C3 Glomerulopathy

Rezan Topaloglu, MD
Hacettepe University
School of Medicine
Department of Pediatric Nephrology
Ankara, TURKEY
Journey in history

• Some diseases have journey
  – Diagnoses may change during this journey by understanding pathophysiology more deeply
    • Eg. Mesangial proliferation+IgA dominancy=IgA nephropathy

• Membranoproliferative glomerulonephritis
  – Thickening of the capillary; «membrane»
  – Mesangial enlargement; «proliferative»

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
Historical classification superseded by pathological classification

• Based on IF staining

• Cases characterized by C3 deposition
  – C3 glomerulopathy

Consensus:
Glomerulonephritis with dominant C3
Intensity of C3 staining at least two orders of magnitude

Kidney Int 2013;84:1079-1089 C3 glomerulopathy consensus report
Primary glomerulonephritis with isolated C3 deposits: a new entity which shares common genetic risk factors with haemolytic uraemic syndrome

Aude Servais, Véronique Frémeaux-Bacchi, Moglie Lequentrec, Rémi Salomon, Jacques Blouin, Bertrand Knebelmann, Jean-Pierre Grünfeld, Philippe Lesavre, Laure-Hélène Noël, Fadi Fakhouri

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
C3 glomerulopathy

- Introduced in 2007
- Glomerulonephritis that is characterized by accumulation in glomeruli of C3 or its metabolites without marked deposition of C1q or C4 and with minimal or no Ig deposits
- Implies activation of alternative complement pathway
- Distinct from aHUS
  - AP activation occurs on glomerular endothelium

In order to keep the «system» in check and to prevent inappropriate activation of the alternative pathway, a number of inhibitory proteins exist. The two most important circulating inhibitors are CFH and CFI.

*Nephrol Dial Transplant 2014 Sep;29 Suppl 4:iv131-41*
• In C3 glomerulopathy, pathogenesis

• Several causes have been identified
  – Congenital absence of factor H
  – Mutations in factor H
  – Autoantibodies against factor H
  – Genetic mutations in C3 that makes it resistant to inhibition by factor H
  – Mutations in CFHR5
  – C3 nephritic factor

*Nephrol Dial Transplant 2013; 28: 1685-1693*
Role of AP in Pathogenesis

• 134 patients with MPGN type1, C3 glomerulopathy and DDD;
  – $CFH$; 16.6%
  – $CFI$; 17.2%
  – $CD46$; 19.6%

Role of C3 nephritic factor

- Autoantibody
- Binds to a neoepitope on the C3 convertase
- Stabilizes C3 convertase against CFH-mediated decay
- Potentiates its C3 cleaving action
- RESULT: Uncontrolled C3 activation and low C3

*Nephrol Dial Transplant 2013; 28: 1685-1693*
C3 nephritic factor

• Common in DDD
  – 80-90% of cases

• Less common in C3 glomerulonephritis
  – 40-60% of cases

Polysaccharide, endotoxin, IgA aggregates
C3
B, D, Mg²⁺
C3bBb
(alternative pathway C3 convertase)
Properdin
stabilize
C3NeF
I, H
C3b
C3 feedback

ReCook HT F1000Res 2017 Mar 10; 6:248
• Not only C3 nephritic factor
• Autoantibodies to factor H, factor B, or C3b have been identified
CFHR5 Nephropathy

- Form of C3 glomerulonephritis
- OD inheritance among Cypriot families (internal duplication within CFHRP5 gene)
- Microscopic hematuria and synpharingitic macroscopic hematuria in half of the affected individuals
- Serum C3 levels were almost normal
- LM; mesangioproliferative/membranoproliferative pattern
- EM; subendothelial, mesangial and occasional subepithelial deposits
- Progression to ESRD is common in adulthood and occurs mostly in males

*Lancet* 2010;376: 794-801
CFHR Mutations

• Mutations in other CFHR genes have also associated with C3 glomerulopathies
  – Hybrid CFHR1-3, familial C3 glomerulonephritis
  – Internal duplication in the CFHR1 gene;

Am J Kidney Dis 2002; 40, E1
J Clin Invest 2013; 132: 2434-2446
C3GP Glomerular lesions

DDD
G3GN
Glomerular Lesions in DDD

68 cases of DDD

4 distinct patterns

MPGN; 25%
Crescentic; 18%
Mesangial proliferative; 45%
Acute proliferative/exudative; 12%

Mod Pathol 2007; 20: 605-616
Nature 2015; 11:11-22
Glomerular Lesions in C3GN

59 cases of C3 glomerulonephritis

- MPGN; 52%
- Crescentic; 5%
- Mesangial proliferative; 24%
- Diffuse proliferative/exudative; 19%

*Mod Pathol* 2007; 20: 605-616
Immunofluorescence

- C3 deposition
- Detected only with antibody against the C3 breakdown fragment, C3c
- Reasons of Ig on C3 glomerulopathy
  - Trapping in sclerotic areas
  - Occurrence of Ig on podocytes
  - Initiation of the disease by IC
- Consensus:
  - Glomerulonephritis with dominant C3
  - Intensity of C3 staining at least two orders of magnitude

*Kidney Int* 2013; 84: 1079-89
For differential diagnosis EM is needed

Very dense deposits in the central part of BM in a ribbon-like fashion
Globular deposits in the mesangium
Similar deposits are seen in Bowman capsules and tubular BM

C3 glomerulopathy that lack distinctive appearance of DDD
ill defined electron densities within the basement membrane or mesangium
Deposits in subendothelial/subepithelial locations

Kidney Int 2012; 81: 434-41
Kidney Int 2013;84:1079-1089 C3 glomerulopathy consensus report
Postinfectious glomerulonephritis

• It is GN with dominant C3
• Self limiting GN
• Bx; diffuse endocapillary GN with subepithelial hump-like deposits
• IF; glomerular staining for IgG and C3 but some cases show C3 only
• EM subepithelial IC deposits

Nephrol Dial Transplant 2013; 28: 1685-1693
Postinfectious glomerulonephritis

• Postinfectious glomerulonephritis patients, with declining renal functions or persistent hypocomplementemia should be investigated for C3 glomerulopathy
Clinical manifestations

<table>
<thead>
<tr>
<th></th>
<th>DDD</th>
<th>C3 glomerulonephritis</th>
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</thead>
<tbody>
<tr>
<td>Pediatric onset (&lt;16 years)</td>
<td>43-58%</td>
<td>25-54%</td>
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<tr>
<td>Mean age at onset (years)</td>
<td>19±18</td>
<td>30±19</td>
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<tr>
<td><strong>Clinical presentation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nephrotic syndrome</td>
<td>38-43%</td>
<td>27-44%</td>
</tr>
<tr>
<td>Microscopic hematuria</td>
<td>76%</td>
<td>65%</td>
</tr>
<tr>
<td>Arterial HT</td>
<td>21-60%</td>
<td>40%</td>
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<tr>
<td>Serum creatinine &gt;1.5 mg/dl</td>
<td>29%</td>
<td>50%</td>
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<tr>
<td>Low C3 (&lt;75 mg/dl)</td>
<td>59-79%</td>
<td>40-48%</td>
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<tr>
<td><strong>Long term outcome</strong></td>
<td></td>
<td></td>
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<tr>
<td>Duration to ESRD (years)</td>
<td>10±11</td>
<td>11±10</td>
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</tbody>
</table>

Clinical manifestations

- Clinical presentations are non-specific, requires high index of suspicion
- Different presentations are also seen
  - CFHR5 associated C3 glomerulonephritis
    - Persistent microscopic hematuria
    - Synpharyngitic gross hematuria
    - Strong family history of ESRD

Hypocomplementemia

- Immune complex MPGN;
  - Complement activation occurs via classical pathway
  - C3, C4, C1q decreases
- Complement mediated MPGN-C3GP
  - Complement activation occurs via alternative pathway
  - C3 usually low,
  - C4 normal

Hypocomplementemia

- French series (n=116)
  - Low C3
    - DDD; 59%
    - C3 glomerulonephritis; 39.6%
  - Low C4
    - DDD; 15%
    - C3 glomerulonephritis; 36.3%

- English series (n=80)
  - Low C3
    - DDD; 79%
    - C3 glomerulonephritis; 48%

Extrarenal findings on C3GN

- Retinal Drusen lipids & proteins
- Acquired partial lipodystrophy is most commonly seen in individuals with C3 nephritic factors

Dalvin LA et al. Retin Cases Brief Rep 2016; 10: 72-78
Treatment

• No standard treatment for patients with MPGN or C3GN
• Mainly based on small-size single center studies/case reports/expert opinions
• Angiotensin converting enzyme inhibitors (ACEi) or angiotensin II receptor antagonists are used in many patients
  – Antiproteinuric
  – Antihypertensive

Immunsuppressive therapy

• Steroids
  – Long term, low dose
  – Only some group of patients (formerly MPGN 1)
  – First line in patients with Ig mediated glomerulonephritis with nephrotic range proteinuria
  – No beneficial effect was shown in DDD

Immunosuppressive therapy

- Mycophenolate mofetil
  - Alone or in combination with prednisone in idiopathic MPGN
  - Steroid resistant primary MPGN, addition of MMF resulted in sustained improvement in renal function and proteinuria
  - Beneficial effects in MPGN 1
  - Effect on DDD or C3 glomerulonephritis?

Immunsuppressive therapy

• Calcineurin inhibitors
  – Prednisone resistant MPGN patients
  – Refractory MPGN, with low dose prednisone therapy, resulted in reduction of proteinuria and stable renal function in 94%
  – In two patients with DDD, low-dose prednisone and cyclosporine A was able to induce remission

• The detection of C3 nephritic factor has leaded the use of B-cell depleting agents
  – Rituximab
  – Several case reports, especially in patients with immune complex mediated disease, RTX resulted in partial/complete remission (in addition to steroids)
  – In DDD, RTX resulted in decrease in C3 nephritic factor but no change in proteinuria or renal functions (both rescued with eculizumab)

Complement targeting therapy

• Therapeutic inhibition of C3 or C5
• In DDD, several cases are reported of successful treatment with eculizumab
• However, unsuccessful treatment with eculizumab was also reported

<table>
<thead>
<tr>
<th>Patient</th>
<th>References</th>
<th>Response</th>
<th>Biopsy</th>
<th>Native/Tx</th>
<th>Gender</th>
<th>Age (y)</th>
<th>Disease duration (y)</th>
<th>UProt/UCreat (mg/mg)</th>
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<tr>
<th>Patient</th>
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<td>ND</td>
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<td>Stable</td>
<td>Improved</td>
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<td>7</td>
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<td>–</td>
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<td>8</td>
<td>McCaughan et al(^{23})</td>
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<td>Bomback et al(^{19})</td>
<td>±</td>
<td>Worsened</td>
<td>ND</td>
<td>More sclerosis</td>
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</table>

• Open label, non-blinded study
• 6 adult C3 glomerulopathy patients
• Total period of 53 weeks
• Eculizumab was well tolerated
• Improvement in renal function was observed in 2/6 patients with elevated sMAC levels

Still debatable

• Treatment with eculizumab for C3 glomerulopathy should be started early before major sclerotic modifications occur
• Elevated C5b-9 levels may be an indicator of patients who can respond to treatment
• Eculizumab may be also beneficial in patients with advanced renal damage
For all patients

- Optimal blood pressure control (suggested blood pressure below the 90% in children and ≤ 120/80 mm Hg in adults)
  - Priority agents include angiotensin converting enzyme inhibitors and angiotensin receptor blockers
- Optimal nutrition for both normal growth in children and healthy weight in adults
- Lipid control

Moderate Disease

- Description
  Urine protein over 500 mg/24 h despite supportive therapy or moderate inflammation on renal biopsy or recent increase in serum creatinine suggesting risk for progressive disease
- Recommendation
  - Prednisone
  - Mycophenolate mofetil
Severe disease

• Description
  1. Urine protein over 2000 mg/24 h despite immunosuppression and supportive therapy OR
  2. severe inflammation represented by marked endo- or extracapillary proliferation with or without crescent formation despite immunosuppression and supportive therapy OR
  3. increased serum creatinine suggesting risk for progressive disease at onset despite immunosuppression and supportive therapy

• Recommendation
  Methylprednisolone pulse dosing as well as other anti-cellular immune suppressants have had limited success in rapidly progressive disease. Data are insufficient to recommend eculizumab as a first-line agent for the treatment of rapidly progressive disease.
C3 targeted intervention
“Achilles heel”

- Anti C5 therapy; not satisfactory in the majority of C3 glomerulopathy patients
- C3; ideal candidate for complement modulation
- Inhibiton abrogates the formation of C3 and C5
- Next generation peptidic C3 inhibitors of the compstatin family
  - Scr1 (CDX-1135, Celldex)
  - Cp40
  - AMY-101 (Amyndas)

Mastellos DC, et al. Trends In Immunology 2017; 38: 383-393
Prognosis

**DDD**
- In a series of 98 patients from North America, 50% progressed to ESRD within 10 years of diagnosis
- Poor prognosis
  - Gender (female>male)
  - Crescent

**C3 glomerulonephritis**
- Similar to DDD
- Depends on underlying pathogenesis

Transplantation

• 18 transplants in DDD, 11 kidneys recurred
• Greater transplant recurrence in DDD compared to MPGN type 1 or MPGN type 3
• Some studies showed similar recurrence of DDD and C3 glomerulonephritis (60% vs 54.5%)

Genetic and clinical characteristics of patients with C3 glomerulopathy

Topaloglu R¹, Gulhan B¹, Korkmaz E², Duzova A¹, Ozaltin F¹,²
C3 Glomerulopathy Study Group*

¹Hacettepe University School of Medicine Department of Pediatric Nephrology
²Hacettepe University School of Medicine Nephrogenetics Laboratory

* C3 Glomerulopathy Study Group: Esra Baskın, Oğuz Söylemezoğlu, Mehmet Bülbül, Nur Canpolat, Osman Dönmez, Gürkan Genç, Nilüfer Göknar, Umut Bayrakçı, Birsin Özçakar, Alper Soylu

To the leading edge... Toward being the best...
At the time of biopsy

- 19 patients with histopathological diagnosis of C3G
  - 9 female, 10 male

- Mean age of biopsy;
  - 12.3±3.6 years

- Electron microscopy was available in 8 patients (42%)
  - C3 glomerulonephritis; 5 patients
  - DDD; 3 patients
At the time of biopsy

- Proteinuria (9 patients)
  - Nephrotic; 6 patients
  - Non-nephrotic; 3 patients
- Serum Albumin levels were low in 14 patients (Range 1.0-3.3 g/dL); in 8 patients ≤ 2.5 g/dL
- Microscopic/macroscopic hematuria in 18 patients
- GFR was low in 6 patients (Range 7.9-65 ml/min/1.73m2)
- Serum C3 level was low in 15, normal in 4
- C3 nephritic factor could be performed in 4 patients
  - found positive in 3 patients
• Genetic analyses were performed in 18/19 patients
• No variation was found in 2 patients for the corresponding genes
• 16 patients had at least one variation
<table>
<thead>
<tr>
<th>Variations</th>
<th>CFB</th>
<th>CFH</th>
<th>CFHR5</th>
<th>CFI</th>
<th>THBD</th>
<th>C3</th>
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<tbody>
<tr>
<td>No. of patients</td>
<td>8</td>
<td>7</td>
<td>6</td>
<td>3</td>
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</table>

- Variation in 1 gene; 10 patients
- Variation in 2 genes; 3 patients
- Variation in 3 genes; 2 patient
- Variation in 4 genes; 1 patient
Treatment

- Mean duration of follow-up was 2.0±1.8 year (Range 3 months-5 year)
- At the last visit
  - Only ACEi or ARB; 4 patients
  - Steroid±ACEi/ARB; 7 patients
  - Steroid+MMF±ACEi/ARB; 3 patients
  - Steroid+Cyclosporine+ARB; 1 patient
  - Steroid+MMF+Eculizumab: 1 patient
  - Eculizumab: 1 patient (transplanted)
Treatment

• Eculizumab (6 patients)
  – Initiated and continued 2 patients
  – Initiated and discontinued; 4 patients
    • Eculizumab was given to patients, 1, 3, 4 and 8 doses each and then stopped
At the last visit

<table>
<thead>
<tr>
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<th>Complete remission</th>
<th>Partial remission</th>
<th>Non-response</th>
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<tbody>
<tr>
<td>Number of Patients</td>
<td>3</td>
<td>7</td>
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<tr>
<td>Most common mutation</td>
<td>CFHR5</td>
<td>CFHR5</td>
<td>CFH</td>
<td>CFB</td>
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</table>
Thank you
1 - Which is **wrong** for C3 Glomerulopathy?

a. Glomerulonephritis with dominant C3 deposition comprise DDD, C3GN and Post infectious GN

b. It Implies activation of Classical Complement pathway

c. Distinct from aHUS because AP activation occurs on glomerular endothelium

d. Mutations in Factor H could cause C3Glomerulopaty
2 - Which is **wrong** for C3 Glomerulopathy?

a. C3 nephritic factor could cause C3 glomerulopathy

b. Not only the mutations but autoantibodies to factor H could cause C3 Glomerulopathy

c. MPGN pattern could be seen in light microscopy

d. e. For differential diagnosis of DDD from C3 Glomerulonephritis Electron microscopy is not needed
3 - Which is wrong for MPGN (Membrano proliferative GN) and C3 glomerulopathy?

a. Historical Classification of MPGN comprise MPGN type 1, MPGN type 2 (DDD) and MPGN type 3

b. In MPGN type 2 immune dense deposits are seen in tubules with ribbon like appearance

c. Post sterptococal Glomerulonephritis consider as self limiting form of C3 Lomerulopathy

d. In C3 glomerulopathy treatment therapeutic inhibition of C3 could be a new treatment options
Conclusion Remarks

• From clinical point of view in our series renal outcome is generally favorable in the patients
• We were able to find several variations in genes encoding complement regulatory proteins with next generation sequencing
• However, further studies are needed to clarify whether these variations are relevant
• As we may miss intronic variations with panel screening, whole genome sequencing may give more precise genetic results