Peer Review File
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Reviewer A

Comment 1:
"*Generalizability, can the findings be applied to other dimorphic fungi such as Histoplasma and Blastomyces in corresponding endemic areas*?"

Reply:
Given some overlap in clinical behavior of these infections with *Coccidioides*, we briefly note this as a possibility in the discussion 'limitations' section, lines 387-89. Nevertheless, we acknowledge our study's conclusions as being limited to a practice within a *Coccidioides*-endemic region.

Comment 2:
"*How might the inclusion of antigen and PCR analysis of respiratory samples have influenced the results and conclusions of the study*?"

Reply:
We thank Reviewer 1 for highlighting this important question. In our 'limitations' section within the discussion, we address this issue. We stress that the goal and focus of the study was to analyze the mutual benefit of lower respiratory secretion and tissue sampling with culture and cytohistology testing, as these are the mainstays of practice, and thus most generalizable—ancillary testing availability, use and performance remain inconsistent (we included several references that demonstrate heterogeneity in performance of ancillary testing). Thus, we did not design our study prospectively to incorporate ancillary testing into our hypothesis, and therefore firm conclusions on this question are not possible.

Nevertheless, since this was a point raised by several reviewers (further discussion below), and we agree it is an important one that many readers will ponder, we added a data table (6) summarizing the results of ancillary testing within our cohort, and added/modified some discussion within the text to highlight some of the limitations of ancillary testing within the context of the clinical situation under study:
Lines 298-300; 328-330; 333-35; 341-43; 401-08

Comment 3:
"*What are the potential benefits and limitations of incorporating bronchial brushings into the study*?"

Reply:
We discussed in our 'limitations' section (lines 409-411) regarding reasons why we did not include bronchial brushings in our study. We do not find them of value in this setting and consider their use superfluous.

Comment 4:
"*How can emerging advanced bronchoscopic modalities, such as robotics, augmented real-time guidance, and transbronchial cryobiopsy, be investigated in the context of atypical respiratory infections?"*

Reply:
These are all excellent questions. We hope our study will fuel future investigation utilizing these even more precise modalities. We mentioned this in our conclusion paragraph, lines 419-422.

Reviewer B

Comment 1:
"*To date, the role of advanced diagnostic bronchoscopy for diagnosing atypical respiratory infections has not been well investigated, thus this large study would provide some useful information to readers.*"

Reply:
We appreciate Reviewer B's positive feedback.

Comment 2:
"*Please describe the study period (start date and end date) in the Method section*"

Reply:
This was added in the Methods section, line 170. It also listed in the flow diagram (Figure 1).

Reviewer C

Comment 1:
"*The study is well written and sites the appropriate references in the field. It is thought provoking for changing the approach to patients with chest imaging and/or clinical characteristics concerning for an infectious process while also acknowledging the limitations of a single center retrospective analysis. Well done*"

Reply:
We appreciate Reviewer C's positive feedback.

Reviewer D

Comment 1:
"*Thank you for this interesting manuscript on a rarely discussed subject*."

Reply:
We appreciate Reviewer D's positive feedback.

Comment 2:
"*I can definitely understand that guided sampling will most likely result in better samples, but in my opinion, this is not shown in your study. Can you perhaps elaborate more clearly on what the benefit of rEBUS and/or EMN have been*."

Reply:
We agree with Reviewer D that this was not the focus of the study—namely that our hypothesis was not that the advanced guidance modalities improved the yield over traditional 'non-guided' bronchoscopy, but that *multimodal sampling <u>during</u>* ADB was the advantage (over single techniques and/or testing methods). This is actually the title of the paper. While elaborating on the benefit of rEBUS or EMN is always a good discussion, it is outside of the scope of this paper and should be assumed by the reader as it is widely accepted that ADB is superior over traditional methods for diagnosing thoracic disease (with data most robust for lung and other thoracic malignancy). We briefly mention in our introduction the central role ADB plays in modern bronchoscopic practice (lines 151-54), while also noting current gaps in evidence (lines 157-59). The novelty of our study is that assessment of atypical infection has not been comprehensively performed in this context.

Comment 3:
"Regarding the different advanced bronchoscopy techniques. In table 1 in the section on bronchoscopy procedures, no differentiation is made between rEBUS and EMN despite there being a significant difference in diagnostic yield, especially if fluorosocopy is not employed (which I can not gather from the text). I would be interested to now if there is a difference in yield between these two modalities."

Reply:
We thank Reviewer D for this astute observation. We respectfully disagree that there is a "significant difference in diagnostic yield" between the two modalities EMN and rEBUS. Among the current literature, both modalities provide yields and diagnostic sensitivities in the ~70-75% range, depending on setting and biased selection of target lesions (please see citations 1-4 below). Furthermore, the bronchoscopists in this study were well-trained and versed with both modalities, and would preferentially choose the technique (rEBUS, EMN or otherwise) best suited to optimize diagnosis for a given clinical context. Therefore, our assumption was that the two peripheral guidance modalities were effectively 'equivalent' for this analysis and we did not aim to further delineate.

However, we did in fact initially plan to include this data, as it supports our assumptions: among our atypical infection cohort radial probe EBUS and electromagnetic navigation were used during 80 and 34 procedures, respectively, and individually each provided a specific cytohistologic diagnosis in 50% of cases. While in the interest of brevity and focus we prefer to exclude this discussion in the final draft of the manuscript, we did add this statement to the legend of Table 1. We hope this will be satisfactory.

Regarding our use of fluoroscopy, for EMN we used the Veran system, which does not require fluoroscopy. Most, but not all rEBUS cases require fluoroscopy, but this approach would be dictated by clinical context. Again, as with above, we deferred to the discretion of the attending bronchoscopist's expertise and did not analyze further, assuming best practice.

Comment 4:
"*I can understand why final malignant diagnoses were excluded as this is not the aim of the study, but were there no false positive microbiological results which turned out to be malignant diagnoses? by using this selection procedure you exclude these patients. Can you elaborate on this?"*

Reply:
We thank Reviewer D for bringing up this important point. Ultimately a patient's final clinical diagnosis explaining the focal lesion in question was dictated by either a final specific result based on a combination of invasive or non-invasive testing, or stability on imaging after 1 year if initial testing did not yield a specific diagnosis (minimizing possibility of malignancy). In other words, any 'positives' that were initially specifically diagnosed as infection by bronchoscopy, but later found to be malignant would have still been counted as an infection if it met our diagnostic criteria as described in the methods section (ie. this would have actually been a true infection, not a false positive—not an uncommon scenario in clinical practice for large/obstructing tumors, though less common for focal peripheral lesions). In fact, this did occur in one case, in which clinically significant atypical infection and a squamous cell tumor were concomitantly discovered. This subject was included in our cohort.

We have added the word "exclusive" to "malignant clinical diagnosis" in the subject selection flow algorithm (Figure 1) to better help clarify the point for the reader. We hope this will be satisfactory.

Comment 5:
"You included 403 patients, of whom 136 were diagnosed with an atypical infection. If I understand correctly, all the statistical analyses have been performed on the 136 patients. This took me a while to understand (assuming I am understanding this correctly). In the results section, only percentages are presented for the sensitivity. I would prefer that these percentages are accompanied with the associated numbers, so it is clearer on which part of the included population the calculations have been performed."

Reply:
In response to Reviewer D's and other reviewers' concerns we made a major revision to focus the outcome on specific diagnostic ***yield***, rather than ***sensitivity*** (rationale is further discussed below in response to other Reviewers' comments, and within the modified text). This means we utilized the entire cohort relevant for a particular analysis as the standard (the denominator). We highlight within the text what the relevant standard is depending on the analysis being performed (and thus the denominator 'N'). For example, overall and method-related diagnostic yields, as described in the modified table 2 (now table 3), utilizes the entire cohort (N=403) as the denominator. As another example, when comparing BAL/BW to TBNA/TBFB among those

with exclusively dual tested samples (N= 252), the denominator for atypical infection yield would be 252 (New figures 2 and 3; lines 275-285 of manuscript). These include subjects from all diagnostic groups. While this modification in analysis changes the absolute proportion results, it does not change the relative proportional synergy (the original hypothesis), and thus does not alter our general conclusions. We did our best to specify these details in the accompanying text and tables, and to include both numbers and corresponding percentages as requested. We hope this will appropriately clarify.

Comment 6:
"Lastly, I would prefer that the impact techniques such as PCR and antigen testing can have on this multimodality approach are discussed more extensively. These tests are standard practice for NTM/opportunistic fungi in my region, while cytohistology is seldomly used for diagnosis of the described pathogens. If data on this is present in your prospectively maintained dataset, this would be greatly appreciated."

Reply:
We very much appreciate Reviewer D highlighting this point, as it is also one brought up by other reviewers and one we think is very important. We prospectively elected to exclude ancillary methods as part of our primary hypothesis testing, for a few reasons:

1- Most importantly, culture and cytohistology testing remain the cornerstone of bronchoscopy practice and provide the most specific and definitive diagnosis when obtained. They also allow for other important applications, such as gold-standard antimicrobial sensitivity testing and demonstration of tissue invasion, the latter of which is not possible with ancillary testing. Consequently, culture and cytohistology testing remain the most widely and consistently employed testing methods with bronchoscopy.
2- The performance of available ancillary tests remains inconsistent, as is mentioned and referenced in our manuscript.
3- The availability and access to ancillary tests varies considerably across regions and models of practice.
4- Not all atypical infections have reliable ancillary tests available for diagnosis (e.g. nocardia, mucormycosis).
5- In real-world practice, when evaluating an undiagnosed focal thoracic lesion, prospectively deciding on when to incorporate ancillary testing remains judgment-based (ie. most patients will receive some combination of cytohistolgy and culture testing, while ancillary testing is selective).

Given the above, we thought the generalizability, and hence value, of our analysis was maximized by focusing on culture and cytohistology. Furthermore, a majority of our subjects did not have a full complement of ancillary testing (as is often real-world practice), and so reaching and discussing specific conclusions about their impact would have been inappropriate. Readers are welcome to (and should) limit our conclusions to the data presented.

Nevertheless, as this is an important and evolving area in bronchoscopy practice, and a point raised by several reviewers that many readers will likely ponder, we added a data table (6) summarizing the results of ancillary testing within our cohort. We also added/modified some

discussion within the manuscript to highlight some of the limitations of ancillary testing within the context of the clinical situation under study:
Lines 298-300; 328-330; 333-35; 341-43; 401-08

We hope these addenda will be satisfactory.

Reviewer E

Comment 1:
"In case of undiagnostic results especially in cocci-endemic area, clinical judgment is important. In this manuscript, 104 non-diagnostic nodules were excluded for sensitivity result, which causes serious bias in benign and malignant disease. Authors should re-evaluate these 104 non-diagnostic nodules."

Reply:
We thank Reviewer E for this important comment. If we understood correctly, there may be some confusion as to how a lesion was defined as non-diagnostic.

First, the 104 subjects were not considered 'non-diagnostic' but rather as not having achieved a *specific* final diagnosis after thorough investigation, which included testing in addition to bronchoscopy. As an example, we considered a granulomatous nodule/lesion in this category, which, by our definition, was not a specific diagnosis unless accompanied by a specific clinical syndrome, such as sarcoidosis or drug induced lung disease.

Second, the lesions that did not have a specific diagnosis during initial evaluation were assessed at one year post incident bronchoscopy. If the lesion had remained stable with imaging surveillance after one year and did not accompany an explanatory clinical scenario, we classified the subject as having a 'non-specific diagnosis.' We believe this minimized the possibility of malignancy. We modified and added clarifying statements in the methods section (lines 172-75, 199-201).

We agree with Reviewer E (and the other reviewers who also bring up this important point) that classifying the diagnostic performance of ADB as a '*sensitivity'* analysis as is presented in our study may be confusing, and even potentially inaccurate. Definitively excluding all infections—particularly self-limiting disease, such as *Coccidioides* (which of course is prevalent in our area) is difficult. While unlikely, despite our relatively comprehensive diagnostics for evaluating infection in this 104-subject 'non-specific diagnosis' cohort, true dimorphic fungal infections may be missed, thus preventing the establishment of a reliable reference standard. We had acknowledged this as a potential limitation, though agree that some readers may not accept this assumption, and this would devalue the study.

Consequently, we modified the description of our analysis from diagnostic '*sensitivity'* to diagnostic '*yield*,' since yield analysis does not require a definitive knowledge of complete disease burden. Accordingly, we reanalyzed the outcomes by using the entire cohort available

for a given analysis as the relevant standard (the denominator), as per 'yield' definition. While this of course affects absolute proportion results, the relative changes are not affected (since the numerators remain the same), and thus also our general conclusions remain the same—our hypothesis was to examine the benefit of technique/test synergy and augmentation of performance over single techniques/tests—these relative proportional improvements are not altered.

Accordingly, numerical and statistical results within the text and tables were changed as applicable. Sections 'Performance of ADB testing methods' and 'Performance of ADB techniques' of the 'Results' were all modified to reflect these changes, and figures 2,3,4,5 were also similarly modified. We also added table 2 to better characterize the evaluation profile of the 104 subjects who had a final non-specific diagnosis. Table 3 (now Table 4) was expanded to include logistic regression modeling of the entire cohort. Finally, we also expanded on the discussion of this limitation in the relevant portion of the 'limitations' section (lines 393-400). Because conclusions remained similar, we did not modify much of the discussion section relevant to this point.

We thank reviewer E's important feedback and hope these changes will enhance the focus of the paper.

Reviewer F

Comment 1:
"*My major concern is the analysis only includes patients undergoing ADB with a first time non-malignant diagnosis and excludes patients that had both cytohistological testing and culture testing but had either non-diagnostic or malignant final diagnoses. This likely will cause these tests to have massively increased sensitivity. I would be interested to see the analysis repeated including patients that underwent ADB had both cytohistological testing and culture testing performed simultaneously regardless of final diagnosis and then use this for the diagnostic sensitivity. This would give a better sense of the diagnostic utility of the testing in showing it performed when there was enough clinical suspicion to send both cytohistological specimen and microbiologic testing*."

Reply:
We thank Reviewer F for this important comment, which was similarly echoed by Reviewer E.

The 104 subjects were not considered 'non-diagnostic' but rather as not having achieved a specific final diagnosis after thorough investigation which included testing aside from bronchoscopy. As an example, we considered a granulomatous nodule/lesion in this category, which, by our definition, was not specific unless accompanied by a specific clinical syndrome, such as sarcoidosis or drug induced lung disease.

We agree with Reviewer F that classifying the diagnostic performance of ADB as a '*sensitivity*' analysis as is presented in our study may be confusing, and even potentially inaccurate.

Definitively excluding all infections—particularly self-limiting disease, such as *Coccidioides* (which of course is prevalent in our area) is difficult. While unlikely, despite our relatively comprehensive diagnostics for evaluating infection in this 104-subject cohort, true dimorphic fungal infections may have been missed in a few subjects, thus preventing the establishment of a reliable reference standard. We had acknowledged this as a potential limitation, though agree that some readers may not accept this assumption.

We do humbly disagree with Reviewer F that a more accurate approach would be to perform a sensitivity analysis on the entire cohort that received culture and cytohistology testing, including those ultimately diagnosed with malignancy. By definition, diagnostic '*sensitivity'* is the proportion of patients with a disease that test positive for that disease (or a positive test divided by number with disease). During our selection process, all patients that received a bronchoscopy for a focal thoracic lesion were screened by the final clinical diagnosis (extending to at least one year) explaining that given lesion. This work-up would have included whatever testing needed to diagnose an etiology for that lesion in question. A subject with a sole final malignant diagnosis was excluded since this explained the lesion. In other words, we minimized (if not entirely excluded) the possibility of a 'true positive' infection being missed in the malignancy cohort. Hence, subjects with a final diagnosis of malignancy should not be included in the denominator of a sensitivity analysis for atypical infection. Doing so would significantly *underestimate* the true sensitivity of ADB in this context. Conversely, we did include in the analysis any subject with a lesion that was concomitantly diagnosed as cancer and atypical infection (this occurred once). We modified and added clarifying statements in the methods section (lines 172-75, 199-201).

We do agree that the 104-subject 'non-specific' cohort should be included in the analysis. Consequently, we modified the description of our analysis from diagnostic '*sensitivity'* to diagnostic '*yield*,' since yield analysis does not require a definitive knowledge of complete disease burden. In essence, this analyzes the question of "how will ADB techniques perform, individually and in combination, in yielding an atypical infection(s) in subjects with non-malignant disease?", which we consider valuable.

Accordingly, we reanalyzed the outcomes by using the entire cohort available for a given analysis as the relevant standard (the denominator), as per 'yield' definition. While this of course affects absolute proportion results, the relative changes are not affected (because the numerators do not change), and thus the general conclusions also remain similar— our hypothesis was to examine the benefit of technique/test synergy and augmentation of performance over single techniques/tests—these relative proportional improvements are not altered.

Numerical and statistical results within the text and tables were changed as applicable. Sections 'Performance of ADB testing methods' and 'Performance of ADB techniques' of the 'Results' were all modified to reflect these changes, and figures 2,3,4,5 were also similarly modified. We also added table 2 to better characterize the evaluation profile of the 104 subjects who had a final non-specific diagnosis. Table 3 (now Table 4) was expanded to include logistic regression modeling of the entire cohort. Finally, we also expanded on the discussion of this limitation in

the relevant portion of the 'limitations' section (lines 393-400). Because conclusions remained similar, we did not modify much of the discussion section relevant to this point.

We thank Reviewer F for this important feedback and hope our modifications are satisfactory.

Comment 2:
"*In the flow diagram, it looks like patients who required repeat ADB were excluded from analysis - it's unclear to me why these patients are excluded. Should these not be included in the analysis as non-diagnostic*?"

Reply:
We thank Reviewer F for pointing out this oversight of unclear terminology to the flow diagram. These 9 subjects were indeed included in the analysis (as not specifically diagnostic) and the flow diagram as shown was inadvertently (and incorrectly) meant to highlight that *repeat* bronchoscopies were not included as original studies in the analysis. The diagram was modified accordingly.

Comment 3:
"There is a typo in the highlight box (an should be and)"

Reply:
We thank Reviewer F for catching this. The typo was corrected.

Comment 4:
"Can you include the data extraction form to the supplement?"

Reply:
Yes, we have added the data extraction form to the data sharing agreement, available upon individual request.

Comment 5:
"In the discussion, you mention that multiple investigators independently valitated the consistency of records -- I would include this in the methods and provide additional detail as to what this means / how this was performed"

Reply:
Two separate investigators independently performed same-subject data collection to ensure/validate that entered data were consistent. Discrepancies were adjudicated by a third investigator. Further validation was performed during statistical analysis (as is routine) to check for missing data, inconsistencies, etc. This description was moved to the method section (lines 178-180).

Comment 6:
"For the limitations, a significant limitation is that patients who had this testing performed but were diagnosed with malignancy and never had a diagnosis identified were excluded, this is a

major limitation and could potentially inflate the value of the testing, would add this to the limitations"

Reply:
In addition to our response to Comment #1 above and associated changes, we also address this in our amended discussion section (lines 393-400).

Comment 7:
"In table 1, how did you define immunosuppressed status? How do you define minor bronchoscopy complication?"

Reply:
We added explanations to the legends of Table 1 and (the new) Figure 2.

Comment 8:
"In Table 3, how was nodule / mass determined versus consolidation / infiltrate? By extractor? Radiology interpretation?"

Reply:
These were interpreted and recorded by investigators per predefined definitions, commonly used in radiology literature/textbooks. Obviously, some subjectivity is inevitable. As with data validation, any inconsistency was adjudicated by a third investigator and/or radiology report.

References:

1- Sainz Zuñiga PV, Vakil E, Molina S, Bassett RL Jr, Ost DE. Sensitivity of Radial Endobronchial Ultrasound-Guided Bronchoscopy for Lung Cancer in Patients With Peripheral Pulmonary Lesions: An Updated Meta-analysis. Chest. 2020 Apr;157(4):994-1011. doi: 10.1016/j.chest.2019.10.042. Epub 2019 Nov 15. PMID: 31738928.

2- Ali MS, Trick W, Mba BI, Mohananey D, Sethi J, Musani AI. Radial endobronchial ultrasound for the diagnosis of peripheral pulmonary lesions: A systematic review and meta-analysis. Respirology. 2017 Apr;22(3):443-453. doi: 10.1111/resp.12980. Epub 2017 Feb 8. PMID: 28177181.

3- Folch EE, Pritchett MA, Nead MA, Bowling MR, Murgu SD, Krimsky WS, Murillo BA, LeMense GP, Minnich DJ, Bansal S, Ellis BQ, Mahajan AK, Gildea TR, Bechara RI, Sztejman E, Flandes J, Rickman OB, Benzaquen S, Hogarth DK, Linden PA, Wahidi MM, Mattingley JS, Hood KL, Lin H, Wolvers JJ, Khandhar SJ; NAVIGATE Study Investigators. Electromagnetic Navigation Bronchoscopy for Peripheral Pulmonary Lesions: One-Year Results of the Prospective, Multicenter NAVIGATE Study. J Thorac Oncol. 2019 Mar;14(3):445-458. doi: 10.1016/j.jtho.2018.11.013. Epub 2018 Nov 23. PMID: 30476574.

4- Folch EE, Labarca G, Ospina-Delgado D, Kheir F, Majid A, Khandhar SJ, Mehta HJ, Jantz MA, Fernandez-Bussy S. Sensitivity and Safety of Electromagnetic Navigation

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