

# Phospholipase A<sub>2</sub> Receptor Antibodies in Membranous Nephropathy: Unresolved Issues

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

In this issue of *JASN*, Hoxha *et al.* report their findings in a large cohort of 163 patients with MN.<sup>8</sup> PLA<sub>2</sub>R 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).<sup>9</sup> PLA<sub>2</sub>R 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<sub>2</sub>R antibodies decreased during follow-up. The decrease in PLA<sub>2</sub>R 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.”<sup>8</sup> These data confirm earlier findings and indicate that an immunologic remission precedes clinical remission in patients with iMN.<sup>6,10</sup> Although no PLA<sub>2</sub>R antibodies were found in patients with complete remission, PLA<sub>2</sub>R 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.

The authors next analyzed the association between antibody levels at baseline and remission at 12 months after presentation. PLA<sub>2</sub>R 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<sub>2</sub>R antibody level was “an independent risk factor for not achieving remission.”<sup>8</sup> 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<sub>2</sub>R ANTIBODIES?

There are three techniques for detecting PLA<sub>2</sub>R 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.*<sup>1</sup> However, the Western blot technique is not suitable in daily practice. Although all techniques can detect PLA<sub>2</sub>R antibodies, some differences are apparent. Dähnrich *et al.* studied 200 patients with PLA<sub>2</sub>R-related MN (defined by positive IFT results) and found that 7 patients had negative ELISA results.<sup>9</sup> 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.<sup>7</sup>

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.<sup>7</sup> The data of Hoxha *et al.* also suggest that the changes in PLA<sub>2</sub>R 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.

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

### **CAN MEASUREMENT OF PLA<sub>2</sub>R ANTIBODIES BE USED TO DIAGNOSE MN?**

Although no prospective study has validated PLA<sub>2</sub>R antibodies as a diagnostic biomarker, we suggest that the time to abandon the kidney biopsy in PLA<sub>2</sub>R-positive patients is near. PLA<sub>2</sub>R antibodies were not found in five studies that evaluated 313 healthy controls with Western blot or IFT.<sup>1,11–14</sup> The ELISA assay always has some background activity, and normal values are established based on the mean+3 SD values in healthy volunteers.<sup>9,15</sup> As a result, few healthy controls are considered positive (e.g., 1 of 291 persons in one study).<sup>9</sup> PLA<sub>2</sub>R antibodies were not detected in patients with other autoimmune diseases (0 of 316),<sup>9</sup> or in patients with nonmembranous GN (0 of 510, although the majority of these patients were non-nephrotic).<sup>1,9,12,14,15</sup> Although some additional data are needed, the available evidence suggests that it may be acceptable to start with the PLA<sub>2</sub>R antibody assay in patients with nephrotic syndrome. A kidney biopsy could be avoided if PLA<sub>2</sub>R 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<sub>2</sub>R 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<sub>2</sub>R antibody-positive patients.

### **CAN MEASUREMENT OF PLA<sub>2</sub>R 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<sub>2</sub>R antibodies.<sup>12</sup> The literature data are equivocal. Antibodies are scarcely found in proteinuric patients with lupus nephritis (2 of 86).<sup>1,11,12,15,16</sup> 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).<sup>11–13,15,17–20</sup> The interesting observation of Debiec *et al.* of a patient with MN secondary to a IgG3 paraprotein deserves special mention.<sup>21</sup> The authors provide evidence that the paraprotein had antibody activity directed against the PLA<sub>2</sub>R antigen. This case report, then, virtually proves the pathogenicity of PLA<sub>2</sub>R antibodies.

For the moment, a secondary cause cannot be fully excluded in PLA<sub>2</sub>R 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<sub>2</sub>R 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<sub>2</sub>R 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%).<sup>7</sup> Oh *et al.* observed spontaneous remission in 17% of patients with high levels versus 45% in patients with low levels.<sup>13</sup> These data require confirmation and large studies are needed to provide meaningful information, including valid accuracy figures. A pilot study showed that PLA<sub>2</sub>R antibodies measured at the end of therapy predicted long-term outcome.<sup>22</sup> After 5 years, 12 of 18 (67%) antibody-negative patients were in persistent remission in contrast with 1 of 8 (13%) antibody-positive patients ( $P<0.01$ ).<sup>22</sup> 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<sub>2</sub>R STAINING IN KIDNEY BIOPSIES?**

Although most studies have evaluated the role of PLA<sub>2</sub>R antibodies that are present in the serum of patients with MN, some authors have pointed to the possibility of using commercially available anti-PLA<sub>2</sub>R antibodies for the detection of the PLA<sub>2</sub>R antigen in a kidney biopsy using an immunostaining procedure. Debiec and Ronco were the first to note that the PLA<sub>2</sub>R antigen could be detected *via* a kidney biopsy in patients without PLA<sub>2</sub>R antibodies.<sup>23</sup> Indeed, a recent study suggested that up to 50% of patients with iMN and negative PLA<sub>2</sub>R antibodies may be positive for the PLA<sub>2</sub>R antigen on a kidney biopsy.<sup>17</sup> Immunostaining of stored kidney biopsy tissue also allows for diagnosis of PLA<sub>2</sub>R-related MN in retrospect.<sup>17</sup> Immunostaining of kidney biopsies was also used in a single-center study that compared patients with iMN and secondary MN. In this study, PLA<sub>2</sub>R expression in kidney biopsies was observed in 64 of 85 patients with iMN and in 14 of 80 patients with secondary MN.<sup>24</sup> 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<sub>2</sub>R-positive patients with secondary MN. These observations confirm the findings of Qin *et al.*<sup>11</sup> and suggest that there may be coexistence of two diseases in such cases.

In the coming years, the role of PLA<sub>2</sub>R 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.

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## DISCLOSURES

None.

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