Peer Review File
Article information: https://dx.doi.org/10.21037/jtd-23-42

Reviewer A

Overall: Kheir and Uribe et al describe their findings on using a plasma proteomics biomarker on clinical management of lung nodules through the view of a patient in their retrospective study. They demonstrate that in patients with lung nodules with a pCA ≤ 50%, the biomarker classifier was associated with fewer invasive procedures and clinic visits without misdiagnosing benign nodules, leading to unnecessary diagnostic interventions. The manuscript is well written and the study design section is nicely presented where the authors describe data collection and processing. While the study is retrospective in nature and overall patient numbers are small to lead to clinical change at this time, the authors make a compelling argument with their data. There are some minor grammatical and spelling mistakes that the authors should carefully re-examine in their revisions. Overall, the manuscript is well-written and a pleasure to read.

Introduction: The authors clearly delineate the clinical challenge they are trying to address and introduce the previous literature results to set the stage for their analysis. The section is written well but I would suggest that authors would further dive into the background in paragraph 2 and explain the biomarkers as the current description leaves the reader (particularly who are not familiar with biomarkers) slightly confused.
-Reply 1: We thank the reviewer for the comment. We have expanded paragraph 2 explaining more about commercially available biomarker. Please see page 3, line 112-125.

Materials and Methods: Line 146 suggest changing “Not Funding” to “No funding”
-Reply 2: Changed

Results: Authors clearly describe the demographics with appropriate statistical analysis.
Line 88: “Number of clinic visits instead of visit.
-Reply 3: Changed

Lines 88-91: Can the authors clarify what they mean without affecting the patients who were diagnosed with malignant nodule?
-Reply 4: We realize it is confusing for readers and changed to (However, the number of clinic visits (2 [IQR, 1-3] vs 2 [IQR,2-3], p=0.004) as well as the procedural utilization (21.6 vs 79.1%; p<0.001) was significantly less in the integrated classifier group without misclassifying patients with malignant nodule in the integrated classifier arm with likely benign results at 1 year follow up.**)**

Questions:

Authors state that the number of clinical visits was less in the integrated classifier arm? Does this include virtual (telemedicine) visits as part of COVID-19 risk mitigation?
-Reply 5: yes, clinic visits include all visits (virtual and in-person)

I think the authors should further expand on why the knowledge and attitude of patient towards biomarker testing in indeterminate lung nodules could lead to clinically relevant changes? Would patients be more amenable for follow up? would patients refuse additional imaging?
-Reply 6: We agree with the reviewer and have elaborated further in the discussion regarding why knowledge of biomarker could lead to clinically relevant changes on page 9, line 398-403. (Furthermore, patients experience distress and inadequate communication about pulmonary nodules and their evaluation.27 Such distress can be associated with decreased adherence for clinic follow up and imaging. However, many clinicians are unaware of the degree to which some patients are affected by the finding of a pulmonary nodule. A decision aid could improve patient-clinician communication regarding lung nodule as well as available biomarker that could help with shared decision making.**)**

Reviewer B

This study retrospectively investigated the usefulness of the integrated proteomic classifier on decision-making in patients with a pretest probability of cancer less than 50% in real word setting. They found that the use of the integrated classifier using biomarkers, clinical and imaging factors could reduce invasive procedures and clinic visits. The manuscript is well written, but there are several concerns.

Major comments
1. The current title is not appropriate because this study used an integrated classifier with two plasma proteomic biomarkers, clinical and imaging factors. The title should be changed to “Impact of an integrated classifier using biomarkers, clinical and imaging factors on clinical decisions making for lung nodules.”
-Reply 1: The integrated classifier consists of combination of protein plasma biomarker, clinical and imaging factors to yield a posttest probability of a lung nodule being benign. We will change the name as suggested by reviewer.

2. The details of the integrated classifier and the weight of each item should be described in a table.
-Reply 2: The integrated classifier is an approved commercially available test which integrated five risk factors (age [in years], smoking status [never, current, or former], nodule diameter [largest diameter], edge characteristics [smooth, spiculated, or lobulated], and location]) with two plasma proteins, LG3BP and C163A to yield a posttest probability of a lung nodule being benign. We have

elaborated more on that in the introduction on page 3,line 112-124 (Several panels of proteins (13-protein blood test) have been proposed to differentiate benign from malignant lung nodules using multiple reaction monitoring mass spectrometry with a 90% negative predictive value (NPV) for benign nodules.[9] Another study evaluated a 5-marker subset of the original 13 proteins together with 6 normalization markers showing clinical utility based on the test's NPV potentially sparing invasive procedures for 31.8% of subjects.[10] Recently, it was shown that the accuracy of two plasma proteins (Galectin-3 Binding Protein (LG3BP) and Scavenger Receptor Cysteine-rich type 1 protein M130 (C163A)) which are independently linked to lung cancer and the inflammatory response to cancer could be optimized for evaluating lower risk nodules by integrating them with five clinical risk factors (nodule location, size, spiculation and patient's age and smoking history) in the intended use population with probability of cancer (pCA) ≤50%.[9-11] Using decision tree analyses, this integrated model, termed Nodify XL2™ test (Biodesix) has been commercially available to provide a post-test probability of a lung nodule being benign.**). The commercially available test will give results as likely benign, indeterminate, or reduced risk along with NPV as explained in the methods section. Regarding the relative contributions of the individual clinical factors and the plasma protein, it has already been published in the supplementary material in the clinical utility study PANOPTIC Trial and is beyond the scope of this study. We elaborated more on this in the introduction section as well on page 3, line 124-125 (**The relative contribution of the component elements of the integrated classifier have already been published previously in the PANOPTIC trial.[12]**).**

3. The integrated classifier was used on 102 patients and compared to 129 patients in standard clinical care. The frequency of malignancy was 8.8% (9/102) in the integrated classifier group compared to 24.0% (31/129) in the standard clinical care, an unnatural and significant difference, suggesting the possible misdiagnosis of malignancy in the integrated classifier group. If so, the conclusion of "the use of the integrated classifier was not associated with misclassifying benign lung nodules" appeared to be an overestimate. This should be discussed.

-Reply 3: We agree with the reviewer that conclusion might be an overstatement. Only patients in the integrated classifier arm who had likely benign results continued to have benign diagnosis at 1 year follow up. There is a possibility that the remaining patients in the integrated classifier arm with indeterminate results or reduced risk results could eventually be diagnosed with cancer after the 1 year follow up. We have modified the conclusion to reflect one year follow up and stressing the fact that it is mainly for patients with benign results in the integrated classifier arm (In patients with lung nodules with a pCA ≤ 50%, use of the integrated classifier was associated with fewer invasive procedures and clinic visits without misclassifying patients with likely benign lung nodules results at 1-year follow-up.**). We have also addressed this in limitation in the discussion section on page 10, line 434-437 (**Third, due to retrospective nature of the study with 1-year follow up, there is a possibility that patients in the integrated classifier arm with reduced or indeterminate risk might eventually had

malignant nodule diagnosis and thus it is essential to continue chest imaging surveillance for such patients.**).**

4. There are significant differences in age and smoking history between the integrated classifier group and standard clinical care (table 1). These differences may affect the probability of cancer. In fact, the integrated classifier used in this study included age and smoking status. This should be discussed.
-Reply 4: We agree with the reviewer about such limitation which was due to retrospective nature of the study. We have acknowledged this in limitation in the discussion section on page 10, line 437-438 (Finally, advanced age and smoking history were more common in the non-integrated classifier arm which might have impacted our results.**).**

Minor concern
1. There was a reduction in outpatient clinic visits in the integrated classifier group compared to the standard clinical care group. What is the impact of the number of follow-up chest CT evaluations?
Reply 5: There was fewer follow up chest imaging without being statistically significant in this study. These are in the result section.

2. How much is the cost per patient for plasma proteomic analysis?
-Reply 6: The commercially available test is fully covered by insurance.

Reviewer C

This study builds on a longstanding work by the authors intended to develop a biomarker-based assessment of pulmonary nodules. Tee study evaluated the impact of the authors' proteomic classifier on management decisions in patients with a pretest probability of cancer (pCA) ≤ 50% in “real-world” clinical setting.
Their finding is that the integrated classifier was associated with fewer invasive procedures and clinic visits without misclassifying benign lung nodules.
In principle, the findings are of interest whether the test is likely to be adopted remains to be determined.
Some discussion regarding other biomarker-based assessment of indeterminate nodules, their potential additive value and additional evidence regarding cost effectiveness would make the paper more interesting to the readers.
-Reply1: We agree with the reviewer and added the following in the Discussion section on page 8,line 345-347 (A biomarker to rule in or out lung cancer among patients with indeterminate pulmonary nodules would have enormous clinical benefit in reducing the rate of unnecessary thoracic surgery on benign nodules, invasive procedures, the time to diagnosis and cost.**), on page 9,line 382-387 (** A recent study assessed the diagnostic costs leading up to a lung cancer diagnosis in patients with abnormal chest imaging. A total of 19% underwent a biopsy and 43% were not diagnosed with lung cancer during follow-up. Among patients with eventually benign lung nodule diagnosis, the median diagnostic cost per patient for those with versus without biopsy was around 28 times higher. Adverse events significantly increased the average cost per biopsy up

to 4-fold.[26]**) and on page 9, line 389-394 (**Another commercially available test that is EarlyCDT-Lung. It consists of seven autoantibody panel that underwent multiple clinical validity studies and in a recent post marketing audit of over 1,600 patients presenting with a nodule of approximately 8–30 mm, showed a sensitivity of 41% at a specificity of 87%.[27] Also, a cost effectiveness study indicated that the use of such test is around $24,000 per quality-of-life adjusted life year gained.[28]**).**