New Autoantibody Diagnostics in Glomerulonephritis
- Anti-PLA2-Receptor

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Glomerulonephritis (GN)

- Constitute some of the major problems in nephrology
- GN may present with isolated hematuria and/or proteinuria, as nephrotic syndrome, nephritic syndrome, acute renal failure or chronic renal failure.
- Categorized into several different pathological patterns. Diagnosing the pattern of GN is important - outcome and treatment differs. **Primary causes** are intrinsic to the kidney. **Secondary causes** are associated with certain infections (bacterial, viral or parasitic pathogens), drugs, systemic disorders
Glomerulonephritis (GN)

Systemic diseases with glomerular involvement

- Systemic lupus erythematosus
- Goodpasture syndrome
- Microscopic polyarteritis/polyangiiitis
- Wegener granulomatosis
- Henoch-Schönlein purpura
- Diabetes mellitus
- Amyloidosis
- Bacterial endocarditis

Rapidly
Progressive
Glomerulonephritis (RPGN)

ANA
dsDNA
GBM
pANCA
cANCA
...

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Glomerulonephritis
- The main clinical syndromes -

**Nephritic syndrome**
- hematuria,
- proteinuria <3g/day,
- oliguria, edema, hypertension

**Nephrotic syndrome**
- proteinuria >3.5g/day,
- hypoalbuminemia,
- hyperlipidemia, lipiduria

Postinfectious GN
- IgA nephropathy
- Lupus nephritis
- Membranoproliferative GN
- Rapidly progressive GN

Membranous glomerulonephritis (MGN)
- Minimal-change GN
- Focal segmental glomerulosclerosis
- Membranoproliferative GN
17% of urban Indians have kidney disease
(The Times of India, June 2013)

"First report of the Indian CKD registry"
(Rajapurkar M et al. BMC Nephrology 2012)

Causes of CKD (Jan 2006- Sep 2010):
- Diabetic nephropathy (16,371 cases)
- Undetermined (8,385)
- Chronic GN (7,217)
- …
The Pattern of GN in the North Indian Gangetic Plain – A 13-Year Epidemiological Study (Mannan R et al. JCDR 2012)
-> 226 biopsied cases of various GD, MGN was the commonest pattern (13.2%)

The spectrum of GD in a single center: A clinicopathological correlation (Golay V et al. Indian J Nephrol 2013)
-> 666 biopsied cases of various GD; Minimal change disease (MCD) was the commonest pattern (20.1%), MGN (12.0%)

Pattern of GD in a tertiary Care Center in South India: A Prospective Study (Jeganathan J et al. Saudi J Kidney Dis Transpl 2013)
-> 75 biopsied cases of various GD; MCD was the common prim. GD, LN most common sec. disorder
Membranous Glomerulonephritis (MGN)
Chronic glomerular disease characterized by in-situ subepithelial immune complex formation and complement activation.

Permeability of the capillary loops is altered leading to proteinuria and frequently to a nephrotic syndrome.
Membranous Glomerulonephritis (MGN)
- Classification -

- ~80% idiopathic or primary form
- ~20% secondary form in other underlying diseases:
  - Malignant tumors (~10%)
  - Infections (Hepatitis B, Malaria, Lues,...)
  - Drugs
  - SLE
  - other autoimmune disorders
Membranous Glomerulonephritis (MGN) - Clinical Features -

- Patients present with nephrotic syndrome or have proteinuria (15%) detected on a routine urinanalysis
- 15-35% have hematuria and mild hypertension
- Important to rule out secondary causes (e.g. SLE, tumor, infection) – treatment can reverse the injury

40-50% of patients with IMN progress to end-stage renal disease
Membranous Glomerulonephritis (MGN) - Morphology -

Pavenstedt et al Nephrologe 2011
Membranous Glomerulonephritis (MGN)
- Formation of immune complexes *in situ* -

Source: www.pgmcqs.com
M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy.

N Engl J Med. 2009 Jul 2;361(1):11-21

“70% of patients with idiopathic membranous nephropathy (IMN) had IgG antibodies (predominantly of IgG4 subclass) that reacted with a conformational epitope present on PLA2-R, a glycoprotein constituent of normal podocytes.”
Anti-PLA$_2$R RC-IFT
- BIOCHIP Technology -

BIOCHIP Mosaic consisting of 2 substrates:

- HEK 293 PLA$_2$R-transfected
- HEK 293 Control plasmid-transfected
Anti-PLA$_2$R RC-IFT

PLA$_2$R-transfected

HEK293 cells
Anti-PLA$_2$R ELISA

Standard ELISA:
- exchangeable reagents,
- incubations 30 / 30 / 15 min,
- semi-quantitative (ratio) or quantitative (5 calibrators, 2 – 1,500 RU/ml)
Anti-PLA2R Ab are specific for primary MGN!
Anti-PLA2-R in membranous Lupus nephritis?

Reactivity to the 185-kD Protein

<table>
<thead>
<tr>
<th></th>
<th>No. of Subjects</th>
</tr>
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<tbody>
<tr>
<td>Idiopathic MN</td>
<td>Reactive serum</td>
</tr>
<tr>
<td></td>
<td>Nonreactive serum</td>
</tr>
<tr>
<td>Secondary MN</td>
<td>Reactive serum</td>
</tr>
<tr>
<td></td>
<td>Nonreactive serum</td>
</tr>
<tr>
<td>Other Diseases</td>
<td>Reactive serum</td>
</tr>
<tr>
<td></td>
<td>Nonreactive serum</td>
</tr>
<tr>
<td>Normal</td>
<td>Reactive serum</td>
</tr>
<tr>
<td></td>
<td>Nonreactive serum</td>
</tr>
</tbody>
</table>

Anti-PLA$_2$R RC-IFT
- Sensitivity & Specificity -

<table>
<thead>
<tr>
<th>Condition</th>
<th>n</th>
<th>Anti-PLA$_2$R positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biopsy-proven primary MGN</td>
<td>100</td>
<td>52</td>
</tr>
<tr>
<td>Secondary form of MGN</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>Non-membranous glomerulonephritis</td>
<td>90</td>
<td>0</td>
</tr>
<tr>
<td>Healthy blood donors</td>
<td>153</td>
<td>0</td>
</tr>
</tbody>
</table>

Sensitivity: 52%   Specificity: 100%

*Retrospective study, includes patients under treatment, remisson!*

Hoxha, Fechner, Stahl et al., *Nephrol Dial Transplant* 26 (2011)
<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Anti-PLA2R positive RC-IFT</th>
<th>Anti-PLA2R positive ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary MN, before treatment, within 8 weeks after biopsy, anti-PLA2R RC-IFT positive</td>
<td>200</td>
<td>200 (100%)</td>
<td>193 (96.5%)</td>
</tr>
<tr>
<td>Secondary MN, before treatment, within 8 weeks after biopsy, other underlying disease known, anti-PLA2R RC-IFT negative</td>
<td>27</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other glomerulonephritides</td>
<td>230</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other autoimmune diseases</td>
<td>316</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Healthy blood donors</td>
<td>291</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Specificity</td>
<td>864</td>
<td>100%</td>
<td>99.9%</td>
</tr>
</tbody>
</table>

*Dähnrich, Stahl, Schlumberger et al., Clin Chim Acta 421 (2013)*
Why are not all patients with pMGN positive for anti-PLA2R antibodies?
Why are not all MN Patients Anti-PLA$_2$R positive?

Almost all prevalence data are based on retrospective studies!

- The antibody levels fluctuate with disease activity and might have disappeared at the time of blood sampling due to:
  a) spontaneous remission
  b) immunosuppressive therapy

- Primary, idiopathic membranous nephropathy is not a uniform disease and might have different target antigens.

- Some patients might have been misclassified as idiopathic when they actually had a secondary form of MN.
Prospective Study on Patients with MN
Hoxha, Stahl et al., Kidney International (2012)

Samples
88 patients with histologic diagnosis of MGN (64 men and 24 women)
78 patients with various diseases
   14 with minimal change disease
   11 with immunoglobulin IgA nephropathy
   9 with primary focal segmental glomerulosclerosis (FSGS)
   8 with sarcoidosis
   8 with diabetic nephropathy
   7 with lupus nephritis type IV
   6 with lupus nephritis type V
   6 with membranoproliferative glomerulonephritis (MPGN) type I
   4 with fibrillary glomerulonephritis (GN)
   5 with normal kidneys
PLA$_2$ Receptor
- Immunohistochemistry and Antibodies -

Hoxha, Stahl et al., Kidney International (2012)
Does the antibody titer correlate with disease activity?
Anti-PLA$_2$R Titer and Clinical Presentation
- Monitoring Response to Treatment -

Relationship between clinical disease and immunological activity

Anti-PLA$_2$R Titer and Clinical Presentation
- Monitoring the Clinical Status -

Hofstra et al., Clin J Am Soc Nephrol 6, 2011
Clinical and Therapeutic Implication

- Anti-PLA2R autoantibodies are highly specific markers of active primary MN.

- Most patients with anti-PLA2R-related MN will have a therapeutic or spontaneous remission.

- Loss of serum reactivity for anti-PLA2R precedes clinical remission of proteinuria.

- High anti-PLA2R titers seem to be associated with a more severe disease course.

- Anti-PLA2R positivity at the time of transplantation is associated with a high risk of recurrent MN.
Summary
- Serological Diagnosis in Autoimmune Nephropathies -

- Primary membranous glomerulonephritis
  → Anti-PLA2R

- Lupus Nephritis
  → Anti-DNA, Anti-Nucleosomes

- Glomerulonephritis in ANCA-associated vasculitis
  → ANCA, Anti-MPO, Anti-PR3

- Goodpasture Disease
  → Anti-GBM
Thank you...