Reviewer's report

Title: A case of atypical anti-GBM disease complicated by CMV pneumonitis and massive hemoptysis

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Reviewer: Dorin Bogdan Borza

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This manuscript describes a case of atypical anti-GBM disease featuring crescentic glomerulonephritis and hemoptysis, with linear IgG (and IgA) staining in the kidney biopsy but negative serology for anti-GBM IgG Abs (as tested using a Bioplex 2200 assay). Hemoptysis improved with immunosuppressive therapy, but severe pulmonary hemorrhage recurred, due to opportunistic CMV pneumonitis.

Major issues:

1) The "take-home message" in the current form of the manuscript is not clear, and the novelty does not come through. Whereas the complication of CMV pneumonitis features prominently in the title and abstract, this is not novel given that two case reports of relapsing anti-GBM disease associated with CMV infection have been previously published (as correctly cited by the authors). Nonetheless, an argument can be made that the most intriguing and novel aspect of this case is the relatively severe kidney and lung phenotype associated with atypical anti-GBM antibodies (which is noted but not emphasized enough). In particular, a severe phenotype is unexpected in light of the predominance of non-inflammatory IgG4 subclass (along with some IgG2 and IgA) in the renal biopsy. This manuscript should be revised to emphasize this point, drawing a contrast with previous reports of atypical anti-GBM disease with predominant IgG4 autoAbs and relatively mild kidney disease (2 of the 4 cases described in ref 16, and also Olaru et al, J. Immunol 2013, vol.190, p.1424). In several of these patients, atypical anti-GBM IgG4 autoAbs preferentially reacted with native NC1 hexamers of a345(IV) collagen and, therefore, may go undetected in anti-GBM Ab immunoassays that use recombinant a3(IV) collagen NC1 monomers as antigen.

2) The intensity of IF staining in Fig 2 does not match the description in the text. Panels B and C (kappa and lambda) appear similar. Also, the text states strong IgG and weak IgA. However, IgA staining in Fig 2/panel D does not appear weaker than IgG in Fig 2/panel A. This implies that anti-GBM IgA may also contribute to pathology in addition to or instead of anti-GBM IgG4 (IgG2), which should be considered and discussed (many cases of atypical anti-GBM disease are IgA-mediated).
3) The authors state on line 171 that "patient may only have had anti-GBM IgG4 which would have been undetectable by our assay (Bio-Rad BioPlex 2200)" and below "the method used for anti-GBM serology described herein detects IgG1, IgG2, and IgG3, but not IgG4 subclasses" (line 174), without providing convincing evidence this is so. These statements should be removed or clearly marked as speculation, unless (preferably) supported by experimental evidence. See for example how Ohlsson et al (ref 16) showed experimentally that their in-house assay for anti-GBM Abs did not detect human IgG4 (beware one cannot extrapolate this finding to different anti-GBM assays). Similarly, while the authors correctly note that IgA anti-GBM is likely to yield false negative results, the statement that the anti-GBM assays are "most specific for IgG1 and IgG3" (line 161) is likely inaccurate about specific IgG subclasses (please revise or cite actual experimental studies to support this statement).

Minor points:

The authors should show the staining for all four IgG subclasses in Figure 3 (to document that IgG1 and IgG3 are negative). Also, in Figure 2, consider showing the negative staining for C3 (since C3 staining is usually positive in typical anti-GBM, the absence of C3 serves to exclude one possible effector mechanisms of atypical anti-GBM in this case), and for IgM (as it rules out glomerular deposition of IgM lambda monoclonal).

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

Yes

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**
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Yes

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