29th Annual Meeting of the Glomerular Disease Collaborative Network

PLA2R in Membranous Glomerulopathy

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Frequency of Renal Biopsy Diagnoses vs Age at UNC from 1985-2007
(Note: The relative frequency in the general population would be very different)

Biopsy Diagnoses in Patients with Nephrotic Syndrome

FSGS
Membranous GN
Diabetic GS
Minimal Change
MPGN
Amyloidosis
DDD

Jennette JC, Falk RJ. NKF Primer on Kidney Disease, 6th Ed, 2014, Chapter 16:152-163
Membranous Glomerulopathy

Thick capillary walls by light microscopy but no hypercellularity

Normal

Membranous glomerulopathy
Membranous Glomerulopathy
Charles Jennette

1) Membranous glomerulopathy in the GDCN
2) Membranous nephropathy serology and pathogenesis
Membranous Glomerulopathy

- Thick walls without hypercellularity by LM
- Granular capillary wall IgG by IM
- Subepithelial dense deposits by EM
Membranous Glomerulopathy

Transmission EM

Scanning EM
Stage Transformations

Stage I

Stage II

Stage III

Stage IV
Primary Membranous Glomerulopathy

- Antibody
- Antigen
- Immune complexes
Subepithelial immune complex containing podocyte antigen (e.g. PLA2R) and antibody
M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy.


A majority of patients with idiopathic membranous nephropathy have antibodies against a conformation-dependent epitope in PLA2R.

PLA2R is present in normal podocytes and in immune deposits in patients with idiopathic membranous nephropathy, indicating that PLA2R is a major antigen in this disease.
Antibody against the M-Type Phospholipase A2 Receptor (PLA2R) and Disease Activity in a Patient with Membranous Nephropathy

# Anti-PLA2R Sensitivity and Specificity for Membranous Glomerulopathy

<table>
<thead>
<tr>
<th>Category</th>
<th>n=</th>
<th>Pan-IgG</th>
<th>IgG4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary MN</td>
<td>200</td>
<td>196</td>
<td>200</td>
</tr>
<tr>
<td>Secondary MN</td>
<td>27</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Other glomerular dz</td>
<td>230</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Other autoimmune dz</td>
<td>316</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Healthy individuals</td>
<td>291</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

Anti-PLA2R Sensitivity and Specificity for Membranous Glomerulopathy

pMN, primary MN; sMN, secondary MN; GN, other renal diseases; RA, rheumatoid arthritis; PSA, psoriatic arthritis; Thyr., autoimmune thyroiditis; Coll., undifferentiated collagenosis; HBD, healthy blood donors.

(EUROIMMUN AG, Lübeck, Germany)
Anti-PLA2R Sensitivity and Specificity for Membranous Glomerulopathy

**Sensitivity (95% CI)**

- Oh2/2013: 0.69 [0.59 - 0.78]
- Oh1/2013: 0.69 [0.59 - 0.78]
- Dahnrich2/2013: 0.96 [0.93 - 0.99]
- Dahnrich1/2013: 0.96 [0.93 - 0.99]
- Svobodova/2013: 0.64 [0.44 - 0.81]
- Zhou2/2012: 0.75 [0.51 - 0.91]
- Zhou1/2012: 0.75 [0.51 - 0.91]
- Hoxha/2012: 0.98 [0.91 - 1.00]
- Murtas2/2012: 0.60 [0.52 - 0.67]
- Murtas1/2012: 0.60 [0.52 - 0.67]
- Hoxha2/2011: 0.49 [0.33 - 0.65]
- Hoxha1/2011: 0.49 [0.33 - 0.65]
- Qin/2011: 0.82 [0.70 - 0.90]
- Beck2/2009: 0.75 [0.43 - 0.95]
- Beck1/2009: 0.75 [0.43 - 0.95]
- **COMBINED**: 0.78 [0.66 - 0.87]

**Specificity (95% CI)**

- Oh2/2013: 0.78 [0.40 - 0.97]
- Oh1/2013: 1.00 [0.77 - 1.00]
- Dahnrich2/2013: 1.00 [0.99 - 1.00]
- Dahnrich1/2013: 1.00 [0.98 - 1.00]
- Svobodova/2013: 0.33 [0.01 - 0.91]
- Zhou2/2012: 0.92 [0.75 - 0.99]
- Zhou1/2012: 1.00 [0.48 - 1.00]
- Hoxha/2012: 1.00 [0.87 - 1.00]
- Murtas2/2012: 0.99 [0.94 - 1.00]
- Murtas1/2012: 0.99 [0.94 - 1.00]
- Hoxha2/2011: 1.00 [0.97 - 1.00]
- Hoxha1/2011: 1.00 [0.98 - 1.00]
- Qin/2011: 0.89 [0.76 - 0.96]
- Beck2/2009: 1.00 [0.88 - 1.00]
- Beck1/2009: 1.00 [0.88 - 1.00]
- **COMBINED**: 0.99 [0.96 - 1.00]

*Q = 220.62, df = 14, p = 0.00*

*Q = 227.01, df = 14, p = 0.00*

*I² = 93.65 [91.49 - 95.82]*

*I² = 93.83 [91.74 - 95.92]*

Membranous Glomerulopathy

Proteinase-treated Paraffin Section Stained with Anti-PLA2R

Photo by Akanksha Gupta, UNC Nephropathology Fellow
Coexistence of different circulating anti-podocyte antibodies in membranous nephropathy.


Coexistence of different circulating anti-podocyte antibodies in membranous nephropathy.

Levels of circulating antibodies in MN patients, controls, and other nephropathies

<table>
<thead>
<tr>
<th></th>
<th>MN</th>
<th>Controls</th>
<th>FSGS</th>
<th>IgAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-AR</td>
<td>37.90</td>
<td>29.67</td>
<td>6.38</td>
<td>9.39</td>
</tr>
<tr>
<td></td>
<td>(7.12–121.90)</td>
<td>(9.39–47.36)</td>
<td>(3.43–9.15)</td>
<td>(4.41–14.46)</td>
</tr>
<tr>
<td>Anti-SOD2</td>
<td>84.34</td>
<td>33.92</td>
<td>6.89</td>
<td>9.09</td>
</tr>
<tr>
<td></td>
<td>(35.51–223.60)</td>
<td>(4.11–62.23)</td>
<td>(4.16–9.69)</td>
<td>(3.93–12.99)</td>
</tr>
<tr>
<td>Anti-αENO</td>
<td>110.40</td>
<td>47.80</td>
<td>5.40</td>
<td>9.31</td>
</tr>
<tr>
<td></td>
<td>(27.84–237.70)</td>
<td>(31.09–67.38)</td>
<td>(3.29–9.43)</td>
<td>(5.06–18.19)</td>
</tr>
<tr>
<td>Anti-NEP</td>
<td>53.52</td>
<td>32.42</td>
<td>4.08</td>
<td>7.05</td>
</tr>
<tr>
<td></td>
<td>(3.52–211.20)</td>
<td>(14.95–54.73)</td>
<td>(2.08–6.92)</td>
<td>(3.67–11.39)</td>
</tr>
<tr>
<td>Anti-PLA2r</td>
<td>40.26</td>
<td>Undetectable</td>
<td>Undetectable</td>
<td>Undetectable</td>
</tr>
<tr>
<td></td>
<td>(13.12–96.76)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Corrado Murtas et al. CJASN 2012;7:1394-1400
Coexistence of different circulating anti-podocyte antibodies in membranous nephropathy.

The horizontal line is set at the 95th percentile of levels titrated in normal controls. AR, aldose reductase; αENO, α-enolase; SOD2, superoxide dismutase 2.
Serum levels of circulating anti-PLA2r and anti-NEP IgG4.

A  anti - PLA2r IgG₄

Optical Density [O.D. unit]

Control n° 61
MN n° 111

B  anti - NEP IgG₄

Optical Density [O.D. unit]

Control n° 96
FSGS n° 32
IgAN n° 60
MN n° 186

Corrado Murtas et al. CJASN 2012;7:1394-1400
Coexistence of different circulating anti-podocyte antibodies in membranous nephropathy.

- Multiple serum anti-podocyte antibodies are increased in MN at diagnosis.

- Follow-up proteinuria is lower in patients who are negative for all antibodies.

- A panel including all antibodies is most promising biomarker to be tested and utilized in prospective studies.

Corrado Murtas et al. CJASN 2012;7:1394-1400
Membranous Glomerulopathy

PRIMARY

- AUTOIMMUNITY TO GLOMERULAR ANTIGENS
  - Anti-phospholipase A2 Receptor Autoantibodies
  - Others (e.g. Anti–neutral Endopeptidase, Aldose Reductase, Superoxide dismutase 2)

SECONDARY

- EXTRARENAL AUTOIMMUNE DISEASE
  - SLE, MCTDz, HUVS

- INFECTIONS
  - Hepatitis B, Hepatitis C, Syphilis

- EXPOSURE TO CERTAIN DRUGS
  - Metals, Penicillamine, Gold, Mercuric Chloride

- MALIGNANCIES
  - Carcinoma, Sarcoma, Lymphoma, Leukemia
Primary Membranous Glomerulopathy

Antibody + Antigen → Immune complexes
Subepithelial immune complex containing podocyte antigen (e.g. PLA2R) and antibody
Secondary Membranous Glomerulopathy

Antibody  Antigen  Immune complexes
Subepithelial, mesangial and rare subendothelial immune complex containing antigen (e.g. HBV ag) and antibody
Primary Membranous Glomerulopathy

Secondary Membranous Glomerulopathy

mesangial immune complex deposits
<table>
<thead>
<tr>
<th>Etiology</th>
<th>PLA2R a+</th>
<th>IgG +</th>
<th>IgA +</th>
<th>IgM +</th>
<th>C3+</th>
<th>C1q +</th>
<th>Mesangial deposits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Idiopathic</td>
<td>64/85 (2.5)</td>
<td>85/85 (2.9)</td>
<td>13/85 (1.4)</td>
<td>14/85 (1.5)</td>
<td>79/85 (1.9)</td>
<td>3/85 (1.2)</td>
<td>25/85 (29%)</td>
</tr>
<tr>
<td>Secondary total</td>
<td>14/80 (2.5)</td>
<td>80/80 (2.8)</td>
<td>27/80 (1.5)</td>
<td>36/80 (1.6)</td>
<td>72/80 (2)</td>
<td>23/80 (1.3)</td>
<td>64/80 (80%)</td>
</tr>
<tr>
<td>SLE</td>
<td>0/33</td>
<td>33/33 (2.8)</td>
<td>17/33 (1.5)</td>
<td>20/33 (1.5)</td>
<td>31/33 (2)</td>
<td>20/33 (1.2)</td>
<td>33/33</td>
</tr>
<tr>
<td>Sjögren’s</td>
<td>1/6 (3)</td>
<td>6/6 (2.8)</td>
<td>1/6 (1)</td>
<td>4/6 (1.5)</td>
<td>5/6 (2.4)</td>
<td>0/6</td>
<td>6/6</td>
</tr>
<tr>
<td>MCTD</td>
<td>0/2</td>
<td>2/2 (2.3)</td>
<td>0/2</td>
<td>1/2 (1)</td>
<td>2/2 (2.5)</td>
<td>1/2 (1)</td>
<td>2/2</td>
</tr>
<tr>
<td>ANCA</td>
<td>0/4</td>
<td>4/4 (2.8)</td>
<td>1/4 (2)</td>
<td>2/4 (1.5)</td>
<td>4/4 (1.3)</td>
<td>0/4</td>
<td>4/4</td>
</tr>
<tr>
<td>Sarcoidosis</td>
<td>3/4 (2.3)</td>
<td>4/4 (3)</td>
<td>1/4 (1)</td>
<td>0/4</td>
<td>3/4 (2)</td>
<td>0/4</td>
<td>2/4</td>
</tr>
<tr>
<td>Neoplasm</td>
<td>3/12 (2.3)</td>
<td>12/12 (2.6)</td>
<td>1/12 (2)</td>
<td>2/12 (2.5)</td>
<td>11/12 (2.3)</td>
<td>0/12</td>
<td>6/12</td>
</tr>
<tr>
<td>IgG4-RSD</td>
<td>0/1</td>
<td>1/1 (3)</td>
<td>0/1</td>
<td>1/1 (1)</td>
<td>1/1 (1)</td>
<td>0/1</td>
<td>1/1</td>
</tr>
<tr>
<td>HBV</td>
<td>0/3</td>
<td>3/3 (2.7)</td>
<td>0/3</td>
<td>1/3 (1)</td>
<td>3/3 (1.3)</td>
<td>1/3 (3)</td>
<td>2/3</td>
</tr>
<tr>
<td>HCV</td>
<td>7/11 (2.6)</td>
<td>11/11 (2.8)</td>
<td>4/11 (1.3)</td>
<td>3/11 (1.7)</td>
<td>9/11 (2.2)</td>
<td>1/11 (1)</td>
<td>5/11</td>
</tr>
<tr>
<td>Syphilis</td>
<td>0/2</td>
<td>2/2 (2.5)</td>
<td>0/2</td>
<td>1/2 (2)</td>
<td>1/2 (2)</td>
<td>0/2</td>
<td>1/2</td>
</tr>
<tr>
<td>RA</td>
<td>0/1</td>
<td>1/1 (3)</td>
<td>1/1 (2)</td>
<td>1/1 (2)</td>
<td>1/1 (3)</td>
<td>0/1</td>
<td>1/1</td>
</tr>
<tr>
<td>HIV</td>
<td>0/1</td>
<td>1/1 (3)</td>
<td>1/1 (2)</td>
<td>0/1</td>
<td>1/1 (1)</td>
<td>0/1</td>
<td>1/1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>% PLA2R +</th>
<th>Age (mean range)</th>
<th>Sex (M/F)</th>
<th>Race (W/B/O)</th>
<th>TBM Deposits (IgG)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-lupus MGN, no mes deposits</td>
<td>22</td>
<td>77%</td>
<td>55-77</td>
<td>13/9</td>
<td>14/4/4</td>
<td>0%</td>
</tr>
<tr>
<td>Non-lupus MGN, with mes deposits</td>
<td>12</td>
<td>67%</td>
<td>63-80</td>
<td>7/5</td>
<td>8/2/2</td>
<td>0%</td>
</tr>
<tr>
<td>Lupus MGN, with mes deposits</td>
<td>7</td>
<td>0%</td>
<td>37-46</td>
<td>1/6</td>
<td>2/5/0</td>
<td>86%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>%+ IgG1/2/3/4</th>
<th>Mean 0-4+ IgG1/2/3/4*</th>
<th>IgA*</th>
<th>IgM*</th>
<th>C3*</th>
<th>C1q*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-lupus MGN, no mes deposits</td>
<td>20</td>
<td>100/100/100/95</td>
<td>2.9/2.0/1.8/3.1</td>
<td>45%</td>
<td>68%</td>
<td>95%</td>
<td>54%</td>
</tr>
<tr>
<td>Non-lupus MGN, with mes deposits</td>
<td>9</td>
<td>100/89/100/78</td>
<td>3.1/1.9/2.5/2.6</td>
<td>83%</td>
<td>83%</td>
<td>100%</td>
<td>67%</td>
</tr>
<tr>
<td>Lupus MGN, with mes deposits</td>
<td>7</td>
<td>100/100/100/100</td>
<td>3.3/3.4/2.5/3.2</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>86%</td>
</tr>
</tbody>
</table>

* % positive and mean intensity when positive (0-4+)

Gasim AM, Rivier LH, Dittrich K, Nachman P, Jennette JC. Mesangial Immune Complex Deposits in Membranous Glomerulonephritis Mediated by Anti-Phospholipase A2 Receptor Autoantibodies. Lab Invest 2014:94,1678A
Anti-phospholipase A2 receptor antibodies and malignancy in membranous nephropathy. 

<table>
<thead>
<tr>
<th>PLA2R Antibody Test</th>
<th>Total (n = 91)</th>
<th>Positive (n = 64)</th>
<th>Negative (n = 27)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male sex</td>
<td>63%</td>
<td>66%</td>
<td>56%</td>
<td>0.5</td>
</tr>
<tr>
<td>Age (y)</td>
<td>53.4 ± 16.2</td>
<td>51.8 ± 15.7</td>
<td>57.3 ± 17.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Anti-PLA2R</td>
<td>70%</td>
<td>76%</td>
<td>81%</td>
<td>0.8</td>
</tr>
<tr>
<td>Proteinuria (g/d)</td>
<td>7.1 ± 4.7</td>
<td>7.3 ± 5.1</td>
<td>6.6 ± 3.4</td>
<td>0.9</td>
</tr>
<tr>
<td>Nephrotic-range proteinuria</td>
<td>77%</td>
<td>76%</td>
<td>81%</td>
<td></td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>1.2 ± 0.9</td>
<td>1.3 ± 1.0</td>
<td>1.1 ± 0.7</td>
<td>0.06</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²)</td>
<td>114 ± 102</td>
<td>118 ± 119</td>
<td>104 ± 43</td>
<td>0.9</td>
</tr>
<tr>
<td>Follow-up</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration (y)</td>
<td>10.7 ± 7.5</td>
<td>11.3 ± 8.1</td>
<td>9.2 ± 5.8</td>
<td>0.4</td>
</tr>
<tr>
<td>Malignancy occurrence</td>
<td>18%</td>
<td>9%</td>
<td>37%</td>
<td>0.005</td>
</tr>
<tr>
<td>Malignancy-free survival (y)</td>
<td>23.3 ± 1.6</td>
<td>26.1 ± 1.7</td>
<td>14.9 ± 2.3</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Note: Continuous variables given as mean ± SD. There were data missing for positive (proteinuria, n = 2; creatinine, n = 1; eGFR, n = 3) and negative (eGFR, n = 1) subgroups.

Absence of anti-PLA2R at the time of biopsy increases the risk of malignancy and merits more careful surveillance for an occult malignancy.
It is likely that the seminal observations of Beck et al. will have a profound effect on how clinicians approach the diagnosis and treatment of membranous nephropathy. Assays for anti-PLA2R autoantibody (and perhaps anti–neutral endopeptidase as well) may permit the noninvasive diagnosis of membranous nephropathy as well as provide a convenient way to follow the activity of the disease in response to treatment. Five decades after its initial recognition, membranous nephropathy is now entering an exciting and dynamic new era.

Assessing circulating anti-PLA2R autoantibodies and proteinuria may help in monitoring disease activity and guiding personalized rituximab therapy in nephrotic patients with primary MN.
Mean±SEM percent changes in proteinuria, serum albumin, and anti-PLA2R autoantibody levels and circulating CD20 cell counts at different time points after rituximab administration compared with baseline (month 0). °P<0.05, *P≤0.01, ***P≤0.001 (all versus baseline).
Proportion of participants with primary MN who progressed to complete or partial remission (left panel) or complete remission (right panel) in three subgroups with or without detectable anti-PLA2R autoantibodies or without anti-PLA2R antibody evaluations at baseline.

Patients with and without anti-PLA2R responded similarly.
Percent changes in proteinuria (highest panel), circulating CD20 cell counts (middle panel), and serum anti-PLA2R autoantibody levels (lowest panel) at different time points after rituximab administration compared with baseline (month 0) in patients who progressed to complete or partial remission, progressed to complete remission, or achieved no remission.

Patients with no remission had more anti-PLA2R and proteinuria.

Piero Ruggenenti et al. JASN doi:10.1681/ASN.2014070640
Proportion of participants with primary MN with a relapse of the NS after initial complete or partial remission considered according to previous anti-PLA2R autoantibody titer increase/re-emergence after initial rituximab-induced reduction/depletion.

Patients with increase in anti-PLA2R after therapy had more relapses

Piero Ruggenenti et al. JASN doi:10.1681/ASN.2014070640
Kidney transplant biopsy on POD 6 reveal no abnormality by LM. IF showed diffuse, finely granular staining of the glomeruli for IgG (1+). EM revealed small subepithelial electron dense deposits (arrows).

Very early recurrence of anti-Phospholipase A2 receptor-positive membranous nephropathy after transplantation.

Autoantibodies specific for the phospholipase A2 receptor in recurrent and de novo membranous nephropathy.


- Some but not all patients with PLA2R1-related idiopathic MN and anti-PLA2R1 antibodies at the time of transplantation will develop recurrence.

- Because PLA2R1 autoantibody is not always associated with recurrence, its predictive value should be carefully analyzed in prospective studies.
Membranous Glomerulopathy

Proteinase-treated Paraffin Section Stained with Anti-PLA2R

Photo by Akanksha Gupta, UNC Nephropathology Fellow