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Review Comments

Reviewer A
very comprehensive review. Just only remark.
In the introduction, medical thoracoscopy should be mentioned. Is't the most largely diagnostic method used with a sensitivity of 95% of malignant pleural effusions.

Reply: The authors thank reviewer A for taking the time to read the manuscript and offer this suggestion.

Changes in the text: The following text has been added to the introduction (page 4, lines 85-86): "Mesothelioma is often diagnosed by medical thoracoscopy which has a high diagnostic yield."

Reviewer B
The paper is well-written and logically organized. References are consistent as the methods stated. Would recommend discussing the potential role of artificial intelligence applied to digital images in order to improve the diagnostic setting also by a cytological point of view as cytology is often the specimen we've available (quote PMID: 34677909, PMID: 32020667)

Reply: The authors thank reviewer B for taking the time to read the manuscript and offer this suggestion.

Changes in the text: The following text has been added (pages 13-14, lines 299-315):
While beyond the scope of this review, it is also important to briefly mention the emerging diagnostic revolution underway brought about by digital pathology and artificial intelligence. These emerging technologies have shown various abilities ranging from basic histologic classification of tumors to prediction of underlying genetic alterations in malignancies.(77-80) One important application of these technologies is in cytopathology. Cytology specimens may represent the only specimen for several patients with malignant pleural effusions. While the application of digital pathology and whole slide imaging (WSI) lags behind what has been done in surgical pathology, WSI can be employed by cytologists for accurate diagnosis and classification of cytologic preparations.(81) Mobile devices may also be of utility in examination of cytology specimens which may improve diagnosis in underserved areas.(82) Some work using artificial intelligence has been done specifically in mesothelioma using WSI, and one of these studies demonstrated that uses of a deep-learning classification of mesothelioma resulted in improved prediction of patient outcome.(24,83) Another recent study highlighted the power of deep learning to identify the transitional pattern in mesothelioma.(25) These are exciting developments, yet the technology and its application to mesothelioma diagnosis and classification is still largely in its nascent form. Additional exciting developments are sure to arise in the coming years.

Reviewer C

The authors summarized here the key diagnostic phases in pleural mesothelioma. The content is clearly fine, but overall the review here overlaps previous ones, even from the same authors.
I would suggest the authors insert some practical comments from their huge experience when facing challenging cases, such as sarcomatoid, unusual features, limitations, and pro of cytology with cell block preparation (particularly in fragile patients requiring a firm diagnosis for medico-legal disputations rather than for therapeutic management).
Since this sort of review is very useful for less expert colleagues when they are called to make some presentations, it could be very useful to organize updated tables summarizing the best mesothelial positive and non-mesothelial negative immunostains or techniques to discriminate benign versus malignant mesothelial proliferations in light of available tissue (cytology/cell block versus histology)

Response: The authors thank Reviewer C for these constructive comments. While we admit there is overlap with other reviews, incorporating all these points into this review article would shift the focus of the paper to a diagnostic review and the aim of the review is to highlight how diagnosis, morphology, and classification may impact therapy and clinical decision making. The authors are not practicing cytologists and only feel comfortable providing basic statements pertaining to the cytologic work-up of mesotheliomas. The authors also feel that there should be no differences in the diagnostic criteria applied to surgical or cytology cases regardless of the scenario (i.e. clinical diagnosis versus medico-legal disputes). In an attempt to provide some key diagnostic practical points and to avoid repeating too much and overlapping with other papers currently under review in other journals, the authors provide the following statements (pages 16-18, Lines 357-411 and Page 21 Table 2, Page 22 Table 3):

Key Practical Points on Mesothelioma Morphologic Considerations

The above sections highlight the importance of accurate morphologic classification of mesothelioma. Unfortunately, achieving an accurate diagnosis and classification is often challenging. In this section, the authors herein offer some practical points to consider when rendering a diagnosis of mesothelioma.

Proving Mesothelial Lineage:

The authors strongly advocate for continuing the practice of an IHC panel approach to establishing mesothelioma lineage (Table 2). Pathologists should be aware of the lack of specificity of the so called “mesothelioma markers.” Many of the markers of mesothelioma lineage stain carcinomas. Pathologists also need to be cognizant of the fact that mesotheliomas may occasionally show reactivity for classic pan-epithelial markers like Ber-EP4 and MOC31. In their everyday practice, the authors frequently rely on claudin 4 as the most specific epithelial marker in excluding carcinoma. Pathologists should move to adopt this marker in their own laboratories or order the IHC using an external reference lab when working on a case in which the differential is carcinoma versus mesothelioma.

Benign Versus Malignant Mesothelial Proliferations:

Separating benign from malignant mesothelial proliferations remains a very challenging task. This is particularly true when dealing with fibrous pleuritis versus mesothelioma. A general rule of thumb is that fibrous pleuritis and other reactive mesothelial proliferations show orderly and predictable arrangement of mesothelial cells. Mesotheliomas often show a full thickness

proliferation of mesothelial cells that is often architecturally complex or haphazardly arranged. The morphologic differences between benign and malignant mesothelial proliferation have been well described elsewhere. The authors feel that a more recent challenge has not been the separation a fibrous pleuritis from mesothelioma, but rather the separation of benign hyperplasia and other tumors and proliferations (well-differentiated papillary mesothelial tumor, etc.) from mesothelioma. In this case, the authors strongly advocate for the use of the markers of malignancy (BAP1, MTAP, *CDKN2A* FISH, Table 3). While these markers are not in widespread use in every laboratory, at least within North America, they are easily orderable through large reference laboratories or academic medical centers. One final note on the morphologic classification of mesothelioma is that the pathologist should not forget about the diagnostic power harbored within a simple cytokeratin IHC. Pankeratins, CK5/6, and CK7 highlight many mesotheliomas. While this is a well-known fact, careful review of cytokeratin staining may reveal very useful information. Cytokeratin may show subtle invasion of individual tumor cells into underlying tissue, proving a mesothelial proliferation is malignant. Cytokeratin may also highlight architectural patterns in a way that may not be obvious on H&E-stained sections. Cytokeratins may show epithelial islands in an otherwise sarcomatoid mesothelioma, allowing for reclassification as biphasic mesothelioma. Also, cytokeratin may highlight subtle spindling of tumor cells in an epithelioid mesothelioma, cluing in the pathologist of the possible presence of transitional or sarcomatoid morphologies.

Diagnosis of Mesothelioma in Cytology:

Recently it has become possible to establish malignancy in mesothelial proliferations on review of serous effusions. The authors have advocated in their own clinical practice, the adoption of BAP1 and MTAP IHC for use in cytology specimens, especially when an adequate cell block is made. Nuclear loss of BAP1 and cytoplasmic loss of MTAP in mesothelial cells is always indicative of a malignant mesothelial proliferation. This is true in both surgical pathology and cytopathology. The one important caveat to the cytologic diagnosis of mesothelioma, is that it is currently unknown how well the cell block is predictive of final subtype. In the limited number of cases in which the authors have helped to establish a diagnosis of mesothelioma on cell block (all of them showing epithelioid morphology), they have advocated for pleural biopsy to subtype the mesothelioma. In a number of these cases, a sarcomatoid component was found in the pleural biopsy, changing the tumor classification from epithelioid to biphasic.