Reviewer's report

Title: Pleural Fluid Cell-free DNA Integrity Index to Identify Cytologically Negative Malignant Pleural Effusions Including Mesotheliomas

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Reviewer: Stefan Holdenrieder

Reviewer's report:

The manuscript of Dr. Sriram reports on the relevance of pleural fluid DNA integrity as a marker for detection of lung malignancy including mesothelioma particularly if cytology is negative. DNA integrity in pleural fluid was found to be elevated in case of malignancy and had a 80% PPV if cytology was negative. For the detection of mesothelioma, similar or even better results were obtained for serum or pleural fluid mesothelin.

This is a very interesting study. However, several points have to be considered:

Major compulsory points for revision:
- Although the pleural fluid samples and serum samples were reported to be matched, there is a considerably lower number of serum samples investigated in the study. This limits the comparison of the findings in pleural and serum markers. For a fair comparison, only those results with markers measurements in both materials should be included.

- The low number of patients in the reference group allows only a first exploratory impression of the markers for the diagnostic purpose. ROC curves should be interpreted with caution. Cutoff calculations or optimization for best sensitivity and specificity should be done with reservation as well. Best criteria for comparison of the markers is AUC of ROC curves plus sensitivity at a defined specificity (e.g. 95th percentile) for all markers.

- It should be clearly indicated how the DNA integrity index is calculated. If it is the ratio of the long to the short fragments, and the short fragments are a part of the long ones (as indicated in the Umetani work), then ratios higher than 1 are unlikely. However, this seems not to be a single event as the medians of several groups are higher than 1. This should be explained and discussed.

- As there are several formula for calculation of DNA integrity index it would be interesting whether there would be different results using the formula of Wang 2003?

- At the moment the DNA integrity assay is only a research tool. It has to be questioned how well it will be suited to be used in clinical routine.

Minor points:
- The table of patients characteristics could be more informative with age, gender etc
- The quality of the figures is poor in the submitted version. Therefore the figures cannot be reviewed.
- Supplemental data could be included in parts in the original paper.

In total a very stimulating study. However, the requested points should be addressed to make the report more informative.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests