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Reviewer A

In this study, the authors conclude that EBUS-TBNB with Acquire is suitable for obtaining core tissue suitable for biomarker analysis in NSCLC while having a good diagnostic rate for lymphadenopathy. In the current clinical situation in lung cancer, where the diagnosis of advanced-stage NSCLC requires not only a histologic diagnosis but also the investigation of indications for molecularly targeted agents and immune checkpoint inhibitors, this article can be significant for clinicians. To improve the quality of this paper, we would like to ask the authors a few questions, as indicated below.

Major comments
Comment 1: Perhaps because the Acquire needle made of cobalt chrome is less accessible to the lesion than the Vizishot needle, the target lesion for the Acquire needle was significantly larger than the Vizishot needle in this study. This point should be added to the Limitation.

Reply 1: We agree that due to the fabrication of the Acquire™ needle with Cobalt Chrome, the Acquire needle has a shallower angle of penetration. For smaller targets this can lead to difficulties with accessibility. Similarly, our experience suggested that the Vizishot™ needle with its reinforced tip was easier to stabilize and hence accessing smaller nodes was easier. For this reason, for smaller targets, the Vizishot™ was often favoured.

Changes in the text: Line 400 to 403:

"In particular, the target lesion for the Acquire™ needle was often larger. Smaller lesions were perceived to be more accessible with the Vizishot™ needle due to stability at the puncture site and steeper needle angulation. The greater capacity for flexion with the Vizishot™ meant it was also favoured for the lymph nodes requiring up-angulation such as 4L and 10R."

Comment 2: In #4L, 5 cases were converted to Vizishot needle because the Acquire needle failed to puncture. How are these cases accounted for in the Acquire needle diagnostic rate calculation? It should be clarified whether the biopsy was recorded as a success because it was possible to biopsy with an Acquire needle from a lymph node lesion other than #4L in the same patient or whether it was treated as a failure. The same is true for the other two cases of #7, one case of #4R, and one case of #10R.

Reply 2: When the Acquire™ needle had to be changed to the Vizishot™ needle (mostly 4L but also 7, 4R and 10R), these were counted as technical failures for the Acquire needle. They were included in the calculation as a "non-diagnostic sample"

Changes in the text: Line 245-246
"These cases were considered to be technical failures and were included in the calculations as non-diagnostic samples."

Comment 3: Although EBUS-Cryo is mentioned by the author in the text, it is better to emphasize that EBUS-TBNB is more cost-effective than EBUS-Cryo and that the technique is almost identical to EBUS-TBNA and easier for the surgeon. EBUS-Cryo is not cost-effective and requires the use of a high-frequency device to create a puncture hole, making it difficult for many respiratory physicians to perform this procedure.

Reply 3: Cryobiopsy requires significant up-skilling and is a more challenging procedural skill. We agree it is more challenging for respiratory physicians to perform this procedure.

Changes in the text: Comment added line 331-333
"EBUS-TBNB is also significantly more cost effective than EBUS-Cryobiopsy and the technique is almost identical to EBUS-TBNA, which therefore makes it easier for respiratory physicians to perform this procedure."

Comment 4: Please add the median and range of the number of punctures in each case (or each lesion) in EBUS-TBNB.

Reply 4: Due to the retrospective nature of this study, the number of punctures in each case is not controlled. However, generally 3-6 punctures of the target lesion are performed with 15-20 passes per puncture. This is on the basis of historical teaching from the primary EBUS-TBNA specialist. This practice did not vary between needles.

Changes in the text: Comment added line 168
"Although not controlled, generally 3-6 punctures of the target lesion and 15-20 passes per puncture are performed at our institution based on historical teaching from the primary EBUS-TBNA specialist"

Minor comments
Comment 1: The order in which Acquire and Vizishot are listed in Table 3 differs from Table 1 and 2, making it difficult for the reader to read. Please correct; the rate of Malignant in Vizishot is also incorrect.

Reply: Corrected

Changes in the text: Table 3. Corrected Malignant diagnoses to 31/101 (30.7%)
The order that the Acquire™ and Vizishot™ are listed has also been amended.
Comment 2: Is the Vizishot used an older model? Please add the model number to Methods. Also, if it is an older model, you should append it to the LIMITATION.

Reply: Model number is NA-U401SX-4021. This is an up to date model and hence has not been added as a limitation.

Changes in the text: line 152 "(Model NA-U401SX-4021)"

Comment 3: Please add the magnification of the microscope in Figure 2.

Reply: Figure 2 has been corrected to include the magnification of the microscope.

Changes in the text: Figure 2a – Histological cores from the Vizishot™ needle. 40x magnification at 0.5mm scale, 200x magnification at 0.1mm scale, 400x magnification at 0.05mm scale.

Figure 2b – Histological cores from Acquire™ needle – demonstrating more abundant tissue obtained. 125x magnification at 1mm scale, 200x magnification at 0.1m scale, 400x magnification at 0.5mm scale.

Reviewer B

Comment 1: As the authors have noted various limitations in this trial, the needle assignment was not randomized and pathologists were not blinded. Bronchoscopists were more likely to use the Acquire™ needle for large and malignant NLs and the VizishotTM needle for small LNs or LNs required up-angulation such as 4L, 10R.

Reply: The Acquire™ was used for larger lymph nodes and malignant Lymph nodes. In these cases, the angle of the needle and stability of the needle were less critical to the sampling process and the perceived increased tissue yield from the Acquire™ was favoured. Conversely, the Vizishot™ was favoured for smaller nodes where accessibility posed a greater challenge. In these instances, the steeper angle of the needle and stability at the puncture site were helpful. For nodes requiring up-angulation such as 4L and 10R the greater flexion of the Vizishot™ needle also made this a favourable needle choice.

Changes in the text: Line 400-403
"In particular, the target lesion for the Acquire™ needle was often larger. Smaller lesions were perceived to be more accessible with the Vizishot needle due to stability at the puncture site and steeper needle angulation. The greater capacity for flexion with the Vizishot™ meant it was also favoured for the lymph nodes requiring up-angulation such as 4L and 10R."

Comment 2: This study was a descriptive study on the use of the Acquire™ needle for EBUS-TBNA. The findings of this investigation provided no proof of ROSE's value. Please edit the abstract and manuscript conclusions.

Reply: Our comments regarding ROSE was based on the fact that the diagnostic yield with the Acquire needle was consistently good with sufficient tissue for PDL1 and ancillary testing. However, we agree the study has not demonstrated proof of the value of ROSE. Therefore, comments regarding the value of ROSE have been removed in the abstract and conclusions.

Changes in the text: Line 84, line 414
Line 84: Deleted "and supports existing literature that ROSE may not be required"
Line 414: Deleted "supporting the existing literature that Rapid On-Site evaluation may not be required and may not necessarily increase diagnostic yield"

Comment 3: Please define 'significant' bleeding. The authors might use the standardized definitions of bleeding after transbronchial lung biopsy (A Delphi Consensus Statement From the Nashville Working Group) [Chest 2020;158:393-400.]

Reply: Significant bleeding was defined as grade 2 bleeding or above as stratified in the Delphi Consensus Statement. Reference added.

Changes in the text: Line 290-291.
"Significant bleeding was defined as grade 2 bleeding or above as stratified in the Delphi Consensus Statement"
Reference added

Comment 4: Please show and compare macroscopic and microscopic samples obtained by each Acquire™ and VizishotTM needles in Figures 1 & 2 as shown in Figures 3 & 4.

Reply: We have obtained images with accurate scaling for both needles and have adjusted figure 2 to be figure 2a and figure 2b.

We do not have access to a macroscopic image of the vizishot™ needle cores.

Changes in the text: See figure 2
Line 209-210: Figure 2a and 2b demonstrates histological samples from the Vizishot™ and Acquire™ needles respectively.
Line 255-258: Figure 2 shows Vizishot™ histological samples and demonstrates that more abundant tissue is obtained with the Acquire™ needle at the lower power view, though we acknowledge the scaling is not equivalent. At the 200x magnification at 0.1mm scaling, there is more detail from the Acquire™ sample.

Comment 5: There is an error in Table 3: 31/101 (3.07%).

Reply: Corrected percentage to 30.7%

Changes in the text: Table 3

Reviewer C

The authors dealt with the first experience of the Boston Acquire Franseen needle for EBUS-TBNA and explored the role of the Acquire™ needle alongside the Vizishot™ standard of care.
This review article provides a lot of helpful information not only to pathologists but also to pulmonologists.

Comment 1: The difference between the Acquire™ pulmonology needle and the existing EBUS-TBNA needle was as follows. (1) 3 symmetrical, fully formed, cutting heels, (2) Electropolished to increase sharpness and improve heel quality, (3) 3 angled points designed to promote tissue stability and reduce the likelihood of passability issues. Does the author think these structural differences in the needle tip can increase the diagnostic yield? I hope more specific explanations about the structural differences and the improvement in diagnostic yield are added to the discussion part.

Reply: There are clear structural differences between the two needles as outlined in the comment. We agree that the 3 symmetrical, fully formed cutting heels and the electropolished tip which creates a smooth cutting surface promote greater tissue capture and minimise tissue tearing and fragmentation. In our experience, the three angled points designed to promote tissue stability did not significantly improve tissue stability and passability. The reinforced, beveled tip of the Vizishot™ needle in addition to the greater flexion of the needle generated greater needle stability.

These differences explain why in our experience, the Acquire™ needle was used for larger targets where the angle of needle penetration was less critical and in situations where the features listed above could be used to maximise tissue capture. Conversely, where stability and predictability were more critical, we tended to favour the Vizishot™ needle. Our data demonstrated that we preferenced the Vizishot™ for smaller nodes and for positions like 4L and 11R.
Changes in the text: Line 365-370
“Other characteristics of the Acquire™ compared to the Vizishot™ needle includes an electropolished needle tip to create a smooth cutting surface and three fully formed heels that promote a circular cut during sampling which maximises tissue capture and minimises tissue tearing and fragmentation. The Acquire™ is also designed to have 3 angled points to promote tissue stability however, in our experience the reinforced, beveled tip of the Vizishot™ needle with greater flexion actually has greater stability.

These technical factors need to be taken into consideration for small nodes and certain anatomical locations. It may be more appropriate to sample the right upper hilar nodes (11R or R11s) with the Vizishot™ needle due to this more predictable angle of entry and the ability to have greater flexion of the EBUS scope (Table 2). For the station 4L node, a steeper angle of entry is also required and hence the Vizishot™ may be preferentially selected. In our experience a conversion from the Acquire™ to the Vizishot™ needle was required in 5/18 (27.8%) of cases and diagnostic tissue was routinely, reliably obtained with the Vizishot™ (13/13, 100%) in this instance (p=0.04)."

Comment 2: Studies dealing with comparisons between products need to be more careful and require caution. The retrospective study design is less convincing when comparing diagnostic yields between products. The persuasiveness of diagnostic yield can be obtained when the study is conducted on the same lesion of the same research subject with the same operator as double-blind. Pleased added these contents in the limitation section.

Reply: We agree that a direct diagnostic comparison between the Acquire™ and Vizishot™ cannot be made. A future study comparing both needles when applied to the same patient and same target lesion with the same operator would be beneficial to answer this question.

Changes in the text: Line 405-406
"Future studies comparing diagnostic yield when both needles are applied to the same patient and same target lesion could be trialed."

Comment 3: Several typos and omissions are observed in the manuscript. Please correct this in the revised manuscript.

Reply: Corrected