New insights in membranous glomerulonephritis: from bench to bedside

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Idiopathic membranous nephropathy (IMN) is responsible for most cases of nephrotic syndrome in adults [1]. Although spontaneous remission may occur in some cases and several therapeutic options are now available [2–4], in a significant number of patients the response to therapy is poor and the risk of cardiovascular events or kidney disease progression remains high. Prognostic markers in IMN would help clinicians identify potential candidates to early intervention and specific strategies.

In general, the development of biomarkers largely depends on the understanding of the pathogenesis of the disease. Until recently, the mechanisms of IMN in humans were translated from animal models, mainly from Heymann nephritis. However, basic differences in autoantigens glomerular expression among mice, rabbits and humans represented the main roadblock to the understanding of mechanisms of IMN in humans [5]. In fact, megalin, the autoantigen of Heymann nephritis, is not expressed in human glomeruli.

The finding that neutral endopeptidase (NEP) is the target antigen of the autoimmune response in neonatal forms of the disease represents a breakthrough in the identification of glomerular autoantigen in human IMN. Elegant works by Debiec et al. [6, 7] showed the presence of anti-NEP antibodies in a woman carrying a homozygous deletion in MME (the gene coding for NEP). After alloimmunization occurred during a former pregnancy, anti-NEP antibodies were transferred to the fetus during a successive pregnancy. This rare form of IMN first proved the role of circulating antibodies against a podocyte protein in determining human IMN.

More recently, new podocyte autoantigens have been identified [8–10] and research is now focused on the development and validation of a panel of antibodies to risk stratify patients and assist clinical decision making. Direct analysis of antibodies eluted from human glomeruli are now possible thanks to advances in technologies of recent application to human pathology, such as laser capture microdissection and proteomics. Potential biomarkers are identified in a three-step process. Firstly, IgG₄ eluted from laser-captured glomeruli of IMN patients are tested for binding to podocyte extracts. Proteins recognized by eluted antibodies become autoantigens candidates [11]. Secondly, these candidate molecules are considered autoantigens if...
they colocalize with IgG4 and C5b-9 in immune deposits and, thirdly, if they are recognized by circulating IgG4 from sera of IMN patients. Only podocyte proteins fulfilling all the three prerequisites should be considered in IMN pathogenesis and may represent good biomarkers.

Considering only data already published or under peer review, at least four autoantigens have been identified. One of them, the phospholipase A2 receptor (PLA2r) is normally expressed in podocyte membrane. The others, i.e. superoxide dismutase 2 (SOD2), aldose reductase (AR) and α-enolase, are cytosol proteins. Antibodies against these antigens have all been detected in the serum of IMN patients in small cohorts of patients and deserve validation in larger studies (Table 1). Cell localization of antigens in podocytes suggests that different mechanisms are possible for autoantibody formation. For membrane protein, such as PLA2r, mimicry may play a relevant role, as circulating antibodies should more easily recognize them. For intracellular molecules, such as SOD2 and AR or α-enolase, mechanisms of delocalization may be key. A temporal hierarchy is also possible, whereby a first autoantigen induces the formation of a prior class of autoantibodies that, in turn, stimulates the expression of other autoantigens. Insights on this aspect should come from the determination of circulating levels of each of the autoantibody and its relation with the disease activity.

The paper by Hoxha et al. [14], published in this issue of Nephrology Dialysis and Transplantation, is an important step on the road that should add clinical value to basic studies on autoantigen/antibodies implication in IMN. These authors utilized and validated an immunofluorescence assay for the analysis of anti-PLA2r in 100 patients with IMN and in 90 patients with other forms of glomerulonephritis. The results show good sensitivity and specificity of the test since they found anti-PLA2r was elevated only in the former group (overall positivity 52%, 66% in patients with proteinuria > 3.5 g/24 h) and it was negative in IMN associated with lupus, cancer and in other secondary forms of the disease. Therefore, determining anti-PLA2r serum levels in patients with nephrotic syndrome should indicate a probable diagnosis of IMN and, in those cases in which IMN had a pathology confirmation, be determinant to exclude secondary forms of the disease.

The relatively large number of patients included in the analysis is the strength of the study. Lack of comparisons of anti-PLA2r performance with that of other autoantibodies (i.e. anti-SOD2, anti-AR and anti-α-enolase) and the relatively incomplete clinical data (50% of patients without reported proteinuria) are the main limitations of the study. Moreover, the inclusion of hemodialysis or transplant patients may be a confounding factor in results interpretation. Overall, the study by Hoxha et al. [14] confirms previous reports on smaller cohorts of IMN patients [8, 12] that used different assays for anti-PLA2r (western blot in both cases) and suffered from the same limitations (Table 1). A second main message from the Hoxha study is that anti-PLA2r may be utilized as a marker of response to therapy. In fact, these authors described five patients who were treated with Rituximab; in two of them disappearance of anti-PLA2r from circulation anticipated a complete or a partial response. On the contrary, antibody levels in patients unresponsive to treatment remained high. A recent paper by Beck et al. [15] focus on the relationship between serum anti-PLA2r and response to Rituximab. These authors followed a cohort of 25 IMN who received two or four Rituximab pulses and found a decline or disappearance of anti-PLA2r in 68% of patients, most of whom had complete or partial remission (88% by 24 months). Interestingly, the fall in anti-PLA2r preceded the improvement of proteinuria, indicating its potential utility in clinical practice.

One main issue that remains unaddressed is the relationship between levels of antibodies anti-PLA2r and the glomerular expression of the antigen. In fact, available data from expression studies are conflicting. Data from Beck et al. [8] indicate that PLA2r is present in normal glomeruli. Conversely, data from Debiec and Ronco [13] suggest a correlation between circulating levels of the antibody and glomerular expression of the antigen, implying variable and, in some cases, negligible renal expression in patients with low circulating levels. That is in contrast with the definition of PLA2r as a structural constituent of podocyte membrane. This crucial aspect requires more investigation and a clear definition.

Despite these inconsistencies, studies on circulating anti-PLA2r are sound and indicate that it is time to apply new technologies to test the prognostic potential of available biomarkers in larger cohorts of IMN patients. New studies should enroll patients with detailed clinical records in order to better characterize patient populations and risk stratify patients. Other autoantigens have to be included in these studies. Multicenter and multinational initiatives are welcome to implement clinical databases, biobanks and laboratory networks. The renal community

### Table 1. Studies reporting the presence of circulating autoantibodies against glomerular antigens in patients with IMN

<table>
<thead>
<tr>
<th>Author</th>
<th>n</th>
<th>Antigen</th>
<th>Technique</th>
<th>Positivity (%)</th>
<th>Clinical correlation(s)</th>
<th>Follow-up</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beck</td>
<td>37</td>
<td>PLA2r</td>
<td>WB</td>
<td>26 (70)</td>
<td>Not reported</td>
<td>No</td>
<td>[8]</td>
</tr>
<tr>
<td>Hofstra</td>
<td>18</td>
<td>PLA2r</td>
<td>WB</td>
<td>14 (78)</td>
<td>With proteinuria and serum creatinine at baseline</td>
<td>Yes</td>
<td>[12]</td>
</tr>
<tr>
<td>Debiec</td>
<td>42</td>
<td>PLA2r</td>
<td>WB/IF</td>
<td>24 (57)</td>
<td>Not reported</td>
<td>No</td>
<td>[13]</td>
</tr>
<tr>
<td>Hoxha</td>
<td>100</td>
<td>PLA2r</td>
<td>IF</td>
<td>52 (52)</td>
<td>Weak with proteinuria</td>
<td>In a small series</td>
<td>[14]</td>
</tr>
<tr>
<td>Beck</td>
<td>35</td>
<td>PLA2r</td>
<td>WB</td>
<td>25 (71)</td>
<td>With proteinuria at follow-up</td>
<td>Yes</td>
<td>[15]</td>
</tr>
<tr>
<td>Prunotto</td>
<td>24</td>
<td>AR</td>
<td>DB</td>
<td>6 (25)</td>
<td>Not reported</td>
<td>No</td>
<td>[9]</td>
</tr>
<tr>
<td>Prunotto</td>
<td>24</td>
<td>SOD2</td>
<td>DB</td>
<td>12 (50)</td>
<td>Not reported</td>
<td>No</td>
<td>[9]</td>
</tr>
<tr>
<td>Bruschi</td>
<td>131</td>
<td>α-enolase</td>
<td>DB</td>
<td>35 (23)</td>
<td>Not reported</td>
<td>No</td>
<td>[10]</td>
</tr>
</tbody>
</table>

*aAbbreviations: α-enolase; WB, western blot; IF, immunofluorescence; DB, dot blot.*
sees these national and international efforts as the necessary condition to understand the mechanisms of renal autoimmunity.

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Conflict of interest statement. None declared.

(See related article by Hoxha et al. An immunofluorescence test for phospholipase-A2-receptor antibodies and its clinical usefulness in patients with membranous glomerulonephritis; Nephrol Dial Transplant 2011; 26: 2526–2532.)

References


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