C3 Glomerulopathy

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The old classification...

**MPGN Type I**
*Subendothelial deposits*
West et al, J Pediatr 1965

**MPGN Type II / DDD**
*Intramembranous deposits*

**MPGN Type III**
*Subendothelial and subepithelial deposits*
Burkholder et al, Am J Pathol 1969
Anders et al, Virchows Arch A Pathol Anat Histol 1997
Strife et al, Clin Nephrol 1984
19 patients with unusual glomerulonephritis and:

- C3NeF positivity (7), CFH (3), CFI (2) or MCP (1) mutations
- overt mesangial and epimembranous (sub-endothelial) C3 deposits
- no dense intramembranous deposits
- no Ig deposition
Complement-mediated MPGN

Inflammation!
Evidence for a role of complement in DDD/C3GN in humans

- Proteomic profile of microdissected glomeruli: 
  C3, C4, C5, C6, C7, C8, CFHR1, CFHR5….

- Very similar profile between DDD and C3GN

Glomerular lesions in DDD

MPGN

Mesangial proliferation

Crescentic

Diffuse endocapillary proliferation

Walker PD et al, Modern Pathol 2007
Glomerular lesions in C3GN

Mesangial proliferation  MPGN  Diffuse endocapillary proliferation
The diagnosis of DDD requires electron microscopy

**DDD**

[Image of DDD]

*Walker PD et al, Modern Pathol 2007*

**C3GN**

[Image of C3GN]

Mesangial proliferative GN and MPGN represent a continuum.

Diffuse proliferative GN

Acute

Chronic

MPGN

Sethi S and Fervenza FC, Semin Nephrol 2011
New classification based on the MPGN pattern

- Positive Igs +/- C3
  - MPGN1
    - Monoclonal gammopathies
    - Dysproteinemia
  - Autoimmune diseases
  - Infections
- Negative Igs + C3
  - Complement-mediated
    - C3 Glomerulopathies
      - DDD
      - C3GN

But can also be something else... (MesProl, encocapillary, crescentic...)

References:
- Sethi S and Fervenza FC, Semin Nephrol 2011
- Sethi S and Fervenza FC, NEJM 2012
MPGN1 can also be secondary to dysregulation of the complement alternative pathway

Table 3 | Complement component analysis and immunofluorescence study of membranoproliferative glomerulonephritis type I cases with positive C3 nephritic factor

<table>
<thead>
<tr>
<th>Patient</th>
<th>C3a (660 to 1250 mg/l)</th>
<th>C4a (90 to 380 mg/l)</th>
<th>CFBa (90 to 320 mg/l)</th>
<th>Immunofluorescence study</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>537b</td>
<td>160</td>
<td>83</td>
<td>MPGN I, IgG, IgM, C3</td>
</tr>
<tr>
<td>26</td>
<td>512</td>
<td>127</td>
<td>50</td>
<td>MPGN I, IgG, IgM, IgA, C3</td>
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<tr>
<td>27</td>
<td>183</td>
<td>178</td>
<td>225</td>
<td>MPGN I, IgG, C3</td>
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<tr>
<td>28</td>
<td>701</td>
<td>233</td>
<td>96</td>
<td>MPGN I, IgG, C3</td>
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<tr>
<td>29</td>
<td>87</td>
<td>202</td>
<td>51</td>
<td>MPGN I, IgG, IgM, C3</td>
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<tr>
<td>30</td>
<td>847</td>
<td>222</td>
<td>71</td>
<td>MPGN I, IgG, IgM, C3, C1q</td>
</tr>
<tr>
<td>31</td>
<td>48</td>
<td>126</td>
<td>89</td>
<td>MPGN I, IgG, IgA, C3</td>
</tr>
<tr>
<td>32</td>
<td>87</td>
<td>309</td>
<td>92</td>
<td>MPGN I, IgG, IgM, C3</td>
</tr>
<tr>
<td>33</td>
<td>293</td>
<td>209</td>
<td>100</td>
<td>MPGN I, IgG, IgM, C3</td>
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<tr>
<td>34</td>
<td>180</td>
<td>248</td>
<td>123</td>
<td>MPGN I, IgG, IgM, C3</td>
</tr>
<tr>
<td>35</td>
<td>193</td>
<td>95</td>
<td>126</td>
<td>MPGN I, IgG, C3</td>
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<tr>
<td>36</td>
<td>275</td>
<td>225</td>
<td>159</td>
<td>MPGN I, IgG, IgM, C3, C1q</td>
</tr>
<tr>
<td>37</td>
<td>1110</td>
<td>162</td>
<td>186</td>
<td>MPGN I, NDc</td>
</tr>
<tr>
<td>38</td>
<td>475</td>
<td>175</td>
<td>155</td>
<td>MPGN I, IgG, IgA, C3</td>
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<tr>
<td>39</td>
<td>741</td>
<td>169</td>
<td>82</td>
<td>MPGN I, IgG, C3</td>
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<tr>
<td>40</td>
<td>875</td>
<td>273</td>
<td>124</td>
<td>MPGN I, IgG, C3, C1q</td>
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<tr>
<td>41</td>
<td>135</td>
<td>182</td>
<td>130</td>
<td>MPGN I, IgG, IgA, IgM, C3</td>
</tr>
<tr>
<td>42</td>
<td>129</td>
<td>227</td>
<td>64</td>
<td>MPGN I, IgG, C3</td>
</tr>
</tbody>
</table>

Abbreviations: CFB, complement factor B; Ig, immunoglobulin; MPGN I, membranoproliferative glomerulonephritis type I; ND, not done.
aLaboratory reference values are indicated in brackets.
bRare variant CFI IVS 12+5 associated.
cBiopsy performed in 1974: lobular MPGN I, no immunofluorescence study available. Cases with genetic abnormality are presented in Table 2.

Servais et al, Kidney Int 2012
• 13-year-old boy
• nephrotic syndrome & hematuria
• markedly low C3 and C4
• initial renal biopsy: MPGN with strong C3 deposition and strong immunoglobulin deposition
• follow-up biopsies (1 and 3 years): MPGN with strong C3 deposition ± no immunoglobulin deposition
• Elevated SC5b-9 treated with eculizumab: decrease in proteinuria

Kerns et al, Ped Nephrol 2013
The value of repeat biopsies

First biopsy

Second biopsy

Positive C3Nef
Elevated C5b9
Atypical APGN

8 y/o girl with recurrent hematuria and persistently low C3 levels (<30 mg/dl) after 8 weeks of the 1\textsuperscript{st} episode of macroscopic hematuria
### Table 3 | Complement abnormalities

<table>
<thead>
<tr>
<th>Patient</th>
<th>CFH</th>
<th>CFHR5</th>
<th>FH antibodies(^b)</th>
<th>Hemolytic assay(^b)</th>
<th>APFA(^c)</th>
<th>C3NeF</th>
<th>sMAC(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>c.2171delC, p.Thr724fsX, 725</td>
<td>No mutations</td>
<td>Negative</td>
<td>ND</td>
<td>ND</td>
<td>Negative</td>
<td>0.24 mg/l</td>
</tr>
<tr>
<td>2</td>
<td>No mutations</td>
<td>c.646-647, AA&gt;TT, p.Asn216Phe</td>
<td>Negative</td>
<td>0%, Normal</td>
<td>63%, Abnormal</td>
<td>ND</td>
<td>0.21 mg/l</td>
</tr>
<tr>
<td>3</td>
<td>No mutations</td>
<td>No mutations</td>
<td>Negative</td>
<td>1%, Normal</td>
<td>63%, Abnormal</td>
<td>Positive (C3CSAP)</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>No mutations</td>
<td>No mutations</td>
<td>Negative</td>
<td>0%, Normal</td>
<td>1% Abnormal</td>
<td>Positive (IFE)</td>
<td>1.23 mg/l</td>
</tr>
<tr>
<td>5</td>
<td>No mutations</td>
<td>No mutations</td>
<td>Negative</td>
<td>12% Abnormal</td>
<td>34% Abnormal</td>
<td>Positive (IFE)</td>
<td>0.48 mg/l</td>
</tr>
<tr>
<td>6</td>
<td>No mutations</td>
<td>No mutations</td>
<td>Negative</td>
<td>0%, Normal</td>
<td>14% Abnormal</td>
<td>Positive (both assays)</td>
<td>ND</td>
</tr>
<tr>
<td>7</td>
<td>c.3350A&gt;G, p.Asn1117Ser</td>
<td>No mutations</td>
<td>Negative</td>
<td>0% Normal</td>
<td>80%</td>
<td>Negative</td>
<td>ND</td>
</tr>
<tr>
<td>8</td>
<td>No mutations</td>
<td>No mutations</td>
<td>Negative</td>
<td>0% Normal</td>
<td>123%</td>
<td>Negative</td>
<td>ND</td>
</tr>
<tr>
<td>9</td>
<td>No mutations</td>
<td>No mutations</td>
<td>Negative</td>
<td>9% Abnormal</td>
<td>77%</td>
<td>Positive (both assays)</td>
<td>ND</td>
</tr>
<tr>
<td>10</td>
<td>c.1699A&gt;G, p.Arg567Gly</td>
<td>No mutations</td>
<td>Negative</td>
<td>0% Normal</td>
<td>0% Abnormal</td>
<td>Positive (both assays)</td>
<td>2.03 mg/l</td>
</tr>
<tr>
<td>11</td>
<td>No mutations</td>
<td>No mutations</td>
<td>Negative</td>
<td>0% Normal</td>
<td>130%</td>
<td>Positive (C3CSAP)</td>
<td>0.21 mg/l</td>
</tr>
</tbody>
</table>

\(^a\) FH antibodies\(^b\) and Hemolytic assay\(^b\): Presence or absence of FH antibodies and complement hemolytic activity, respectively.

\(^c\) APFA: Anti-Polyepitope fluorescent antibodies.

\(^d\) sMAC: Soluble MBL-associated complement C1r/C1s, Human Normal Serum, and MASP-1.
### Atypical APGN

#### Light microscopy

<table>
<thead>
<tr>
<th></th>
<th>PIGN</th>
<th>aPIGN</th>
<th>C3GN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diff. prol.</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Mes. prol.</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>MPGN</td>
<td>-</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>Crescentic</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

#### Immunofluorescence

<table>
<thead>
<tr>
<th></th>
<th>PIGN</th>
<th>aPIGN</th>
<th>C3GN</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3 capill.</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>C3 mesang.</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>IgG</td>
<td>++</td>
<td>+</td>
<td>+/-</td>
</tr>
</tbody>
</table>

#### Electron microscopy

<table>
<thead>
<tr>
<th></th>
<th>PIGN</th>
<th>aPIGN</th>
<th>C3GN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Humps</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Mesangial</td>
<td>+/-</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Sub-endoth.</td>
<td>+/-</td>
<td>++</td>
<td>+++</td>
</tr>
</tbody>
</table>

*Adapted from Sethi et al, Kidney International 2013*
Does C1q and C4d staining help?

Classical Pathway
- Ig bound to antigen
  - C1
  - C4
  - C2

Lectin Pathway
- MBL/ficolin bound to carbohydrate on pathogen
  - MASP
  - C4
  - C2
  - C3 activation

Alternative Pathway
- Presence of pathogen
  - C3
  - B
  - D
Does C1q and C4d staining help?

Sethi et al, JASN 2015

Proliferative glomerulonephritis

- **Immune-complex mediated GN**
  - Ig ++/+++  
  - C3 +/++/+++  
  - C1q +/+/-/+  
  - C4d +/-/+/-
  - CP activation (Eg: Autoimmune disease, monoclonal Ig, infections*)

- **Complement-mediated GN**
  - C3 ++/+++  
  - Ig 0/+
  - C1q 0/trace
  - C4d ++/+++  
  - LP activation (Eg: Infections, IgA nephropathy, membranous nephropathy, monoclonal Ig**)
  - LP/CP activation, possibly in addition to AP (Eg: C3 glomerulopathy triggered by infections, autoimmune disease, monoclonal Ig***)

- AP activation (Eg: C3 glomerulopathy driven by AP abnormalities***)

* Eg: Autoimmune disease, monoclonal Ig, infections
** Eg: Infections, IgA nephropathy, membranous nephropathy, monoclonal Ig
*** Eg: C3 glomerulopathy triggered by infections, autoimmune disease, monoclonal Ig
**** Eg: C3 glomerulopathy driven by AP abnormalities
# Immunofluorescence studies in C3G

<table>
<thead>
<tr>
<th>C3</th>
<th>IgG</th>
<th>C1q</th>
<th>C4d</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>+++</td>
<td></td>
<td></td>
<td></td>
<td>C3G</td>
</tr>
<tr>
<td>+++</td>
<td></td>
<td></td>
<td>+/-</td>
<td>Probable C3G triggered by infection</td>
</tr>
<tr>
<td>+++</td>
<td>+/-</td>
<td></td>
<td></td>
<td>Possible C3G – consider 2\textsuperscript{nd} biopsy if no response to treatment or if persistently low C3</td>
</tr>
<tr>
<td>+++</td>
<td>+/-</td>
<td></td>
<td>+/-</td>
<td>C3G unlikely – consider 2nd biopsy if no response to treatment or if persistently low C3</td>
</tr>
<tr>
<td>+++</td>
<td>+++</td>
<td></td>
<td></td>
<td>C3G very unlikely – consider 2nd biopsy if no response to treatment or if persistently low C3</td>
</tr>
<tr>
<td>+++</td>
<td>+++</td>
<td></td>
<td></td>
<td>C3G extremely unlikely</td>
</tr>
</tbody>
</table>
C3 glomerululopathy

✓ **spectrum** of related kidney disorders

✓ autoimmune or genetic causes

✓ unifying glomerular finding: predominant deposition of C3 cleavage fragments

✓ deregulation of the alternative complement pathway

✓ distinct from classical post infectious glomerulonephritis

✓ includes different histological subgroups

Zipfel et al, Molecular Immunology (2015)

D’Agati and Bomback, 2012; Fakhouri et al., 2010; Sethi and Fervenza, 2011; Chen et al., 2014; Martinez- Barricarte et al., 2010; Servais et al., 2012; Sethi et al., 2012a; Tortajada et al., 2013; Zhang et al., 2012; Alchi and Jayne, 2010; Kanjanabuch et al., 2009; Rodriguez-Iturbe and Musser, 2008; Tejani and Ingulli, 1990; Walker et al., 2007
C3 glomerulonephritis with predominant C3 depositions

Adapted from Zipfel et al, Molecular Immunology (2015)
Extrarenal features

Partial lipodystrophy

Macula

Drusen

Lipids & proteins
A simplified view of the complement system

OFTEN INFECTION

Zipfel and Skerka, Nat Rev Immunol 2009
C3 convertase

TED : thioester-containing domain
fB - SP: serine protease domain
fB - VWA:von Willebrand factor type A domain

Rodríguez de Córdoba S et al., Biochem Biophys Acta, 2011
Control of the C3 convertase
Autoimmune forms of C3G

C3 nephritic factor (C3NeF):
- Differences in the stabilization of the C3 convertase
- May disappear spontaneously

CFH (mini-)antibody

CFB autoantibody
## Genetic forms of C3G

<table>
<thead>
<tr>
<th>Gene / Protein</th>
<th>Mutation / Variant</th>
<th>Function</th>
<th>Phenotype</th>
<th>Reference</th>
</tr>
</thead>
</table>
| CFH            | Mutations:        | - Intact surface binding  
- Reduced C3b binding  
- Loss of CFH cofactor and decay accelerating activity | DDD C3GN | Levy, Kidney Int 1986  
Vogt, Pediatr Nephrol 1995  
Ault, J Biol Chem 1997  
Dragon-Durey, J Am Soc Nephrol 2004  
Licht, Kidney Int 2006  
Habbig, Kidney Int 2009 |
| CFH            | Polymorphisms:    | - Impaired C3b / heparin binding  
- Impaired CFH cofactor activity | DDD | Hageman, Proc Nat Acad Sci 2005  
Abrera-Abeleda, J Med Genet 2006  
| CFHR3-1        | CNV:              | - Not tested  
- Dominant negative effect | C3GN | Malik, J Am Soc Nephrol 2012 |
| CFHR5          | CNV:              | - Not tested  
- Dominant negative effect | CHFR-GN | Gale, The Lancet 2010 |
| CFHR5          | Polymorphisms     | - Not tested | DDD | Abrera-Abeleda, J Med Genet 2006  
| C3             | Mutations:        | - C3\textsubscript{mut} resistant to cleavage by C3bBb  
- C3\textsubscript{mut} convertase – resistant to CFH inactivation | DDD | Martinez-Barricarte, J Clin Invest 2010 |
| C3             | Polymorphisms     | - Not tested | DDD | Smith, J Am Soc Nephrol 2007  
Risk of developing Ig-MPGN or C3G according to genotype

### Physiopathology of DDD/C3GN

<table>
<thead>
<tr>
<th>C3 Nephritic factor</th>
<th>Anti Factor H autoantibody</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Diagram of C3 Nephritic factor" /></td>
<td><img src="image2" alt="Diagram of Anti Factor H autoantibody" /></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Genetic deficiency of Factor H</th>
<th>Gain of function C3 mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image3" alt="Diagram of Genetic deficiency of Factor H" /></td>
<td><img src="image4" alt="Diagram of Gain of function C3 mutations" /></td>
</tr>
</tbody>
</table>

Adapted from Smith et al. Mol Immunol 2011
CFHR5 Nephropathy

• **Clinical presentation**
  - Persistent microscopic hematuria
  - Recurrent macroscopic hematuria
  - Progressive renal failure

• **Genetics**
  - Autosomal dominant transmission pattern
  - Identified in families of Cypriot origin
  - Heterozygous duplication of exons 2-3 of CFHR5

• **Histology**
  - Strong glomerular C3 deposition
  - No Ig deposition
  - Similar to C3GN

• **Pathophysiology**
  - C3 convertase stabilization (in the GBM)

Gale et al, The Lancet 2010
GC3 and SC5b-9 according to C3NeF positivity

C3 levels in C3G

C3 convertase dysregulation: DDD>C3GN
C5 convertase dysregulation: C3GN>DDD

Servais et al, Kidney Int 2012
Yuzhou Zhang et al. CJASN 2014
Complement dysregulation in kidney diseases

Systemic

Local

DDD

CFHR-GN
C3GN
aPIGN

aHUS

C3

C5bC9
Treatment of DDD / C3GN

- No established therapy

- Some cases may spontaneously improve
  Some patients have a relapsing course

- Genetic forms:
  - immnosuppressive drugs (PDN, CsA, MMF) may be beneficial in some cases, in particular if evidence of renal inflammation
  - anecdotal reports and retrospective cohort studies;
    1 relatively large Spanish cohort showing benefits with MMF (Rabasco et al, KI 2015)

- Autoimmune forms:
  respond to PEX, rituximab, immunosuppression

- Anti-C5 may be beneficial in some, but not all patients and is very expensive
Complement-targeting therapies

- Classical
- Lectin
- Alternative

DISEASE
- IC-MPGN
- C3G
- IgAN
- IMN

COMPLEMENT-TARGETING THERAPIES
- Soluble CR1
- Anti-Factor B
- Anti-Factor D
- Anti-Properdin
- Anti-C3 (Comstain)
- Aurin tricarboxylic acid

- AAV
- GPA
  - Anti-C5
  - C5 siRNA
  - C5aR antagonist (CCX168)

- aHUS
  - Anti-C5a
  - Anti-C5
  - Aurin tricarboxylic acid

Courtesy Christoph Licht
Not so good, but cases of permanent remission exist
Nearly 50% of patients have CRF after 10 years

- Negative prognostic factors:
  - nephrotic range proteinuria
  - CRF
  - DDD

- High risk of relapse after transplantation
  If relapse: 50% of graft losses within 5 years
Risk factors of poor long-term outcome

Multivariate analysis of the association of long-term renal outcome with clinical, laboratory and genetic features.

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>HR</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absence of mutations or C3NeFs</td>
<td>7.1</td>
<td>1.9–26.3</td>
<td>0.004</td>
</tr>
<tr>
<td>Sclerotic glomeruli (% of glomeruli)</td>
<td>69.3</td>
<td>3.1–1553</td>
<td>0.008</td>
</tr>
<tr>
<td>Crescents (% of glomeruli)</td>
<td>39.7</td>
<td>3.3–481</td>
<td>0.004</td>
</tr>
<tr>
<td>Nephrotic syndrome at onset</td>
<td>10.9</td>
<td>2.5–47</td>
<td>0.002</td>
</tr>
</tbody>
</table>

HR: hazard ratio calculated by Multivariate Cox proportional-Hazards analysis. CI: confidence Interval. nc: not calculable. Nephrotic syndrome was defined as: 24-h proteinuria exceeding 3.5 g in adults or 40 mg/h/m2 in children together with albuminemia ≤3 g/dL. Intensified immunosuppression was also included in multivariate Cox Regression analysis but was not significantly associated with progress to ESRD (HR = 3.9, 95%CI 0.65–23.9, p = 0.138).
## C3G: OPBG experience on 32 pediatric patients

<table>
<thead>
<tr>
<th>Clinical presentation (number of patients)</th>
<th>Urine</th>
<th>Biopsy</th>
<th>Therapy</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M.E.</td>
<td>C3GN</td>
<td>DDD</td>
<td>NoR</td>
</tr>
<tr>
<td>Acute GN (13)</td>
<td>100%</td>
<td>92%</td>
<td>8%</td>
<td>0%</td>
</tr>
<tr>
<td>Nephrotic syndr. (11)</td>
<td>18%</td>
<td>91%</td>
<td>9%</td>
<td>0%</td>
</tr>
<tr>
<td>Random urine (8)</td>
<td>0%</td>
<td>50%</td>
<td>50%</td>
<td>13%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>None</th>
<th>ACEi</th>
<th>PDN</th>
<th>MMF/CsA</th>
<th>ECUL.</th>
<th>PR</th>
<th>NoR</th>
<th>CR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute GN (13)</td>
<td>31%</td>
<td>62%</td>
<td>77%</td>
<td>0%</td>
<td>0%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nephrotic syndr. (11)</td>
<td>0%</td>
<td>100%</td>
<td>100%</td>
<td>82%</td>
<td>18%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Random urine (8)</td>
<td>13%</td>
<td>88%</td>
<td>50%</td>
<td>13%</td>
<td>0%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Thank you!