Author's response to reviews

Title: Anti-hLAMP2-antibodies and dual positivity for anti-GBM and MPO-ANCA in a patient with relapsing pulmonary-renal syndrome.

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Author's response to reviews: see over
Dear Mrs Chap

Thank you for the constructive comments on our manuscript “Anti-hLAMP2-antibodies and dual positivity for anti-GBM and MPO-ANCA in a patient with relapsing pulmonary-renal syndrome”. Following the reviewers’ comments we added a considerable amount of new data and rewrote the manuscript. The suggestions allowed us to improve the quality of the paper and the new data fortunately support our hypothesis.

Please find enclosed the new version of the manuscript with changes from the original highlighted in red font and our replies to the comments of the reviewers.

We hope that the extensively revised paper is now acceptable for publication in BMC Nephrology.

Thank you for your time and consideration.

Sincerely yours,

Christoph Etter and Clemens D. Cohen for all co-authors

Reviewer 1: Thomas Hellmark

Major Compulsory Revisions

1. As the study is described only one sample is analyzed for the presence of hLAMP2 antibodies. I would like the authors to provide data for the other samples as well. As long as this data is not provided it is not possible to speculate in any additional value of the anti-hLAMP2 ab. Thus, I would like anti-hLAMP2 ab included in Table 1.

Thank you for this suggestion. We collected additional archival serum samples of the patient from 2007 until 2009 and tested them for hLAMP-2 antibodies. Unfortunately, no serum was available for the initial presentation but the subsequent anti-hLAMP2-antibody levels were highest at time of relapse of disease. The data have now been included in Table 1 and Figure 2.

2. The authors conclude that the patient probably don’t have anti-GBM nephritis but instead SVV. I agree, but from this conclusion to say that the anti-hLAMP2 antibodies “strongly support this hypothesis” is not logical. There is no evidence in the manuscript that substantiates this notion and should be rephrased both in the abstract and the conclusions.

We are happy to read that you agree on the SVV-like presentation. Our statement might have been too strong. We deleted it and rephrased several parts of the manuscript. We also state now clearly that no firm conclusion can be made from a single case-report.

Minor Essential Revisions

3. On page 4, first paragraph, 4th row: It is unclear how many biopsies that were taken and when. On row 3 there is a first biopsy taken and on row 4 there is a second but both are described as if they where taken at the same time.
Two kidney biopsies were performed: a first biopsy at initial presentation (month 0) and a second at relapse of disease (month 23). We give this now more clearly in the case presentation, table 1 and figure legends and hope that the reader can follow the presentation in the current version of the manuscript more easily.

Discretionary Revisions

4. How was the anti-GBM antibodies measured? It is written that anti-alpha3(IV) antibodies where measured and found positive but in fact most commercially available kits contains all alpha-chains. Could this perhaps be the reason for finding anti-GBM antibodies that obviously do not bind to the GBM? Can the patient have antibodies against the alpha1(IV) chain and not the alpha3(IV) chain as reported elsewhere?

The reviewer is right and the initial statement was too strong. We made use of the the Anti-GBM ELISA from Euro Diagnostica. This kit contains purified NC1 domain of the type IV collagen containing mainly alpha 3 chains. However, other alpha chains are also present but to a much lesser extent. As we cannot exclude the existence of antibodies against other type IV collagens we changed the sentence accordingly.

Reviewer: Alenka Vizjak

This is a well written case report documenting a patient with relapsing pulmonary-renal syndrome and anti-GBM antibodies, MPO-ANCA and anti-hLAMP2 antibodies. It is very unusual that no linear IgG deposits along glomerular basement membrane were found despite convincingly positive anti-GBM antibodies in serum.

Thank you for your supportive comment.

Major Compulsory Revisions

1. The statement that so far no reports on double-positive patient with a relapsing course of the disease have been published, as mentioned by the authors in Conclusion and some other places in the manuscript, does not hold. Some studies have been published reporting clinical relapses and reappearance of ANCA positivity in patients with coexistent anti-GBM antibodies and ANCA, similarly to typical relapsing course of ANCA vasculitis (Markowitz et al., Am J Kid Dis 2004; Lionaki et al., Semin Immunopathol 2007; Lind# et al., Ther Apher Dial 2009)

The reviewer is correct and we deleted the statement. In other parts of the manuscript we incorporated the two most recent references suggested by the reviewer.

2. It is very unusual that no linear IgG deposition in kidney biopsy was found in a patient with positive anti-GBM antibodies in serum. I would like to see a discussion and possible explanation of this unusual finding. May be the reason was not well preserved frozen kidney specimen for immunofluorescence in the first biopsy. Also subepithelial deposits observed by electron microscopy, were not seen by immunofluorescence. In the second biopsy, linear deposition could be absent due to only low value of anti-GBM antibodies in serum or could it be that scanty linear deposition was covered by fine granular IgG deposits?
We state now clearly that the initial negative immunofluorescence might have been caused by the not well-preserved frozen tissue specimen. The well preserved frozen sample from the second biopsy showed only small segmental areas of linear positivity (see also comments to reviewer 3).

3. The authors’ assumption in Conclusion: “The presence of anti-hLAMP2 antibodies in this patient’s serum might be in line with the primary event being a SVV associated with MPO-ANCA and anti-hLAMP2 antibodies with secondary development of anti-GBM antibodies.” is not understandable and needs some further explanation.

The respective paragraph was rewritten. We hope the statement is now clearer.

Minor Essential Revisions

1. Was immunofluorescence done in the lung biopsy? Particularly in absence of linear deposits in the kidney, IF results in lung should be given if available.

Routine diagnostics in the clinical center performing the bronchoscopy do not include immunofluorescence on lung biopsies. Hence, no immunofluorescence on lung tissue is available. We give now in the text that no immunofluorescence was performed on the lung biopsy.

2. I would suggest adding data on antihLAMP2 antibodies in Table 1.

We analyzed archival serum samples of the patient for presence of hLAMP-2 antibodies and included the results in Table 1 and Figure 2. Unfortunately no serum was available for the initial presentation. However, the titers correlate with disease activity with highest values at relapse of disease.

Reviewer 3: Agnes Fogo

This is an unusual kidney biopsy where the authors have performed analysis for anti-hLAMP2 antibodies, and draw conclusions that are rather sweeping and beyond what the data support. There are several major issues:

1) First, the authors state there was no linear anti-GBM staining, but in the picture submitted, there are small segmental areas where the IF positivity might possibly be linear - such a linear pattern can be segmental and may be difficult to assess underneath the granular pattern. Please examine the IF again under oil and determine this issue.

The IF picture was taken under oil and actually we did notice some segmental linear positivity, somewhat accentuated in the mesangial basement membranes. However, under the microscope this finding isn't as prominent as it appears in the photograph. Compared to a typical very intense linear staining in "usual" anti-GBM disease, it was of lower intensity. The finding was described in the original renal biopsy report but was found not to be “diagnostic” for anti-GBM disease on morphological grounds alone. But we agree that the finding may have been underestimated and we changed the interpretation accordingly.

2) Be that as it may, if indeed there really is no anti-GBM staining, then the patient does not have anti-GBM disease and the serologic positivity is just that, a positive serum test without
pathogenic effect in the kidney. The discussion point on this issue then should not be comparison with the literature with patients with antiGBM disease and positive ANCA; those patients have positive antiGBM linear staining in the tissue, and presumably disease caused at least in part by this injury mechanisms, with added injury by ANCA.

It is not known how much each antibody contributes to the clinical syndrome. As such we still reference the literature on other double-positive patients but added some additional references to show the heterogeneity of the course of disease in these patients.

3) The authors strongly suggest that anti-hLAMP2 AB specifically indicates a relapsing course. This is an overstatement. There is no comparison with patients with MPO positivity, or with any other patients with antiGBM Ab and ANCA, (other reported patients had kidney disease related to anti-GBM Ab, with linear staining).

We made several changes to the main text and believe that we have removed all statements which can be interpreted as overstatements.

4) The confounding issue of the MN is not even discussed- the coexistence of MN with anti-GBM disease (or antibody) albeit rare, is well described, with evidence supporting a pathogenic link. These incongruent elements should be integrated into the discussion, and the conclusions tempered.

The membranous nephropathy is indeed an additional interesting aspect. In the current version of the manuscript we make now clear reference to two reports describing the coexistence of CGN with MN. However, we tried to be focused on the clinical course and the serological findings.

Minor points:

In the background section, line 8, it should be "latter antibody", not "later".

Thank you. The correction has been made in the current version of the manuscript.

Please describe EM findings in more detail. Were there any mesangial deposits?

Indeed the EM findings were not described in detail in the initial version of the manuscript. We have expanded it accordingly and included the lack of mesangial deposits specifically. There were no subendothelial deposits, no deposits in the tubular basement membranes and no reticular aggregates in the cytoplasm of endothelial cells.

Please be explicit in describing the "different ages of glomerular lesions" in the text- this is detailed in the figure legend, but the main text is cryptic.

This part was also kept short in the initial submission but is described more precisely now.

In the EM picture, please reposition the arrows to point directly to and abut the deposit.

Thank you for addressing this, the arrows were indeed not placed perfectly. This was corrected.

Reviewer 4: Abraham Rutgers
The authors report on a patient with a pulmonary renal syndrome who is not only anti-GBM positive, but also ANCA positive. This is a rare but pathologically interesting observation and has been previously published. New in their data is the positivity for hLAMP2 as well. This very intriguing new autoantibody in AAV has been characterized by the same group and could help understand the development of AAV by molecular mimicry with bacterial proteins. The finding would have been even more relevant if hLAMP2 positivity could have been demonstrated also in the first presentation of the disease.

Thank you for your interest and the positive comment. We agree that the antibody titer at the time of presentation would be most interesting. We tried hard to get a sample from this time point in year 2007 but were not successful. However we have now included new ELISA, western blot and immunofluorescence data from archival serum samples of the patient. The results show that the anti-hLAMP2 antibody positivity was highest at the time of relapse.