Phospholipase A_2 Receptor Antibodies in Membranous Nephropathy: Unresolved Issues

Julia M. Hofstra and Jack F.M. Wetzels
Department of Nephrology, Radboud University Medical Centre, Nijmegen, The Netherlands

J Am Soc Nephrol 25: 1137–1139, 2014.
doi: 10.1681/ASN.2014010091

The discovery of the M-type phospholipase A_2 receptor (PLA_2R) as a major antigen in idiopathic membranous nephropathy (iMN) was a breakthrough and established iMN as an autoimmune disease.[1] Subsequent studies confirmed that antibodies against PLA_2R were present in approximately 70% of incident iMN patients (reviewed by Hofstra and Wetzels[2]). The potential role of measuring PLA_2R antibodies for clinical practice was suggested by studies showing that the presence of PLA_2R antibodies supported a diagnosis of iMN,[2–4] changes in antibody levels paralleled clinical disease activity,[5] disappearance of antibodies preceded and predicted subsequent decrease of proteinuria,[6] and high titers of antibodies were associated with a low likelihood of spontaneous remission.[7]

In this issue of *JASN*, Hoxha *et al.* report their findings in a large cohort of 163 patients with MN.[8] PLA_2R antibodies (both IgG and IgG4) were measured in serum obtained within 6 months from kidney biopsy. The authors used an ELISA assay and immunofluorescence testing (IFT) (both commercially available in Europe).[9] PLA_2R antibodies were detected in 133 patients (82%). The median follow-up was 12 months, and the majority of patients (101 of 133) started immunosuppressive therapy within 3 months after presentation.

Hoxha *et al.* show that PLA_2R antibodies decreased during follow-up. The decrease in PLA_2R antibodies preceded the decrease in proteinuria. The authors concluded that "there was a remarkable time lag between the rather rapid fall in antibody levels at 3 months and the protracted reduction in proteinuria."[8] These data confirm earlier findings and indicate that an immunologic remission precedes clinical remission in patients with iMN.[6,10] Although no PLA_2R antibodies were found in patients with complete remission, PLA_2R antibodies were still present in a low titer in 50% of patients with a partial remission. These data indicate that a partial remission may not always reflect the absence of disease activity.

Published online ahead of print. Publication date available at www.jasn.org.

Correspondence: Prof. Dr. Jack F.M. Wetzels, Department of Nephrology 464, Radboud University Medical Centre, PO Box 9101, 6500 HB Nijmegen, The Netherlands. Email: jack.wetzels@radboudumc.nl

The authors next analyzed the association between antibody levels at baseline and remission at 12 months after presentation. PLA_2R antibody levels were significantly higher in 28 patients without remission than in 39 patients with remission. The median time to remission was significantly longer in patients with antibody levels above versus below the median (15 versus 9 months). The authors concluded that the PLA_2R antibody level was "an independent risk factor for not achieving remission."[8] Such a conclusion, if valid and applicable to untreated patients, could improve individualized patient care. However, the data from the study by Hoxha *et al.* do not allow to conclude that antibody levels can help to accurately identify patients who will develop a spontaneous remission because most of Hoxha's patients were treated. Moreover, because treated patients nearly all developed a remission, the antibody levels merely predicted the time to remission.

Although their patient cohort is large, the study by Hoxha *et al.* is limited because of the relatively short follow-up period, the use of various immunosuppressive treatment regimens, and the unnecessary early start of immunosuppressive therapy in many patients. The reported findings cannot change current guidelines for diagnosis and treatment of patients with iMN. Evidently, more rigid study protocols are needed to reliably answer the most relevant questions. Certainly, Hoxha *et al.* could perform additional analyses to answer some of the following unresolved questions.

WHICH ASSAY SHOULD BE USED TO MEASURE PLA_2R ANTIBODIES?

There are three techniques for detecting PLA_2R antibodies in serum: the Western blot technique, IFT, and an ELISA assay. The Western blot was the first technique and was used in the pivotal study by Beck *et al.*[1] However, the Western blot technique is not suitable in daily practice. Although all techniques can detect PLA_2R antibodies, some differences are apparent. Dähnrich *et al.* studied 200 patients with PLA_2R-related MN (defined by positive IFT results) and found that 7 patients had negative ELISA results.[9] We also observed good agreement between an ELISA assay and IFT (94%; $\kappa=0.85$), with some discrepancies. For example, of 117 patients, 2 had negative IFT results but positive ELISA results, whereas 5 had positive IFT staining results but negative ELISA results.[7]

The results are quite different if we compare the quantitative assays. Although we observed a reasonable correlation between antibody levels measured with IFT or ELISA, within-patient variation was quite high.[7] The data of Hoxha *et al.* also suggest that the changes in PLA_2R antibody levels over time may be different depending on the assay used. Moreover, although there is a high correlation between total IgG and IgG4, subtle differences may again exist, with some patients having a negative result using a total IgG assay and a positive result using an IgG4 assay.

Prospective studies directly comparing and calibrating the available assays in a quantitative manner are warranted.

CAN MEASUREMENT OF PLA_2R ANTIBODIES BE USED TO DIAGNOSE MN?

Although no prospective study has validated PLA_2R antibodies as a diagnostic biomarker, we suggest that the time to abandon the kidney biopsy in PLA_2R-positive patients is near. PLA_2R antibodies were not found in five studies that evaluated 313 healthy controls with Western blot or IFT.[1,11–14] The ELISA assay always has some background activity, and normal values are established based on the mean+3 SD values in healthy volunteers.[9,15] As a result, few healthy controls are considered positive (*e.g.*, 1 of 291 persons in one study).[9] PLA_2R antibodies were not detected in patients with other autoimmune diseases (0 of 316),[9] or in patients with nonmembranous GN (0 of 510, although the majority of these patients were nonnephrotic).[1,9,12,14,15] Although some additional data are needed, the available evidence suggests that it may be acceptable to start with the PLA_2R antibody assay in patients with nephrotic syndrome. A kidney biopsy could be avoided if PLA_2R antibodies are present and the patient is at low risk of progression. A kidney biopsy could be performed in the case of progressive disease when immunosuppressive therapy is warranted. If a kidney biopsy is considered a high risk (*e.g.*, in a patient treated with anticoagulant drugs for a recent pulmonary embolus), the finding of PLA_2R antibodies would suffice to make a diagnosis of MN with enough certainty to also avoid the biopsy. We expect that future studies will provide further data on diagnostic accuracy, so that a kidney biopsy may no longer be needed in 2015 to diagnose MN in all PLA_2R antibody–positive patients.

CAN MEASUREMENT OF PLA_2R ANTIBODIES BE USED TO EXCLUDE SECONDARY CAUSES OF MN?

In an earlier study, Hoxha *et al.* did not observe secondary causes of MN in patients with PLA_2R antibodies.[12] The literature data are equivocal. Antibodies are scarcely found in proteinuric patients with lupus nephritis (2 of 86).[1,11,12,15,16] By contrast, small case series, allowing for significant publication bias, suggest that antibodies can be detected in approximately 20% of patients with hepatitis B virus, hepatitis C virus, sarcoidosis, malignancy, or hematologic disorders (P. Brenchley, personal communication).[11–13,15,17–20] The interesting observation of Debiec *et al.* of a patient with MN secondary to a IgG3 paraprotein deserves special mention.[21] The authors provide evidence that the paraprotein had antibody activity directed against the PLA_2R antigen. This case report, then, virtually proves the pathogenicity of PLA_2R antibodies.

For the moment, a secondary cause cannot be fully excluded in PLA_2R antibody–positive patients, although it seems reasonable to limit the search for secondary causes to patients with a high risk of underlying disease (*e.g.*, elderly patients, patients from areas with endemic hepatitis B virus).

CAN QUANTITATIVE MEASUREMENT OF PLA_2R ANTIBODY LEVELS PREDICT OUTCOME AND/OR GUIDE THE TYPE AND DURATION OF IMMUNOSUPPRESSIVE THERAPY?

Hoxha *et al.* should be credited for trying to provide some answers. As indicated, the PLA_2R antibody level was associated with the time to remission. We showed that patients with high titers of antibodies were less likely to develop a spontaneous remission (4% versus 38%).[7] Oh *et al.* observed spontaneous remission in 17% of patients with high levels versus 45% in patients with low levels.[13] These data require confirmation and large studies are needed to provide meaningful information, including valid accuracy figures. A pilot study showed that PLA_2R antibodies measured at the end of therapy predicted long-term outcome.[22] After 5 years, 12 of 18 (67%) antibody-negative patients were in persistent remission in contrast with 1 of 8 (13%) antibody-positive patients ($P<0.01$).[22] If validated, these data suggest that the treatment duration may be guided by a change in antibody levels during therapy in the future.

WHAT IS THE VALUE OF PLA_2R STAINING IN KIDNEY BIOPSIES?

Although most studies have evaluated the role of PLA_2R antibodies that are present in the serum of patients with MN, some authors have pointed to the possibility of using commercially available anti-PLA_2R antibodies for the detection of the PLA_2R antigen in a kidney biopsy using an immunostaining procedure. Debiec and Ronco were the first to note that the PLA_2R antigen could be detected *via* a kidney biopsy in patients without PLA_2R antibodies.[23] Indeed, a recent study suggested that up to 50% of patients with iMN and negative PLA_2R antibodies may be positive for the PLA_2R antigen on a kidney biopsy.[17] Immunostaining of stored kidney biopsy tissue also allows for diagnosis of PLA_2R-related MN in retrospect.[17] Immunostaining of kidney biopsies was also used in a single-center study that compared patients with iMN and secondary MN. In this study, PLA_2R expression in kidney biopsies was observed in 64 of 85 patients with iMN and in 14 of 80 patients with secondary MN.[24] The sensitivity and specificity for detecting iMN were 75% and 83%, respectively. Certainly, these values are too low for clinical care. Of note, this study showed that IgG4 was the predominant subclass in PLA_2R-positive patients with secondary MN. These observations confirm the findings of Qin *et al.*[11] and suggest that there may be coexistence of two diseases in such cases.

In the coming years, the role of PLA_2R antibodies as a biomarker for diagnosis, prognosis, and treatment guidance will be validated. We envisage that the availability of a calibrated assay will profoundly change nephrology practice in patients with MN.

ACKNOWLEDGMENTS

J.M.H. is supported by a grant from the Dutch Kidney Foundation (KJPB 11.021).

DISCLOSURES

None.

REFERENCES

1. Beck LH Jr, Bonegio RG, Lambeau G, Beck DM, Powell DW, Cummins TD, Klein JB, Salant DJ: M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy. *N Engl J Med* 361: 11–21, 2009
2. Hofstra JM, Wetzels JF: Anti-PLA_2R antibodies in membranous nephropathy: Ready for routine clinical practice? *Neth J Med* 70: 109–113, 2012
3. Debiec H, Ronco P: Nephrotic syndrome: A new specific test for idiopathic membranous nephropathy. *Nat Rev Nephrol* 7: 496–498, 2011
4. Hoxha E, Kneißler U, Stege G, Zahner G, Thiele I, Panzer U, Harendza S, Helmchen UM, Stahl RA: Enhanced expression of the M-type phospholipase A2 receptor in glomeruli correlates with serum receptor antibodies in primary membranous nephropathy. *Kidney Int* 82: 797–804, 2012
5. Hofstra JM, Beck LH Jr, Beck DM, Wetzels JF, Salant DJ: Anti-phospholipase A_2 receptor antibodies correlate with clinical status in idiopathic membranous nephropathy. *Clin J Am Soc Nephrol* 6: 1286–1291, 2011
6. Beck LH Jr, Fervenza FC, Beck DM, Bonegio RG, Malik FA, Erickson SB, Cosio FG, Cattran DC, Salant DJ: Rituximab-induced depletion of anti-PLA2R autoantibodies predicts response in membranous nephropathy. *J Am Soc Nephrol* 22: 1543–1550, 2011
7. Hofstra JM, Debiec H, Short CD, Pellé T, Kleta R, Mathieson PW, Ronco P, Brenchley PE, Wetzels JF: Antiphospholipase A2 receptor antibody titer and subclass in idiopathic membranous nephropathy. *J Am Soc Nephrol* 23: 1735–1743, 2012
8. Hoxha E, Thiele I, Zahner G, Panzer U, Harendza S, Stahl RAK: Phospholipase A2 receptor autoantibodies and clinical outcome in patients with primary membranous nephropathy. *J Am Soc Nephrol* 25: 1357–1366, 2014
9. Dähnrich C, Komorowski L, Probst C, Seitz-Polski B, Esnault V, Wetzels JF, Hofstra JM, Hoxha E, Stahl RA, Lambeau G, Stöcker W, Schlumberger W: Development of a standardized ELISA for the determination of autoantibodies against human M-type phospholipase A2 receptor in primary membranous nephropathy. *Clin Chim Acta* 421: 213–218, 2013
10. Beck LH Jr, Salant DJ: Membranous nephropathy: Recent travels and new roads ahead. *Kidney Int* 77: 765–770, 2010
11. Qin W, Beck LH Jr, Zeng C, Chen Z, Li S, Zuo K, Salant DJ, Liu Z: Anti-phospholipase A2 receptor antibody in membranous nephropathy. *J Am Soc Nephrol* 22: 1137–1143, 2011
12. Hoxha E, Harendza S, Zahner G, Panzer U, Steinmetz O, Fechner K, Helmchen U, Stahl RA: An immunofluorescence test for phospholipase-A_2-receptor antibodies and its clinical usefulness in patients with membranous glomerulonephritis. *Nephrol Dial Transplant* 26: 2526–2532, 2011
13. Oh YJ, Yang SH, Kim DK, Kang SW, Kim YS: Autoantibodies against phospholipase A2 receptor in Korean patients with membranous nephropathy. *PLoS ONE* 8: e62151, 2013
14. Murtas C, Bruschi M, Candiano G, Moroni G, Magistroni R, Magnano A, Bruno F, Radice A, Furci L, Argentiero L, Carnevali ML, Messa P, Scolari F, Sinico RA, Gesualdo L, Fervenza FC, Allegri L, Ravani P, Ghiggeri GM: Coexistence of different circulating anti-podocyte antibodies in membranous nephropathy. *Clin J Am Soc Nephrol* 7: 1394–1400, 2012
15. Kanigicherla D, Gummadova J, McKenzie EA, Roberts SA, Harris S, Nikam M, Poulton K, McWilliam L, Short CD, Venning M, Brenchley PE: Anti-PLA2R antibodies measured by ELISA predict long-term outcome in a prevalent population of patients with idiopathic membranous nephropathy. *Kidney Int* 83: 940–948, 2013
16. Gunnarsson I, Schlumberger W, Rönnelid J: Antibodies to M-type phospholipase A2 receptor (PLA2R) and membranous lupus nephritis. *Am J Kidney Dis* 59: 585–586, 2012
17. Svobodova B, Honsova E, Ronco P, Tesar V, Debiec H: Kidney biopsy is a sensitive tool for retrospective diagnosis of PLA2R-related membranous nephropathy. *Nephrol Dial Transplant* 28: 1839–1844, 2013
18. Knehtl M, Debiec H, Kamgang P, Callard P, Cadranel J, Ronco P, Boffa JJ: A case of phospholipase A_2 receptor-positive membranous nephropathy preceding sarcoid-associated granulomatous tubulointerstitial nephritis. *Am J Kidney Dis* 57: 140–143, 2011
19. Huang X, Qin W, Zhang M, Zheng C, Zeng C, Liu Z: Detection of anti-PLA2R autoantibodies and IgG subclasses in post-allogeneic hematopoietic stem cell transplantation membranous nephropathy. *Am J Med Sci* 346: 32–37, 2013
20. Ronco P, Debiec H: Pathogenesis of membranous nephropathy: Recent advances and future challenges. *Nat Rev Nephrol* 8: 203–213, 2012
21. Debiec H, Hanoy M, Francois A, Guerrot D, Ferlicot S, Johanet C, Aucouturier P, Godin M, Ronco P: Recurrent membranous nephropathy in an allograft caused by IgG3κ targeting the PLA2 receptor. *J Am Soc Nephrol* 23: 1949–1954, 2012
22. Hofstra JM, Bech AP, Brenchley PE, Wetzels JF: Measurement of anti-PLA2R antibodies predicts relapse rate after immunosuppressive therapy in patients with idiopathic membranous nephropathy [Abstract FR-OR049]. *J Am Soc Nephrol* 24: 47A, 2013
23. Debiec H, Ronco P: PLA2R autoantibodies and PLA2R glomerular deposits in membranous nephropathy. *N Engl J Med* 364: 689–690, 2011
24. Larsen CP, Messias NC, Silva FG, Messias E, Walker PD: Determination of primary versus secondary membranous glomerulopathy utilizing phospholipase A2 receptor staining in renal biopsies. *Mod Pathol* 26: 709–715, 2013

See related article, "Phospholipase A2 Receptor Autoantibodies and Clinical Outcome in Patients with Primary Membranous Nephropathy," on pages 1357–1366.