

Peer review file

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Reviewer A

Comment 1: If patient was diagnosed with no mediastinal affection by the deferred pathological assessment, how long was the interval between the VAM and surgery?

Reply 1: Mean time between last mediastinal exploration (EBUS or VAM) and surgery was 26,9 days (+/- 15,24). (L233)

Comment 2: Some studies had reported that the tumor markers might have some value in predicting the mediastinal lymph node metastasis. If the preoperative tumor markers were available in the two centers, please enrolled them into the multivariate analysis.

Reply 2: Patients were referred from four different centers, staged by EBUS in two and by VAM in one. Unfortunately, we have no homogeneous data regarding tumor markers.

Comment 3: The variables enrolled in the Cox hazard model were not comprehensive. Plenty of perspective studies had found that the clinical and pathological characteristics had significantly effects on the overall survival of lung cancer.

Reply 3: To evaluate influence of invasive staging on survival we decided to include each one of the inclusion criteria (tumor size, tumor location and cN1) and gender because it was detected as a risk factor for pN1 upstaging in our previous study.

Comment 4: The mean values and standard deviations presented in the main text and Tables should keep to the same digits after decimal point.

Reply 4: Standard deviations values have been adjusted to two digits.

Comment 5: The abbreviations of ASTER (page 8, line 168) and ESTS (page 10, line 219) should be defined the first time it was used within the text.

Reply 5: Definitions have been added. (L242), "ASTER 3 (Assessment of Surgical Staging vs Endosonographic Ultrasound in Lung Cancer: a Randomized Clinical Trial)" and (L311), "European Society of Thoraci Surgery (ESTS)"

Comment 6: The format of references did not comply with the author instruction

(<http://cdn.amegroups.cn/journals/pbpc/public/system/jtd/jtd-instruction-for-authors.pdf>).

Reply 6: Changes have been made as advised. The number of authors has been adjusted as required. (see pages 18 to 21)

Comment 7: All tables should be showed as three-line form.

Reply 7: Table form has been changed.

Reviewer B

Comment 1: There are a few typographical errors such as in the abstract 60,7% should be 60.7% for example and line 137 "opered" should be "operated".

Reply 1: See corrections in L47 and L124.

Comment 2: Were there any patients who were negative by EBUS positive by mediastinoscopy?

Reply 2: No, preoperatively there were not. 2/5 patients were cN2 by EBUS and 3/5 were positive by VAM without previous EBUS.

Reviewer C

I congratulate the Authors for this nice paper. It is important to identify patients who could benefit of neoadjuvant chemotherapy. The advent of immunotherapy makes this effort necessary to offer the best treatment for NSCLC.

Reviewer D

Thank you for the opportunity to review this manuscript. The authors, a group experienced in publishing on the subject of NSCLC staging, attempt to refine the recommendation to invasively stage patients with CT and PET negative mediastinum if they carry 3 risk factors:

1. T>3cm
2. N1 involvement
3. Central location

The biggest contribution of this study to the literature is the finding that, of the above

3 risk factors, only clinical N1 involvement emerged as significantly predictive of incidental N2 disease at surgery. This single-center study, and even more so the manuscript itself, is quite flawed, but it's of sufficient value to merit publication. I have the following comments to bring to the authors' attention for addressing:

Abstract

There are many awkwardly worded statements in the abstract. I suggest the following version:

BACKGROUND Tumor involvement of mediastinal lymph nodes impacts management and prognosis in non-small cell lung cancer (NSCLC). Invasive mediastinal staging is recommended in selected patients without evidence of mediastinal involvement on staging by imaging. In the present study, we aimed to evaluate the effectiveness of invasive staging in reducing pN2, its impact on survival, and risk factors for occult pN2.

METHODS. Patients with NSCLC tumors larger than 3 cm, central tumors or cN1 cases treated in our institution between 2013 and 2018 were prospectively included in the study. Incidence of pN2 and overall survival was compared among invasively staged (IS) and non-invasively staged groups (NIS). Multivariate analysis was performed to identify risk factors for pN2.

RESULTS. A total of 201 patients were included in the study, 79 (39.3%) of whom were non-invasively staged (NIS group) and 122 (60.7%) were invasively staged (IS group). Incidence of cN1 and mean PET/CT uptake was different between the two groups. Prevalence of pN2 was similar in both groups (7.6% in NIS vs 12.6% in IS; $p>0.05$). Median survival in IS-pN2 patients was 11 months longer than in NIS-pN2 group (33.6 months vs 22.5 months; $p=0.245$). cN1 emerged as the only risk factor for pN2.

CONCLUSIONS. Invasive staging does not reduce the incidence of pN2. However, this finding could be biased because in our series cN1 patients were more often staged and cN1 has been established as a risk factor for pN2. In addition, better patient selection resulting from invasive staging might have an impact on overall survival. To conclude, invasive mediastinal staging in intermediate risk patients for positive mediastinal nodes is justified.

Reply: Thank you for your suggestions. Changes have been made.

Major points:

Comment 1: How long was the follow up of the patients in this study? Did all of the study patients remain under the care of the study investigators for the entire duration of follow up?

Reply 1: Mean follow up was 25,31 (+/- 18) months. Follow up was done on referral center and information updated on a shared clinical course. (L239)

Comment 2: The authors frequently use the terms “staged” and “non-staged” when I believe they are referring to those who underwent and did not undergo invasive staging, respectively. This should be made clear in the first instance of using the terminology and then followed consistently throughout.

Reply 2: Changes have been done as suggested.

Comment 3: P6: in the Surgery subsection, the authors use terminology such as pN2 and cN2 but define them only in the subsequent subsection. Without the definitions that follow, interpreting these terms as used in this study is challenging. Recommend moving the definitions found in the Mediastinal involvement terminology subsection to an earlier point in the manuscript.

Reply 3: Subsection has been moved as suggested.

Comment 4: On the issue of definitions, it appears that pN2 includes the two patients with pre-operatively known N2 disease who underwent neoadjuvant therapy. Should they be included if the pN2 group is meant to correspond to occult N2 disease? Unless these two patients were found to have N2 disease at a different location intraoperatively...

Reply 4: pN2 includes all patients who had positive mediastinal lymph nodes at surgery. We decided to include induction therapy patients to pN2 because none of them downstaged to ypN0-1. Mediastinal affection characteristics are detailed on table 3, one patient had N2 disease at same location (4L) and one patient at different location (4L to 5).

Comment 5: P9L188-91: The authors correctly mention that in 10/21 incidental N2 patients, the pN2 node would have been inaccessible to EBUS and VAM. It would be interesting to report the risk factors for pN2 that these 10 patients had (i.e., how many had cN1 vs. T>3cm vs. central tumors)

Reply 5: Characteristics of all N2 patients are summarized on table 3. Regarding unreachable N2: 6 cases were T> 3cm, 6 were cN1 and only 2 were central.

Comment 6: P10L217-22: please provide the fraction that yielded the result of 10.4%. I needed to refer to figure 1 and perform the calculation to understand the numerator and denominator, which is too much work for a reader. The same thing goes for the origin of the denominator of 18 in L220—takes careful review of Figure 1 to understand how that number was obtained.

Reply 6: Requested information have been added to clarify the data. "Postoperative mediastinal lymph nodes affection (pN2) was detected in 21 cases (21/201; 10,4%)" (L308) and " Mediastinal involvement (N2) on invasively staged intermediate risk patients was detected on 18 patients, 5 out these 18 (27,8%) histologically confirmed N2 patients were identified preoperatively (cN2) in our series. " (L311)

Comment 7: P10L217-22: It seems that inclusion of some sort of overall comparison between the detection of N2 by invasive staging vs. by eventual surgery would be helpful; perhaps a presentation of these two fractions side by side: 5/122 vs. 15/119, assuming that the two patients who received neoadjuvant therapy for known N2 were found to have occult N2 at other stations.

Reply 7: in our opinion this data could be confusing in this paragraph. Nonetheless, a sentence like "detection of cN2 on IS group was 5/122, after negative IS the incidence of unexpected pN2 was 14/117" could be added.

Comment 8: P13L278-9: It's not clear what is meant by the phrase "clinical criteria" in that sentence.

Reply 8: The decision to stage or not cN1 patients was based on multidisciplinary board opinion and experience.

Comment 9: P13L277-80: To me it seems that the uneven distribution of cN1 (more in the invasive group) should have favored N2 occurrence and detection in the invasive group, so the absence of a statistically significant difference in occult N2 between the groups seems even more striking as a result.

Reply 9: We totally agree at this point, absence of differences "could be attributed to asymmetric distribution of cN1 patients that is indeed the only independent risk factor for pN2" (L415).

Comment 10: P13L282-7: Not sure this is a relevant weakness since the study was not meant to assess EBUS performance. The authors may wish to list other weaknesses, of which the study has its share.

Reply 10: The value of EBUS compared to VAM especially on these patients is a hot topic on debate. Many will ask for this specific results that unfortunately we cannot provide. In this paragraph, we want to clarify why we centered the study on mediastinal staging.

Comment 11: In the Discussion, the authors may want to put the survival figures for their occult N2 patients in the context of that of prior studies: i.e., comparable to figures for cN1 patients who undergo resection or not? Examples: PMID 23423241, and in this journal PMID 30233853.

Reply 11: We decided to compare pN2 survival because it would highlight the importance of selection. Survival could be better because 3 patients out of 18 N2 were discarded for surgery because of different reasons.

Comment 12: Regarding Table 3, it would be useful to report, in the text perhaps, the % of occult N2 cases detected at surgery that were found to have single station involvement—presumably the single-station occult N2 differs in terms of prognosis and management from multi-station occult N2.

Reply 12: text has been added (L274)" Finally, 2 patients (2/21; 9.5%) had multistation mediastinal involvement at surgery, all of them at NIS group"

Minor points:

1. P2L34: “discarded” is the wrong word usage in that context.
2. P4L90: ROSE was available to ensure “sample quality.” One might take that to mean that the cytotechnologist was only assessing adequacy (i.e., presence of lymphocytes), whereas it appears from a subsequent statement that there was also a diagnostic assessment performed. The authors should clarify the role of the cytotechnologist in their procedures.
3. P5L107: “of” should be changed to “with”
4. P6L126: would switch the order of cN2 and pN2 in the parentheses
5. P6L137: awkward phrasing; consider: “...regardless of whether they underwent surgery.”
6. P8L178: “two only” should be “only two”
7. P8L182-3: “...but it did not alter incidence...” should be rephrased to something like “...there was no difference in the incidence...”
8. P9L195: replace “was not detected” with “did not emerge”
9. P10L212: “has been” should be “was”
10. P10L214: “on” should be “in” and “among” should be “between”
11. P10L220: I believe authors meant “were” rather than “are”
12. P10L227-9: awkward sentence; consider: “Our previously published work from 2012 showed that tumor size is an independent risk factor for upstaging; however, we analyzed only clinical stage I NSCLC and excluded central tumors (14).”
13. P11L236-7: awkward sentence; consider: “In summary, our findings support the notion that only cN1 status should be considered an independent risk factor for pN2.
14. P11L240: “worth” should be “worthwhile”
15. P11L243: “of” should be “from”
16. P11L250: Here and elsewhere the term “recommendable” is awkward; consider “advisable” or rephrasing statements that use the word “recommendable” altogether
17. P11L251-2: consider: “However, considering the results of the current study, invasive efforts may not lead to a reduction in incidental N2.”
18. P12L260: “consider” instead of “considered”
19. P12L261: replace “because of” with “by”
20. P12L270: replace “due to” with “because”
21. P12L271-2: better phrasing: “...experience, so further studies are necessary in this

specific patient category.”

22. P13L290-1: consider: “In our series of intermediate-risk patients, (A) there was no statistically significant difference in PN2 incidence between the invasively staged and non-invasively staged groups.”

Reply 1-22: changes were made in the text.

23. Table 1: what does “no feet” mean? Also should be “excluded from” rather than “discarded”.

Reply 23: no feet change to not fit and discarded to excluded from.

Reviewer E

The authors retrospectively investigated the role of invasive mediastinal staging (EBUS-TBNA or VAM) in patients with intermediate risk of mediastinal metastasis (tumors larger than 3 cm, central tumors or cN1 cases). There was no significant difference of pN2 between invasively staged (IS) and not invasively staged groups (NIS). Median survival of IS-pN2 group was 11 months longer than that of NIS-pN2 group, which was not statistically significant ($p=0.245$). The authors considered it as clinically significant and concluded that invasive mediastinal staging in intermediate risk patients for positive mediastinal node is recommendable.

Major comments:

Comment 1: I think the authors need to provide the detailed information about the diagnostic performances of invasive mediastinal staging (VAM or EBUS-TBNA). In this study, 122 invasive staging detected only 5 mediastinal metastases and missed 13 mediastinal metastases. Therefore, the diagnostic sensitivity is just 33.3% (5/15). This means that about 24.4 invasive mediastinal staging was conducted to find one case of mediastinal metastasis while they missed 2.6 cases of mediastinal metastases. Ten of 12 pN2 was non-reachable by invasive mediastinal staging in this study. Although major practices guidelines (ie, NCCN, ACCP, ESTS) recommended invasive mediastinal staging for N1, tumors larger than 3 cm, or central tumors, the role of invasive mediastinal staging is still debatable. Shin SH, et al recently published the similar issue and they reported the limited role of endosonography (EBUS-TBNA ± EBU-B-FNA) in patients with radiological N0 (Lung Cancer 2020; 139: 151-156). In

their study, diagnostic sensitivity of endosonography was only 47% in whole study population and diagnostic performance of endosonography did not differ according to tumor centrality or diameter. Interestingly, pN2 was not associated with centrality and size of the tumor like this study.

Reply 1: we did not detail the information about invasive mediastinal staging because two reasons. First, EBUS was performed in two different centers and they are not comparable. Second, we did not want to evaluate if EBUS is better than VAM and when to use each technique. However, in table three you can see how each of the N2 patients were staged.

Comment 2: The authors concluded that invasive mediastinal staging in intermediate risk patients for mediastinal metastasis is recommendable although the survival difference between IS-pN2 group and NIS-pN2 group was not statistically significant. However, among 122 IS group, only 2 patients with cN2 which were detected by invasive mediastinal staging received neoadjuvant therapy followed by surgery. Did the authors believe that the marginal survival benefit of IS group over NIS group is due to the effect of neoadjuvant therapy? Considering low diagnostic sensitivity and marginal survival benefit of IS group, I cannot agree with authors' conclusion. Given the very low diagnostic yield of invasive mediastinal staging in detecting occult mediastinal metastases in cN0 patients, the optimal management strategy of occult N2 patients (neoadjuvant therapy followed by surgery vs upfront surgery followed by adjuvant therapy) should be clarified first in the future.

Reply 2: It is, indeed, a very interesting point. We believe that differences on survival are not related to neoadjuvant therapy, in fact they are related to better patient selection. As far as we know exclusion of multistation patients, cN3 patients and those who won't tolerate chemotherapy would make the difference on survival.

Comment 3: In this study, cN1 was more frequent in IS group compared with NIS group. Moreover, cN1 was an only independent predictor of pN2. Therefore, the authors need to do survival analysis according to cN1 status.

Reply 3: We decided not to do the cN1 survival because of two reasons. First, it is well known and established the impact of cN1 on Survival. Secondly, because it was not the main goal of the study and could make the final result confuse.

Minor comments:

Comment 1: This was a retrospective study. However, the authors also described that all participants signed informed consent (line 62-63). How is this possible? Please, explain this point.

Reply 1: Patients signed conventional informed consent for mediastinal exploration (EBUS/VAM) or surgery, not to be included to the study.

Comment 2: There were several typos (ie, line 131: witch, etc).

Reply 2: text has been reviewed.

Reviewer F

The authors are investigating an important area in lung cancer - utility of invasive mediastinal staging prior to lung cancer resection. This is a small retrospective study of 200 patients that attempts to compare two groups: those with and without invasive mediastinal staging.

Comment 1: Unfortunately, this is small sample size that does not enable an almost doubling in the detection of N2 disease to be statistically significant (12.6 vs, 7.6%, $p>.05$). This study is underpowered to detect meaningful differences.

Reply 1: it is true that differences on N2 were not statistically significant. However, we strongly believe that the results of the study would help to better decide the strategy on this subgroup of patients.

Comment 2: The inclusion criteria for this study are unusual. There are prospective studies investigating clinical stage I disease (ACOSOG Z0050) and larger and central tumors (Fernandez J Thorac Cardiovasc Surg. 2015 Jan;149(1):35-41). The presence of clinical stage II disease is a very different cohort with a much higher propensity for N2 disease. Every guideline recommends invasive mediastinal staging for this group so they should not be included in this study.

Reply 2: We individually analyzed the three main indications for mediastinal staging in PET negative for N2 patients. According to our results only cN1 is a risk factor for pN2 and not the tumor size. In L342 we hypothesize that tumor size and centrality are risk factors for pN1 and, in consequence, indirect risk factor for pN2.

Reviewer G

The authors tried to evaluate the effect of invasive mediastinal staging on prevalence of unexpected N2 (can reduce?) and survival (can increase?).

This attempt should be highly appreciated, however, there are serious problems with the analytical method.

Major

Comment 1. The authors tried to evaluate the effect of invasive mediastinal staging (including EBUS and VAM) on prevalence of unexpected N2 (confirmed by surgery, mediastinal LN dissection) in NSCLC patients with > 3 cm of tumor, or central tumor, or cN1 (n = 201).

Of these 201 patients, 79 patients underwent non-invasive staging (NIS group) and 122 patients underwent invasive staging (IS group).

This study was not RCT, so true N2 (cN2 + pN2) prevalences were 7.6% (6/79) in NIS group and 14.8% (18/122) in IS group.

In IS group, the prevalence of N2 was 11.1% (13/117) after excluding 5 patients who confirmed by EBUS and/or VAM (before MLND).

But, the authors evaluated there was no reduction on prevalence of pN2 with presenting "7.6% in NIS vs 12.6% in IS; $p > 0.05$ ".

There are several critical problems.

First, why you compare the prevalence between two groups? It is not RCT. The true N2 prevalences (7.6% in NIS and 14.8% in IS group) and baseline characteristics are not same. If you want to evaluate the effect of invasive mediastinal staging on prevalence of unexpected N2, you only need the IS group (14.8% (18/122) before invasive staging to 11.1% (13/117) after invasive staging).

Reply 1.1: It is true. However, we wanted to know why were similar in both groups. As it was pointed out, it is because cN1 was asymmetrically distributed.

Second, of 18 patients with true N2 in IS group, you were able to detect only 5 patients with cN2 by EBUS and/or VAM.

The sensitivity of invasive staging was only 28% (5/18).

Considering the sensitivity of EBUS is usually 50% in patients with radiological N0 patients, 28% is so low.

Reply 1.2: It is true, however when we evaluate sensitivity of EBUS on "reachable stations" it is 50% (5/10).

Comment 2: There are many problems with statistical methodology.

First, you used multivariate logistic regression to investigate risk factors of occult pN2 and Cox regression to evaluate relation among invasive staging and survival.

How did you choose variables? ($P < 0.20$ in univariable analysis?, backward selection with $P < 0.05$ for entry of variables and $P > 0.10$ for removal of variables?, select clinically meaningful variables?)

Reply 2.1: We decide to use Cox regression to evaluate the impact of staging on survival. Only clinical meaningful variables were selected for analysis.

Second, in Table 4, it is OR (odds ratio), not HR (you mentioned you used logistic analysis for evaluate risk factor for pN2 in Methods section).

you presented only P value (not HR) in univariate analysis in Table 4.

You should present univariate HR in Table 4.

I can't believe the two P values in univariate and multivariate analyses are the same in Table 4.

Reply 2.2: We absolutely agree, we use OR in a retrospective analysis. It was a writing mistake. We apologize for p values, evidently the multivariate p values were copied to univariate column. Right values are now on table 4. We don't consider that only p values are necessary on univariate analysis.

Third, in Table 5, you should present univariate and multivariate HR and P value.

What is the subject of this analysis? total 201 patients? 21 patients in Figure 2?

Reply 2.3: All surgically treated patients (198) were included to determine relation between staging and survival (L566).

Minor

Comment 3: line 34. ...whereas patients with mediastinal affection (cN2 or cN3) are

discarded for surgery and treated with radical chemo-radiotherapy.

"cN2 are discarded for surgery" seems to be too extreme. cN2 can consider Op after neoadj Tx.

Reply 3: we changed discarded for initially excluded from (L78)

Comment 4: line 59. How many patients were excluded by previous lung cancer treatment, not suitable for surgery, with unresectable pathology, and suspicion of cN2-3 or cM1, respectively.

Reply 4: Unfortunately, we cannot provide this information to reviewer F. First because these patients were not included to the study and in consequence we did not register them, and second, because some of them were not even referred to our department.

Comment 5: Figure 2A. Why number at risk (0 month) are 22 for pN2-3 and 175 for pN0-1?

In Table 1, they are 21 and 177, respectively.

Reply 5: Transcription error from a preliminary analysis. Changes have been done in figure 1.

Comment 6: Figure 2B. Why number at risk (0 month) are 77 for non-staged and 120 for staged?

In Figure 1, they are 79 and 122, respectively.

Reply 6: Transcription error from a preliminary analysis. Changes have been done in figure 1. Patients at risk on staged group are 119 because 3 were not operated and survival can not be calculated.

Comment 7: Table 1. Why total number of "Pathological stage" is not 201? $(1+9+11+32+15+55+6 = 129)$ IIB = 0?

Reply 7: Stage IIB was missing (69 cases, 34,3%).