Peer review file

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Reviewer A

Comment 1: The diagnostic accuracy/results depended on patient selection criteria. The study included 87.7 % patients undergoing most previous unsuccessful diagnostic procedures. And, the diagnosis accuracy was still 75%. I considered it was acceptable. But, two-thirds of all patients with negative or inconclusive ENB pathology underwent a diagnostic re-intervention, namely traditional bronchoscopic procedures (n=13), CT-guided TTNA (n=12) or video-assisted thoracoscopic surgery (VATS) (n=15), which revealed another 20 malignant pathologies. Most patients have received procedures before ENB. After ENB failed, two-thirds of all patients have received re-intervention again. The authors should explain.

Reply 1: We recognise that a considerable proportion of patients with negative or inconclusive ENB pathology underwent diagnostic re-intervention based on a new MDT decision. If the patient in spite of increased perioperative risks was found eligible for surgery, a VATS procedure was the natural next step of the diagnostic algorithm (n=15). However, if the patient was deemed a poor surgical candidate and the probability for malignancy was considered high, re-intervention with CT-guided TTNA (n=12) or traditional bronchoscopic procedures (n=13) was elected to be the best possible minimal invasive option to get diagnostic reassurance despite previous failure or reservation regarding low yield. We acknowledge that the pathway of this patient subgroup is far from ideal, nonetheless, it reflects daily clinical diagnostic challenges and dilemmas that we believe many units can relate to. We admit that used term "traditional bronchoscopic procedures" is potentially misleading because only EBUS or pEBUS was used for re-intervention in these patients. We have revised the later (Page 6, line 106 + Page 10, line 169) and added the following explanation to the discussion:

"Moreover, two-thirds of the patients with negative or inconclusive ENB pathology underwent diagnostic re-intervention based on a new MDT decision. If the patient in spite of increased perioperative risks was found eligible for surgery, a VATS procedure was the natural next step of the diagnostic algorithm (n=15). However, if the patient

was deemed a poor surgical candidate and the probability for malignancy was considered high, re-intervention with CT-guided TTNA (n=12) or EBUS/pEBUS (n=13) was elected to be the best possible minimal invasive option to get diagnostic reassurance despite previous failure or reservation regarding low yield. We acknowledge that the pathway of this patient subgroup is far from ideal, nevertheless, we believe it reflects daily clinical diagnostic challenges and dilemmas that many units can relate to." (Page 14, line 279-288)

Comment 2: As we know, learning cured is a crucial role in ENB. Please showed the learning curve.

Reply 2: We agree and a learning curve comparing the first 40 consecutive procedures versus the last 41 procedures has been added to the manuscript (figure 2).
Furthermore, we have added the following paragraphs to the results and the discussion section:
1) "The learning curve of our initial series is illustrated in figure 2 comparing the results based on diagnostic accuracy from the first consecutive 40 ENB procedures with the last 41 procedures. An increase in the diagnostic accuracy from 67.5% to 82.9% and almost a half in the number of false negatives (13 vs. 7) was observed, however, the difference was not statistically significant (p-value: 0.11)." (Page 10, line 191-195)
2) "The impact of the learning curve when introducing ENB must also not be underestimated. Lamprecht et al. (18) studied the diagnostic yield of 112 ENB procedures and observed a steep learning curve with an increase in diagnostic yield of 80% and 87.5% for, respectively, the first 30 and last 30 procedures. Likewise, the learning curve of our initial series showed a non-significant but substantial improvement in diagnostic accuracy and almost a half in the number of false negatives when comparing the results from the first 40 ENB procedures with the last 41 procedures (p-value: 0.11)." (Page 12, line 233-239)

Comment 3: What was proportion of bronchial sign, consolidation/diameter ratio.

Reply 3: As stated in the discussion the proportion of bronchus sign (or the C/T-ratio) was not recorded, but from experience we concur with its positive impact on the

diagnostic yield. We retrospectively acknowledge that this information would help interpretation of the results.

As an explanation we have modified and added the following paragraph in the discussion:

"…although we did not record the presence of bronchus sign (or CT-ratio and distance from the pleura), we do from experience concur with its positive impact on the diagnostic yield. Nevertheless, in this initial series of highly selected patients (generally not obvious surgical candidates) these factors had no impact on the MDT decision because a minimal invasive biopsy was desired. In this context, we highlight that we were not in a position where we could select the patients based on factors that would positively influence the diagnostic yield. But we were keen to know if the addition of ENB would be a true clinically applicable asset to our minimal invasive diagnostic algorithm and subsequently help us reduce potentially harmful or futile diagnostic surgical resections in frail patients. Consequently, we actually predisposed ENB to the most diagnostically challenging cases regardless of conventional recommendation of ENB suitability and obviously these data cannot be extrapolated to population screening or algorithms with ENB as the first choice of the diagnostic work up." (Page 13, line 256-267).

Reviewer B

Comment: I have a few important questions to clarify. It seems that the majority of the patients had a previous history of malignancy. So the lesions that had been sampled by ENB should be thought to be a metastasis. So according to the previous malignancy history the diagnosis for metastatic diseases to the lung might be done clinically (other organs involvement). Or the surgical resection of the lesion would be helpful for both diagnosis and also help for treatment. Needle biopsy for differential diagnosis for a new primary tumor or recurrence –metastasis would be difficult with such small amount of aspiration material (the need for immunohistochemically staining etc). The further approach (surgery for metastatic lesion etc.) of these patients needs to be mentioned. If the aim to give a diagnosis lymph nodes (mediastinal) would also be helpful there is no data about that. Honestly, I am feeling happy with the patient selection which is the key point of the study. Do you have any data with patients of no previous cancer history?

(potential candidate for surgical lung resection) Would like to use your data about those patient group?

Reply 1: We acknowledge that some patients in this series may just as well have been under investigation for pulmonary metastatic disease as well as primary lung cancer or recurrence. If metastatic disease is the main suspicion of a pulmonary lesion, we agree that VATS resection is a good diagnostic and therapeutic option, and we perform many of these procedures at our institution on a weekly basis. The present group of patients are unfortunately not that straight forward due to either high risk of surgical complications (previous radiated field, tumor location etc.), poor performance status or co-morbidity, which is why this approach was preserved in favour of less invasive diagnostic techniques. A tissue diagnosis via a minimal invasive approach would allow other treatment options such as stereotactic radiation therapy, which may be a better option for this category of patients.

Reply 2: A minimum of three forceps biopsies were part of our standard biopsy protocol, which often revealed the diagnosis and usually gave the pathologist enough tissue to perform immunochemical staining if necessary.

Reply 3: We appreciate the reviewers understanding of the patient selection, which absolutely is a key point of this study and underlines the diagnostic dilemmas/challenges we are all facing in these difficult cases. Please see the following addition to the manuscript:

“Moreover, two-thirds of the patients with negative or inconclusive ENB pathology underwent diagnostic re-intervention based on a new MDT decision. If the patient in spite of increased perioperative risks was found eligible for surgery, a VATS procedure was the natural next step of the diagnostic algorithm (n=15). However, if the patient was deemed a poor surgical candidate and the probability for malignancy was considered high, re-intervention with CT-guided TTNA (n=12) or EBUS/pEBUS (n=13) was elected to be the best possible minimal invasive option to get diagnostic reassurance despite previous failure or reservation regarding low yield. We acknowledge that the pathway of this patient subgroup is far from ideal, nevertheless, we believe it reflects daily clinical diagnostic challenges and dilemmas that many units

can relate to." (Page 14, line 279-288)

The majority of patients included in this study are as mentioned not obvious candidates for major lung resection. Nonetheless, 22 patients in this series had no previous cancer. Nine out of the 22 had a malignant ENB biopsy and 6 of these patients went on to surgical treatment (lobectomy or wedge resection).

We are always happy to share data and collaborate on interesting research projects – please contact me about the study you have in mind. Email: Michael.stenger@rsyd.dk

Reviewer C

Comment 1: Their population is rather special. 71.6% had a history of prior cancer. This is different from the usual lung cancer screening population. Please address this in the discussion, how this may impact the diagnostic yield. The results obtained may not be extrapolated to the population screening.

Reply 1: We concur and thank reviewer C for this comment: We have added the following paragraph to the discussion:
"In this context, we highlight that we were not in a position where we could select the patients based on factors that would positively influence the diagnostic yield. But we were keen to know if the addition of ENB would be a true clinically applicable asset to our minimal invasive diagnostic algorithm and subsequently help us reduce potentially harmful or futile diagnostic surgical resections in frail patients. Consequently, we actually predisposed ENB to the most diagnostically challenging cases regardless of conventional recommendation of ENB suitability and obviously these data cannot be extrapolated to population screening or algorithms with ENB as the first choice of the diagnostic work up." (Page 13, line 256-267).

Comment 2: Please provide details and the exact diagnosis obtained under the "benign" category.

Reply 2: The benign category comprises findings of fibrosis, inflammation/pneumonia, inconclusive/non-representative tissue and atypical cells. We have added this to the

manuscript (Page 9, line 174)

Comment 3: Please discuss the limitation of observing for an average of 11 months. Slow growing neoplasm maybe missed. Therefore, the so-called "true negative" results should only be provisional.

Reply 3: We acknowledge that an average follow-up period of 11 month is a limitation of the study, which may impact the results. We have modified and added the following to the discussion:

"The true prevalence of malignancy in the present patient population remains undetermined due to the average follow-up duration of 11 months. This must be noted as a limitation of the study because slow growing tumors might be missed on consecutive CT-scans and a longer follow up period would potentially impact the proportion of "true and false negatives"." (Page 15, line 295-298)

Comment 4: Please provide statistical analysis regarding whether prior malignancy predicts a biopsy result of malignant lesion.

Reply 4: We appreciate that the suggestion made by reviewer C may be interesting to explore, and we have performed a logistic regression analysis accordingly. The following three sentences have been added to the manuscript:

1) "The relationship between previous cancer diagnosis and malignant ENB pathology was explored through logistic regression analysis." (Page 8, line 152-154)

2) "Finally, logistic regression analysis revealed that previous cancer diagnosis does not predict a malignant ENB biopsy (p-value: 0.874)" (Page 10, line 196-197)

3) "But previous cancer history did not prove to be a predictor for malignant ENB pathology in our study. This result must, nevertheless, be interpreted with caution given the learning curve and the small number of procedures." (Page 15, line 302-304)

Reviewer D

Comment:

1. The determining factor for ENB is how deep is your lesion. The information is not present.

2. Since most patients in the cohort are of a history of cancer, what C/T ratio are those tumors carrying? I mean, people with a suspicion of metastatic lung tumor are looking different with the ones without previous cancer history. They are more solid and kind of higher C/T ratio.

3. What percentage do these tumors have a route? The bronchial sign is important to decide it is suitable or not to take biopsy by ENB.

Reply: Bronchus sign, C/T-ratio and distance from the pleura was not recorded, but all lesion had a route and from experience we concur with the positive impact of bronchus sign on the diagnostic yield. We retrospectively acknowledge that this information would help interpretation of the results. Nevertheless, in this initial series of highly selected patients (generally not obvious surgical candidates) these factors had no impact on the MDT decision because a minimal invasive biopsy was desired. Furthermore, we highlight that the context and the patient selection of this study underlines that we had no interest in performing a constructed ENB success story, but we were keen to know if the addition of ENB would be a true clinically applicable asset to our minimal invasive diagnostic algorithm and subsequently help us reduce potentially harmful or futile diagnostic surgical resections in frail patients. Consequently, we actually predisposed ENB to the most diagnostically challenging cases regardless of conventional recommendation of ENB suitability in respect of bronchus sign etc. We have modified the text and added this explanation to manuscript:

"…although we did not record the presence of bronchus sign (or CT-ratio and distance from the pleura), we do from experience concur with its positive impact on the diagnostic yield. Nevertheless, in this initial series of highly selected patients (generally not obvious surgical candidates) these factors had no impact on the MDT decision because a minimal invasive biopsy was desired. In this context, we highlight that we were not in a position where we could select the patients based on factors that would positively influence the diagnostic yield. But we were keen to know if the addition of ENB would be a true clinically applicable asset to our minimal invasive diagnostic algorithm and subsequently help us reduce potentially harmful or futile diagnostic surgical resections in frail patients. Consequently, we actually predisposed ENB to the most diagnostically challenging cases regardless of conventional recommendation of

ENB suitability and obviously these data cannot be extrapolated to population screening or algorithms with ENB as the first choice of the diagnostic work up." (Page 13, line 256-267).

Reviewer E

I have reviewed your manuscript carefully. You perform the procedure of ENB for small pulmonary nodule biopsy (mean target diameter 15.5 mm, smaller than previous studies) without radial EBUS / fluroscopy/ ROSE assistance. So the lower value of sensitivity/NPV/NLR in your study is predictable. You rely on only ENB, one tool, to take a small pulmonary nodule biopsy. But I have some concerns requiring your answers.

Comment 1: true negative: If ENB revealed benign or inconclusive pathology, pulmonary lesions with consistent benign pathology on several consecutive biopsies or with no signs of growth or morphological changes on repeated CT scans were considered as true negative. " No signs of growth or morphological changes on repeated CT scans" is debatable. Your follow up duration is only 11 months. Some cancers are indolent. You will make an error in the short follow up period.

Reply 1: We thank the reviewer for pointing this out and we agree that the follow up duration is a limitation of the study. We have added the following paragraph to the discussion:

"The true prevalence of malignancy in the present patient population remains undetermined due to the average follow-up duration of 11 months. This must be noted as a limitation of the study because slow growing tumours might be missed on consecutive CT-scans and a longer follow up period would potentially impact the proportion of "true and false negatives"". (Page 15, line 295-298).

Comment 2: Patients selection following two criteria: A: previous unsuccessful traditional diagnostic procedure including flexible bronchoscopy. B: low yield rate of traditional diagnostic procedure including flexible bronchoscopy. But in your 60 cases (ENB revealed benign or inconclusive pathology), you use traditional flexible bronchoscopy for diagnostic re-intervention in 13 patients. Why?

Reply 2: Correct, but as also stated in the manuscript "…considerations regarding tumour size and location, patient co-morbidities including emphysematous changes around small peripheral lung lesions, pulmonary function, and the clinical probability of malignancy were all part of the MDT discussion before offering ENB."
We admit that used term "traditional bronchoscopic procedures" is potentially misleading because only EBUS or pEBUS was used for re-intervention in these patients. We have revised the later (Page 6, line 106 + Page 10, line 169) and added the following explanation to the discussion:

"Moreover, two-thirds of the patients with negative or inconclusive ENB pathology underwent diagnostic re-intervention based on a new MDT decision. If the patient in spite of increased perioperative risks was found eligible for surgery, a VATS procedure was the natural next step of the diagnostic algorithm (n=15). However, if the patient was deemed a poor surgical candidate and the probability for malignancy was considered high, re-intervention with CT-guided TTNA (n=12) or EBUS/pEBUS (n=13) was elected to be the best possible minimal invasive option to get diagnostic reassurance despite previous failure or reservation regarding low yield. We acknowledge that the pathway of this patient subgroup is far from ideal, nevertheless, we believe it reflects daily clinical diagnostic challenges and dilemmas that many units can relate to." (Page 14, line 279-288).

Comment 3: How many percentage of bronchus sign in your study? How about the distance between pleura and target? How about the distance between target center and ENB sensor tip? Do you use the mode of ENB cross country? How about your learning curve in the ENB procedure?
I was very interested in your work and want to know the limitation of ENB.

Reply 3: As stated in the discussion, the proportion of bronchus sign (including C/T-ratio or distance from the pleura) was not recorded, but from experience we concur with its positive impact on the diagnostic yield. We retrospectively acknowledge that this information would help the interpretation of the results. Nevertheless, in this initial series of highly selected patients (generally not obvious surgical candidates) these

factors had no impact on the MDT decision because a minimal invasive biopsy was desired. Furthermore, we highlight that the context and the patient selection of this study underlines that we had no interest in performing a constructed ENB success story, but we were keen to know if the addition of ENB would be a true clinically applicable asset to our minimal invasive diagnostic algorithm and subsequently help us reduce potentially harmful or futile diagnostic surgical resections in frail patients. Consequently, we actually predisposed ENB to the most diagnostically challenging cases regardless of conventional recommendation of ENB suitability in respect of bronchus sign etc.

We have added the following paragraph to the manuscript:

"…although we did not record the presence of bronchus sign (or CT-ratio and distance from the pleura), we do from experience concur with its positive impact on the diagnostic yield. Nevertheless, in this initial series of highly selected patients (generally not obvious surgical candidates) these factors had no impact on the MDT decision because a minimal invasive biopsy was desired. In this context, we highlight that we were not in a position where we could select the patients based on factors that would positively influence the diagnostic yield. But we were keen to know if the addition of ENB would be a true clinically applicable asset to our minimal invasive diagnostic algorithm and subsequently help us reduce potentially harmful or futile diagnostic surgical resections in frail patients. Consequently, we actually predisposed ENB to the most diagnostically challenging cases regardless of conventional recommendation of ENB suitability and obviously these data cannot be extrapolated to population screening or algorithms with ENB as the first choice of the diagnostic work up." (Page 13, line 256-267).

We did not routinely record the distance from the sensor tip to the target centre, but we had a general consensus that the distance should ideally be between 5-8 mm. From experience, we believe that the importance of the distance from the sensor tip to the target centre may also largely depend on the size/shape/formation of the lesion.

We have not used the cross-country mode, because this was not commercially available at this point in time.

We have added the learning curve of this series to the manuscript (Figure 2) including the following paragraphs:

1) “The learning curve of our initial series is illustrated in figure 2 comparing the results based on diagnostic accuracy from the first consecutive 40 ENB procedures with the last 41 procedures. An increase in the diagnostic accuracy from 67.5% to 82.9% and almost a half in the number of false negatives (13 vs. 7) was observed, however, the difference was not statistically significant (p-value: 0.11).” (Page 10, line 191-195)

2) “The impact of the learning curve when introducing ENB must also not be underestimated. Lamprecht et al. (18) studied the diagnostic yield of 112 ENB procedures and observed a steep learning curve with an increase in diagnostic yield of 80% and 87.5% for, respectively, the first 30 and last 30 procedures. Likewise, the learning curve of our initial series showed a non-significant but substantial improvement in diagnostic accuracy and almost a half in the number of false negatives when comparing the results from the first 40 ENB procedures with the last 41 procedures (p-value: 0.11).” (Page 12, line 233-239)