Peer Review File
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Review Comments:

The case is very interesting and reasonably well written.

Some of the highlights about the case:

• Usually pulmonary amyloidosis is a post-mortem diagnosis, in this case this was diagnosed ante-mortem. Pulmonary involvement rarely dominates the clinical picture in systemic amyloidosis.

Reply: Thank you for your insightful comment. We acknowledge that pulmonary amyloidosis is typically a postmortem diagnosis and that pulmonary involvement rarely dominates the clinical picture in systemic amyloidosis. However, in this unique case, we were able to diagnose the condition antemortem through transbronchial biopsies. We believe that reporting this case is valuable as it highlights the potential for early diagnosis and intervention in cases of rare pulmonary amyloidosis, which can contribute to improved patient outcomes. We appreciate your attention to this aspect of our manuscript.
Changes to Text: We have added this discussion to our manuscript (page 7, lines 113-126).

• Congo's stain was negative, which is unusual. I am wondering if it was false-negative as amyloid P and thioflavin T were positive! Please discuss.

Reply: Thank you for your excellent comment. You rightly point out an unusual finding in our case where Congo red staining was negative, despite positive amyloid P and thioflavin T staining. This discrepancy indeed raises the possibility of a false-negative result for Congo red staining, or it may suggest the presence of amyloid-like fibrils that do not exhibit the typical Congo red birefringence under polarized light microscopy. Such a scenario might indicate the presence of amyloid-related proteins or structures that share biochemical and morphological characteristics with amyloids but do not fulfill all the criteria for classical amyloid deposition. To address this discrepancy and validate our findings, we forwarded the pathology samples to a comprehensive center for amyloidosis for confirmation. This step ensures that our diagnostic interpretation is as accurate and robust as possible, providing a valuable learning point for similar future cases.
Changes to Text: We have added this discussion to our manuscript (page 8-9, lines 148-156).

Following points need further explanations.

• Was there a concern for pulmonary HTN? Please provide information on RVSP on echo or right heart catheterization.

Reply: Thank you for your comment regarding the concern for pulmonary hypertension (PH) in our patient. Indeed, the mean pulmonary artery (PA) pressure was recorded at 32 mmHg (systolic/diastolic 48/26 mmHg with a mean of 32 mmHg), indicating the presence of pulmonary hypertension. Additionally, the pulmonary capillary wedge pressure was measured at 22 mmHg, further suggesting the involvement of post-capillary element contributing to the PH.
Given these findings, along with the biopsy results pointing towards a specific pathology, we chose to pursue an experimental treatment option aimed at addressing the underlying cause and alleviating respiratory symptoms. The patient's remarkable response to this treatment strategy highlights the importance of tailoring therapy based on a comprehensive assessment of the underlying disease.
Changes to Text: We have updated the manuscript to include this information, providing clarity on the cardiac and the pulmonary evaluation and its outcomes (page 4-5, lines 64-72).

• There are non-calcified, solid centrilobular nodules. Is there a possibility patient also had nodular pulmonary amyloidosis

Reply: We appreciate your keen observation regarding the presence of non-calcified, solid centrilobular nodules in the imaging findings. While the possibility of nodular pulmonary amyloidosis was initially considered due to the imaging characteristics, we would like to clarify that the nodules observed in our patient's imaging studies were not consistent with the typical findings associated with nodular pulmonary amyloidosis. Further evaluation, supported by pathology, suggested diffuse alveolar septal amyloidosis, which is indeed a rare antemortem pathology. However, we acknowledge the significance of maintaining a broad differential diagnosis in such cases and value your input in exploring potential differential diagnoses.
Changes to Text: We have updated the Discussion of manuscript to include this information (page 8, lines 136-145).

• If cardiac biopsy revealed amyloidosis, is a bronchoscopy needed? The symptoms could just be from cardiac amyloidosis and pulm HTN if patient had it

Reply: Thank you for your insightful comment questioning the necessity of bronchoscopy after a cardiac biopsy revealed amyloidosis. We agree that the symptoms presented by our patient could indeed be attributable to cardiac amyloidosis and pulmonary hypertension, which often share clinical manifestations. However, given the identification of newly recognized pulmonary nodules in our patient, we believed it prudent to proceed with a bronchoscopy. This approach was aimed at providing a comprehensive evaluation of the respiratory system, ensuring

that no concurrent pulmonary conditions were overlooked, and to precisely characterize the nature of these nodules. Our decision was guided by the principle of thorough investigation to rule out or confirm any additional pulmonary pathology that could influence the patient's management plan.
Changes to Text: We have updated the Discussion of manuscript to include this information (page 8, line 136-144).

• What did pathology show? [Usually - The lesions are typically hypocellular, but scant plasma cells may be present. Giant cells are not usually seen with diffuse alveolar-septal amyloidosis] Please discuss.

Reply: Thank you for your inquiry regarding the pathological findings in our case study. The histopathological examination indeed revealed lesions that were consistent with the characteristics of diffuse alveolar-septal amyloidosis. We observed the deposition of amyloid material within the alveolar septa and surrounding pulmonary tissues. While the lesions were predominantly hypocellular, there was a presence of scattered plasma cells, aligning with the expectation for this condition. Notably, giant cells, which are not typically associated with diffuse alveolar septal amyloidosis, were absent in our findings.

Further to your query, Congo red staining and AA amyloid testing yielded negative results. However, the amyloid P component and thioflavin T stain were positive, supporting the diagnosis of amyloidosis. Electron microscopy provided additional insights, revealing expanded alveolar septa and focal nodules containing amorphous material consistent with amyloid deposition. The fibrillary material, deposited randomly within the alveolar septa, formed non-branching fibrils measuring 7 to 12 nm in diameter, which is characteristic of amyloid fibrils, thereby disrupting the normal pulmonary architecture.

Complementary to these findings, bone marrow examination revealed 6% to 10% plasma cells with lambda light chain restriction, indicating a monoclonal gammopathy associated with amyloidosis. This comprehensive analysis allowed us to better understand the extent and nature of amyloid deposition in our patient and to correlate these findings with the clinical presentation.
Changes to text: We appreciate your insightful comments, which have guided a more detailed discussion of our pathological findings and their significance in the context of diffuse alveolar septal amyloidosis and have made changes to the text accordingly (page 5, lines 79-86).

• Line 100-101 mentions patient had MM disease. There is no CRAB criteria. On BM biopsy, plasma cells are less than 10%. This looks like MGUS

Reply: Thank you for your insightful comment regarding the characterization of the patient's disease status. Upon careful review of the patient's medical records and comprehensive diagnostic evaluations, we acknowledge and appreciate your observation regarding the absence of CRAB criteria and the relatively low percentage

of plasma cells observed on bone marrow biopsy, which indeed suggests a presentation more aligned with monoclonal gammopathy of undetermined significance (MGUS) rather than multiple myeloma (MM). However, it is important to note that the diagnosis and classification of the patient's condition were extensively reviewed and confirmed by our hematology/oncology department. This determination took into account not only the bone marrow biopsy results but also a comprehensive evaluation of the patient's clinical presentation, laboratory findings, and overall disease progression. The multidisciplinary approach to diagnosis in this case allowed for a thorough consideration of all potential differential diagnoses, including MGUS and MM, to ensure the most accurate and appropriate classification of the patient's condition.

Changes in text: We understand the significance of your point and have clarified this aspect within our manuscript to better communicate the basis upon which the diagnosis was established, emphasizing the collaborative, multidisciplinary diagnostic process that was undertaken (page 6, lines 100-101).

• It could just be primary amyloidosis (AL type)

Reply: Thank you for your observation. It is essential to consider the clinical presentation and diagnostic findings to distinguish between primary amyloidosis (AL type) and diffuse alveolar amyloidosis in our patient's case. Upon thorough consideration of the patient's clinical presentation and diagnostic findings, we believe that the diagnosis of diffuse alveolar amyloidosis is more appropriate. The patient predominantly presented with respiratory symptoms and localized pulmonary findings, without systemic symptoms or multi-organ involvement typically associated with primary amyloidosis (AL type). Additionally, imaging studies, including the CT scan, revealed diffuse reticular or nodular opacities predominantly affecting the lung parenchyma, consistent with diffuse lung involvement. Importantly, histological examination confirmed the presence of amyloid deposits within the alveolar septa and surrounding tissues, supporting the diagnosis of diffuse alveolar amyloidosis. Therefore, based on the clinical presentation and diagnostic findings, we maintain that the diagnosis of diffuse alveolar amyloidosis is accurate in this case.

Changes to Text: We have updated the Discussion of the manuscript to include this information (page 8, lines 157-167).

• Treatment is usually CyBorD. Why was cyclophosphamide not started?

Reply: Thank you for your inquiry regarding the choice of treatment regimen in our patient's case. Cyclophosphamide was not initiated as part of the treatment protocol due to our decision to commence therapy with bortezomib, a proteasome inhibitor that has been investigated in clinical trials for the treatment of amyloidosis, particularly in light chain (AL) amyloidosis. Clinical trials exploring bortezomib-based regimens often in combination with other agents such as dexamethasone or alkylating agents, have assessed outcomes such as hematologic response, organ response, progression-

free survival, and overall survival, and reported favorable outcomes in patients. Additionally, given the atypical presentation and respiratory symptoms confirmed by transbronchial biopsy, as well as the findings from the bone marrow examination, we opted to initiate this treatment regimen after consultation with the hematology/oncology specialists. This decision was made based on the potential benefits of bortezomib therapy in amyloidosis, supported by existing clinical evidence and in collaboration with the multidisciplinary care team. As notes, the patient responded remarkably well to this treatment.
Changes to Text: We have updated the Discussion of the manuscript to include this information (page 10, lines 176-192).

• Why was she being evaluated for lung transplant? Her symptoms could just have been from cardiac amyloidosis

Reply: Thank you for your valuable input, which has contributed to the refinement of our manuscript. Our patient presented to us with significant respiratory symptoms, including dyspnea and cough, which prompted further investigation into the possibility of pulmonary involvement. These symptoms were not solely attributable to cardiac amyloidosis, as they were primarily respiratory in nature. Furthermore, objective assessments, including pulmonary function tests and imaging studies, revealed evidence of significant pulmonary involvement, with findings consistent with pulmonary amyloidosis. This suggested that the patient's respiratory symptoms were likely related to pulmonary amyloid deposition rather than isolated cardiac involvement.

While medical treatment may have initially improved the patient's symptoms, the progressive nature of pulmonary amyloidosis raises concerns about long-term management and the potential for disease progression despite medical therapy. Lung transplantation was considered as a potential option for definitive treatment and long-term management, particularly if the patient's symptoms were to worsen over time. Hence the decision to evaluate for lung transplant was made in collaboration with the patient's healthcare team, with careful consideration of the potential benefits and risks associated with this intervention.
Changes to Text: We have updated the Discussion of the manuscript to include this information (page 10, lines 187-192).

• Line 101: Page 3. lamba is incorrectly spelled

Reply: Thank you for bringing this to our attention. The spelling error has been corrected.
Changes to the text: Page 6 (line 100)