a working group established by the European Commission on sleep apnea and driving (which is expected to lead to an EU Directive). Could you share with us more information about the Directive?

Prof. Mcnicholas: Firstly the EU directive is not issued and it needs to be finalized. There is an agreement on the part of the Transport Commission that the directive needs to be implemented. But the EU is a conversing democracy where each of the member states have to be given the opportunity to comment before it is issued as a directive. But almost certainly there will be an EU directive in 2014 because the Transport Commission is quite keen that sleep apnea will be specified. It is awaiting formal approval in the individual governments (the consensus has been reached). As regards what this is going to result in, an approach will likely be taken whereby patients with moderate or severe sleep apnea, particularly associated with significant sleepiness, will be prevented from driving, or at least will be prevented from holding a driver's license, until the condition is successfully treated. The overall emphasis of our working group has been put on motivating patients with symptoms of sleep apnea to seek medical attention. The important reason for that is the very clear demonstration that where patients with untreated sleep apnea run a risk on the highway when driving. Patients with treated sleep apnea are at no risk, at least no greater risks than the general population. So patients should be strongly encouraged to seek treatment rather than to avoid the doctors because of the concern that their driver license would be removed.

JTD: In the treatment of sleep apnea, CPAP is currently the most widely used therapeutic method. What do you think of the future development of this therapy?

Prof. McNicholas: I think the traditional CPAP is likely to remain the mainstay of treatment for the average patients with sleep apnea. More sophisticated forms of CPAP are other modalities of pressure support largely confined to patients with complex disorders. For example, the patients with overlap syndromes of COPD and sleep apnea where there is a prominent component of chronic hypoventilation may be better managed by other forms of pressure supports such as Bi-level. Patients with complex sleep apnea or central sleep apnea are possibly suitable for other forms of pressure supports such as adaptive servo ventilation (ASV). In a sense, the ordinary form of CPAP remains the primary modality of therapy and there are other more sophisticated and complex pressure supports modalities restricted to small sub-populations of patients with complex disorders.

JTD: What are the challenges and opportunity in the future research on sleep apnea?

Prof. McNicholas: I think there are developments in the broad ranges of areas, for example in the diagnosis. My presentation in the conference showed some of novel approaches that are either already developed or evaluated, and there are some very novel devices such as devices we are working on in our own university relating to a campus company, which is becoming commercialized and they involve the use of completely non contact device. It is potentially an exciting development maybe leading towards the ability to do widespread screening of sleep apnea, as a preliminary move before the determination and the assessment of sleep apnea.

JTD: Currently it seems hard to cure the disease. What are your advices for patients with sleep apnea to improve their life quality?

Prof. McNicholas: The approach to the management of sleep apnea should not be simply focused on the CPAP machine. There are life style measures that would be helpful—the potential role of exercise as I referred in my presentation which is likely to do some benefit. Clearly, obese patients with sleep apnea should be strongly encouraged to lose weight, and also overall measures to improve sleep quality, sleep hygiene measures can also be

beneficial. CPAP will eliminate sleep apnea, but the other measures can help to further improve the overall life quality of the patients.

JTD: Thank you very much for your time!

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Prof. Klaus F. Rabe: COPD as a systematic disease

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Klaus F. Rabe, professor of Pulmonary Medicine at the University of Kiel and Director of the Department of Pneumology at Clinic Grosshansdorf (Figure 1). He has been active in various fields of Respiratory Medicine worldwide, predominantly asthma, COPD and lung cancer. Prof. Klaus F. Rabe has served on various editorial boards. He was the first European Associate Editor of the American Journal of Respiratory and Critical Care Medicine and has been Chief Editor of the European Respiratory Journal until recently. His current interests are related to large clinical trials in COPD and asthma, the mechanisms of airway inflammation, and the endoscopic staging of lung cancer. Prof. Rabe has served on GINA and GOLD, and is a member of the German and Dutch Chest Societies, the British Pharmacological Society, the American Thoracic Society, and he served as President of the European Respiratory Society 2011-2012. In the 3rd international Conference on Respiratory Disease, we are honored to have an interview with Prof. Rabe (Figure 2).

Figure 1 Prof. Klaus F. Rabe.

JTD: Good afternoon, Prof. Rabe. Thank you so much for sparing your time for this interview. You have given an excellent presentation regarding COPD as a systematic disease. Would you please further introduce the characteristic of defining COPD as a systematic disease in terms of its mechanism?

Prof. Rabe: Well, quite interesting question. First of all, it is the disease that occurs in the individuals who are older and who are likely to have other conditions as well. Secondly the risk factor for COPD in a large part of the population with the disease is cigarette smoking, which effects on the organ system like vascular system and heart. It will incapacitate the individual, by making them physically inactive and bringing the consequence for metabolic disorders, overweight and diabetes. There are systemic markers of the disease, the inflammation found in the blood for example, which makes it likely that this is a chronic inflammatory process that affects not only some parts of lung but also other organ systems.

Figure 2 Prof. Klaus F. Rabe with *JTD* Editor.

JTD: COPD as a systematic disease can lead to comorbidities, How to deal with the comorbidities in treating COPD patients?

Prof. Rabe: Well, I think first of all, individuals, physicians and people who care for the patients need to be aware that individuals with advanced COPD are very likely to have something else. That means in management of the disease, you need generalist approach. That would obviously mean that in treatment of advanced COPD we should recognize other conditions thought in understanding of the outcome of COPD, identification of the comorbidities is important, which means patients with advanced COPD need as much pulmonary medication as the other medication.

JTD: We understand that COPD and asthma have many similar symptoms. How to identify them at the early stage?

Prof. Rabe: Well, that's an interesting question. There are individuals who have both diseases. If they get more severe, it is something that probably, in terms of treatment, is not that different anymore. However, for the mild disease, the differentiation is important, which means someone with mild asthma clearly needs steroids, and someone with mild COPD should not have them probably. Then how do we differentiate them? First of all, we should look at the age and onset of disease, which means the time when the symptoms start. If someone starts at middle age, it is very important to know whether there is a former history of it. Secondly, we need to understand if there is there a family history of allergy and asthma. Thirdly, we have to identify the present allergies. Fourthly, we should pay attention to whether there are any seasonal changes of symptoms or are there any daily changes of symptoms as a clinical presentation. With information of all these, there is still something which needs testing. The patients that have an obstructive lung disease pattern with asthma only sometimes have a normalization of lung function which you would not expect with the patient with COPD, but there will remain a small proportion of individuals where whatever you do, there will be very difficult to make the distinction and I think these are the few patients where you can rightly say that they have both diseases, namely a chronic obstructive disease with an asthmatic component.

JTD: Are there any effective tools to monitor the progress of the disease?

Prof. Rabe: I think it is next to symptomatic assessment because breathlessness has an exercise primarily unaddressed, and it is one of the hallmark symptoms. People have to understand the role of chronic bronchitis, cough and sputum production seems to be more and more obviously a bad component, because it relates to more outcome and it is however classically. I believe, that the lung function assessment still plays a central role in increasing the severity of the disease and this seems that patients and doctors need to know to monitor and safeguard exacerbation which is expected to be prevented and the results of people that have many of those episodes fare worse than people just do not.

JTD: Is there any bio-markers used for evaluation of COPD?

Prof. Rabe: Unfortunately, no. People do try to look for bio-markers, like serum bio-markers but with poor results in differentiating aspects of COPD though some people ever get to use nitric-oxide, an exhalation. I believe for the initial assessment nitric-oxide can be helpful and if you have accessibly high level of it and the fitting clinical presentation is witnessed, it is likely the patient have asthma. Eosinophilia in the sputum or in the peripheral blood is also the indicator for asthma. In a sense, there are some bio-markers that give you a hint into what direction the diagnosis will take, but the validity of them is still questionable.

JTD: As you said that, when we try to identify COPD patient we will check the family history, should the genetics of COPD also be considered?

Prof. Rabe: Well, this is an emerging field. There is no question that there are environmental influences and early life environmental influences that could not use epigenetic changes in risk population. Currently, there is research of genetic COPD which are not yet conclusive. Despite the fact that some people may be more enthusiastic, that we are looking for epigenetic modification or genomic and genetic asthma, it is very difficult to find genetic risks in the genetic background. Therefore, I am not too optimistic what will come out of it, clearly not for clinical practice, but genetic research in COPD is currently an ongoing field.

JTD: In the drug development for COPD patients, what would be the challenges and opportunities in the field?

Prof. Rabe: Obviously, since COPD is a disease

characterized by increasing mortality, it will be likely to change mortality issues. Basically, there are some indications that long-acting bronchodilators like anti-cholinergic may do something on this. I do believe the evidence that steroid changes mortality is very slim, and I actually think that the trials trying to demonstrate this have not been convincing for the change of mortality in COPD. But there is what we want to achieve with new development. Unfortunately, there is very little new literature in the field. There are lots of developments that have copied from one another. They are just new range of long-acting bronchodilators drugs, beta-agonist, anti-cholinergics, but not really new drugs and surprisingly, we do not have an effective treatment for chronic bronchitis, which is a very prevalent disease. In summary, there are large gaps in treatment and we need some better new drugs.

JTD: Where do you see the future development of COPD research is going?

Prof. Rabe: First of all, I think you have to understand more about the risk factors in it and which is on the top. Cigarette smoking is an important factor but there are many other risk factors that contribute to the prevalence of this disease. Secondly I think in terms of drug development, as I said, drugs that treat chronic bronchitis or signs or symptoms of this and/or severe dyspnea and/or emphysema need to be developed and still there is long way to go. Thirdly for the management, people need to realize that rather than just treating lung function impairment and symptoms, it is a holistic approach that the disease needs. It needs recognizing of the other underlying conditions and comorbidities, and you have to treat them as a standard treatment of COPD. It seems to me that since this disease is so much linked to other diseases, it needs a treatment algorithm that does not stop by inhaler medication for the lung period. It needs to be managed in a much more broad sense, where we also need more studies obviously to see what would a cardiac and metabolic medication do for a patient with COPD that also have a sort of respiratory medication. Finally, it is a change of life style, which depends on not only reducing risk factors like smoking, but also in fact the diet and exercise. The concept of exercise takes care of the lot of aspects that are inherent in the morbidity as well including insulin resistance and positive cardio-vascular outcomes. Still, that is something have not been educated enough.

JTD: But patients with advanced COPD, maybe some exercises are not suitable, right?

Prof. Rabe: That is sure. But it is a matter of level of exercise. The problem is people need to get educated with rehabilitation training programs, which need to be adapted to the individual level. However, I think there is no single level of COPD that is inappropriate. It is a matter of the mild or severe depending on the individual constitution.

JTD: How do you foresee the international cooperation, especially the cooperation between China and Europe, in the treatment research of COPD?

Prof. Rabe: I do believe that lots of things have changed. There are more and more trials done in China, and logistic of the former clinical science in this country is improved dramatically. There is more personal interchange of individuals and for example, if you look into the European Respiratory Society meeting, there are more and more Chinese delegates going there. And what I still believe is that there is a language barrier that we have to overcome. That should begin in schools, and continue to university. So the structured programs for medical or clinical science are in English. I think the cooperation can be improved much by the improvement English abilities. On the other side, westernized countries need to understand where the science in east or Asia is, but the things happening last year have seen great increase in this level of this project. I am here to talk at Chinese conference and I have come to China three times this year. We talk at the meeting and I think it is the good side. There are lots of mutual agreements. To solve specific problems, we need international efforts to understand risk, genetic and environmental profiles. I am very hopeful and optimistic that we have bright future in cooperation, which I believe will lead to better science result in the end.

JTD: Thank you so much for your informative talk!

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Prof. Denis E. O'Donnell: personalized treatment of COPD

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Denis E. O'Donnell, MB, BCh., (NUI), MD (NUI), FRCP(I), FRCP(C) is a professor of Medicine, with cross appointments to the Departments of Biomedical & Molecular Sciences (Physiology), Rehabilitation Medicine and Kinesiology & Health Studies, Queen's University, Kingston, Ontario, Canada (Figure 1). Dr. O'Donnell's main research focus is clinical integrative physiology and specifically, exercise pathophysiology and mechanisms and management of dyspnea in respiratory disorders. His main clinical focus is COPD and pulmonary rehabilitation (PR).

Dr. O'Donnell had a leadership role (Chair) in the development of best practice guidelines for the management of COPD in Canada. He has been a senior author in over 260 scientific publications. He has co-edited a book on Dyspnea, now in its third edition. He has lectured extensively on these topics both at a national and international level.

He serves on several national and international scientific panels on respiratory diseases and sits on the Editorial Boards for CHEST, Journal of Applied Physiology, Journal of COPD, the International Journal of COPD, and is an Associate Editor of the Canadian Respiratory Journal. He is the Past President of the Canadian Thoracic Society and is a member of the Institute Advisory Board (IAB) for the Canadian Institutes of Health Research (Circulatory and Respiratory Health). He is currently the National Canadian Delegate for the European Respiratory Society.

In the Thirst International Conference on Respiratory Diseases, Prof. O'Donnell gave a speech on "When obesity and COPD collide: physiological implications". In the following interview, Prof. O'Donnell further introduces the detection and treatment of COPD.

Figure 1 Denis E. O'Donnell, MD, FRCPI, FRCPC.

JTD: What are the current methods in early detection of COPD?

Prof. O'Donnell: We really have to depend on the screening procedure, such as spirometry, to make a diagnosis. We have to select individual sectors at risk, so smokers generally. Those who have persistent symptoms, have a high pre-tested probability of having airway obstruction which can be confirmed by spirometry. At present, spirometry is the best the methods we have to make the diagnosis. We can't make a conclusion based on clinical evaluation and we have no biomarkers so we have to rely on breathing tests. Therefore, for early detection, we first need to select the individuals at risk, especially smokers usually over 55 years of age and particularly those who are beginning to develop symptoms of cough, sputum, and breathlessness.

JTD: In your presentation, you have introduced the management of COPD combined with obesity. Has obesity been established as a high risk factor for COPD?

Prof. O'Donnell: The link between obesity and COPD is complex. Some European studies show that patients with early COPD had a higher rate of obesity, but we did not find this condition. Inactivity may play a main role in the association between COPD and obesity. In other words, it is because patients began to get symptoms avoiding activity so they put on weight. Nevertheless, there is no other

clear association, other than the two diseases are common. Obesity is not a concern in China but in western world it takes up 30% of the population while COPD is just about 10%. Both conditions are rising so the coexistence has both increased. But one point is related to diagnosis of COPD and the obese as obesity affects lung mechanics and it changes the vital capacity, with the indicators as FEV1, FEC, SN1, 7. Thus if the denominator that effects the vital capacity is diminished because of obesity, we can make an over-estimation of airways obstruction. Sometimes the diagnosis is not made or not appreciated because of the mechanical effect on lung volume. That's why obesity considered as an important factor. Certainly, it is more difficult to diagnose COPD combined with obesity in the individuals.

JTD: Are there any difference between the treatment on obese patients with COPD and nonobese COPD patient?

Prof. O'Donnell: The presence of obesity, at the moment, does not alter the treatment. We have no proof that the usual treatments we offer are less affective on obesity. So there is no difference in treatment with combination of obesity and COPD and their response to treatment seems to be the same. If you give bronchodilators to a patient with obesity and COPD, he has similar response as a patient with just COPD.

JTD: What would be the effective tools to evaluate the progress of COPD?

Prof. O'Donnell: Traditionally, we just measure the breathing tests seriously and we look for the declination in FEV1. It is still the simpliest way; however, it is oversimplistic because it doesn't consider the fact that FEV1 does not correlate with the quality of life, the activity levels, exercise capacity and the shortness of breath. So if you really want to mark the progress of the disease, there are a few things. The first one is spirometry, e.g., FEV1. The second is the assessment of dyspnea such as shortness of breath, such as the MRC dismissed skills. The third would be the frequency of exacerbation.

JTD: Pulmonary Rehabilitation (PR) is now widely used as treatment COPD patients. How you do see the application of this PR and where do you see its future is leading to?

Prof. O'Donnell: Rehabilitation is critical intervention in COPD because even in early stage of disease, inactivity is becoming measurable. Patient avoids activity because shortness of breath and this is a so slowly suggestive process over a long period of time that they don't even notice it. They are just in behavior and life style to avoid shortness of breath and they become more and more inactive.

So at present we offer rehabilitation to people with more advanced disease, but these people may have other comorbidities like obesity and it's hard to get sustained effect that lasts overtime. There is new information coming out that if rehabilitation under activity promotion occurs earlier at the disease, the effects are more pronounced. Overall, I think PR is playing a very important role, but it is challenging to get sustained effects in people with advanced disease.

JTD: Is there any other treatment to combine with PR?

Prof. O'Donnell: It's all about behavioral therapy to try to motivate people to maintain the program. We have only begun to learn how to do that. There are agencies to training where we give oxigent-driven training, to some people to allow them to train to higher levels, and they got more successful effects. There are other experimental approach to try to improve their ability to do the higher levels of exercise such as non-invasive ventilator assistance and even relaxations techniques. I think Chinese have a lot of teachers there. Relaxation exercises are the area neglected and there are some paper showing they are effective. So I think we have to learn new techniques to encourage and sustain the program. We have program for 12 weeks so we can measure all these improvements. We have to draw the baseline there unless we have some ways of making sure people have kept up home-based maintenance program. That's the big challenge because if they are not supervised, they easily retreat to their old habits.

JTD: As the chair of the best practices guidelines treatment in COPD and relative respiratory system medicine, would you like to share with us your experience in leading such a program?

Prof. O'Donnell: Guideline development is complex. Strictly speaking, it should be exclusively evidence-based. Unfortunately we don't have a great deal of evidence for many recommendations and they become best practice suggestions if there is clear evidence to support them. I chair COPD guidelines in Canada and I think it is the most frustrating part because when we look at different interventions for the evidence, though there are some

strong cases, many cases were based on clinical experiences of the group, and that was not the best type of guidelines to offer.

In my opinion, in COPD we are moving towards more personalized approach rather than as used to happen based on gold guidelines of treatment choices. Pharmacotherapies were based exclusively on measurement of breathing tests, and now we have moved away from that to the treatment of shortness of breath and dyspnea and the prevention and treatment of exacerbations. There has been a revolution. With new treatment of COPD, and much greater choice of different therapies, we are going to have to learn to individualize and personalize the choices, so if we have three long-acting anticholinergic choices, we are going to be guided more by the patients as to what their preferences are, and we are going to get better at phenotype of these individuals and recognizing clinical characteristics that would demand particular treatment. Therefore, we have a long way to go, but we are beginning, for example, there is a patient with chronic bronchitis who have COPD responds to one treatment, e.g., PD4-inhibitors and people without chronic bronchitis do not respond. This is the first time in the treatment of COPD that we realized selective treatment that works in one group but not in another!

I am influenced by the new Chinese study which is the first convincing study showing that in certain patients with COPD, they are likely to respond in terms of reduced exacerbation, so as we move forward, our effort would be to recognize the clinical characteristics of patients who will respond. We are learning now that inhaled corticosteroid should not be given to everybody: the worry about pneumonia is real. So we have to reevaluate this whole question. By knowing that there are a subset of patients who do respond to steroid, we have to identify those people and give the medication to people who will not be caused side effects and be more sure that we are getting responses.

I think the revolution of guidelines will result in a more personalized approach and the guideline I was developing is too dogmatic because we have only a few medications. As now we have all range of them overnight, we cannot use the same for the original approach.

JTD: Which aspects of COPD should be addressed in future conferences?

Prof. O'Donnell: I think one of the questions should be the COPD phenotype as such a heterogeneous, broad diseases will be oversimplified based on some common characteristics such as airways obstruction. We are realizing that we have neglected systematic disease, the systematic aspect of the condition. We have to think beyond just trying to improve airway function and recognize that there is other very important comorbidity that we cannot neglect, such as sleep apnea which is not common in patient with COPD. Also, the obese COPD patients pose particular challenges and we have to understand why they are more limited in activity than the normal COPD patient.

In treating the patient with cardiovascular disease and other metabolic comorbidity, we also cannot neglect the other comorbidity that are contributing, particularly cardiovascular diseases, so it is the most common study that gives us more careful characterization phenotype in COPD that we can begin to test individualized approaches in therapies.

In terms of future of the study, we should focus more on early and mild COPD, we have neglected this completely. The average of FEV1 in patient entering the clinical trials is 44%. In my own study, I am recognizing that these people with early disease have physiological impairment that is in their small airways and small vessels and their lungs are infected by inflammation. This cannot be measured by our traditional method of spirometry, so we need to develop new test which will give us information of small airways function so that we can introduce the treatment earlier in the cause of disease and hopefully has much bigger effect on modifying disease that we have at the moment.

Therefore, the new edge of COPD is the neglected mild disease who do not receive treatment, but if you do the right test, you will see that it has extensive damage to the small airways. We are getting new methods, new imaging techniques, possibly new biomarkers which will help us to identify the smokers who are going to get into trouble like rapid progress of the diseases and hopefully we can intervene much earlier than we are now. Because we don't really identify a patient with COPD until they have lost nearly 50% of their lung capacity. Here we get back to the previously discussed question of early detection. With spirometry as our best approach on screening so far, we have to do selective screening as there is no proof that global screening of all smokers has any impact on long progress of disease in healthy economics. However, there is more proof that if you recognize a smoker, who has symptoms and is over 55 years of age, the risk of them to get COPD is much higher, so we need to test them.

JTD: So what would be your suggestions to the young clinicians?

Prof. O'Donnell: Young clinicians have to recognize that the disease starts much earlier than we knew before and it is a challenge for them to screen patients and understand the disease much better. They have to be aware that is such a slow progressive disease, which unless you are very careful, watching and vigilant, you won't pick up until is too late.

We need young scientists dedicated to better understanding the pathophysiology of the disease, better measurement of pulmonary function that we are not able to do with reposition. We have to understand the genetic basis of the disease. We have to understand how the different phenotypes progress and what makes them different. We also have to understand how people who smoke very heavily do not get the disease. These are questions totally unanswered. We need a new group of scientists maybe from China to solve them.

JTD: Thank you very much for your time!

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Preface

Review Article

Original Article

Visualized Surgery