CLINICAL PHENOTYPE & ASSESSMENT

Fernando C. Fervenza, MD
Division of Nephrology and Hypertension
Mayo Clinic College of Medicine
Rochester, MN
Disclosure of Interests

In the last 12 months I have received unrestricted research grants/support from

- Genentech Inc
- Biogen Idec
- Mallinckrodt Pharmaceuticals
- Sanofi Pharmaceuticals

Honorarium

- Up-To-Date
- American Board of Internal Medicine
- Journal of the American Society of Nephrology
Clinical Phenotype & Assessment

1. What tests are required to diagnose C3G?
2. How should these patients be followed?
3. Can we predict disease course?
4. How does pathology correlate with phenotype?
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2. How should these patients be followed?
3. Can we predict disease course?
4. How does pathology correlate with phenotype?
C3 Glomerulopathy
Acquired and genetic complement abnormalities play a critical role in dense deposit disease and other C3 glomerulopathies

Aude Servais¹,², Laure-Hélène Noël³,⁴, Lubka T. Roumenina⁵, Moglie Le Quintrec⁵, Stephanie Ngo⁶, Marie-Agnès Dragon-Durey⁵,⁷, Marie-Alice Macher⁸, Julien Zuber²,⁹, Alexandre Karras¹⁰, François Provoit¹¹, Bruno Moulin¹², Jean-Pierre Grünfeld¹,², Patrick Niaudet²,⁷, Philippe Lesavre¹,² and Véronique Frémeaux-Bacchi⁵,⁶

C3 glomerulonephritis: clinicopathological findings, complement abnormalities, glomerular proteomic profile, treatment, and follow-up

Sanjeev Sethi¹,⁶, Fernando C. Fervenza²,⁶, Yuzhou Zhang³, Ladan Zand², Julie A. Vrana¹, Samih H. Nasr¹, Jason D. Theis¹, Ahmet Dogan¹ and Richard J.H. Smith³,⁴,⁵

¹Division of Anatomic Pathology, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, USA; ²Division of Nephrology and Hypertension, Department of Internal Medicine, Mayo Clinic, Rochester, Minnesota, USA; ³Molecular Otolaryngology and Renal Research Laboratories, Divisions of Nephrology, Carver College of Medicine, Iowa City, Iowa, USA; ⁴Department of Internal Medicine, Division of Nephrology, Carver College of Medicine, Iowa City, Iowa, USA and ⁵Department of Pediatrics, Division of Nephrology, Carver College of Medicine, Iowa City, Iowa, USA
Complement component assessment according to histological type

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>MPGN 1</th>
<th>DDD</th>
<th>GNC3</th>
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<tbody>
<tr>
<td>N</td>
<td>115</td>
<td>41</td>
<td>22</td>
<td>53</td>
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<tr>
<td>C3 (660 to 1250 mg/l)</td>
<td>621.91 ± 339.5</td>
<td>583.1 ± 360.7</td>
<td>492.8 ± 337.7</td>
<td>705.4 ± 305.2</td>
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<tr>
<td>Low C3 (&lt;660 mg/l)</td>
<td>53 (46.1%)</td>
<td>19 (46.3%)</td>
<td>13 (59.1%)</td>
<td>21 (39.6%)</td>
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<td>C4 (93 to 380 mg/l)</td>
<td>227.9 ± 86.3</td>
<td>198.4 ± 65.7</td>
<td>204.8 ± 88.9</td>
<td>260.8 ± 89.3</td>
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<tr>
<td>Low C4 (&lt;93 mg/l)</td>
<td>2 (1.7%)</td>
<td>1 (2.4%)</td>
<td>1 (4.5%)</td>
<td>0</td>
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<tr>
<td>Factor B (90 to 320 mg/l)</td>
<td>116.4 ± 49.3</td>
<td>110.9 ± 42.2</td>
<td>112.6 ± 39.9</td>
<td>122.2 ± 57.7</td>
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<tr>
<td>Low factor B (&lt;90 mg/l)</td>
<td>34 (29.6%)</td>
<td>14 (34.1%)</td>
<td>6 (27.3%)</td>
<td>14 (26.4%)</td>
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<tr>
<td>Low factor H (&lt;338 mg/l)</td>
<td>8 (6.9%)</td>
<td>2 (4.9%)</td>
<td>4 (18.2%)</td>
<td>2 (3.8%)</td>
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<tr>
<td>Low factor I (&lt;42 mg/l)</td>
<td>3 (2.6%)</td>
<td>3 (7.3%)</td>
<td>0</td>
<td>0</td>
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<tr>
<td>C3NeF</td>
<td>65 (58.6%)</td>
<td>22 (53.6%)</td>
<td>19 (86.4%)</td>
<td>24 (45.3%)</td>
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<tr>
<td>Unexplained C3 (&lt;660 mg/l)</td>
<td>6 (5.2%)</td>
<td>1 (2.4%)</td>
<td>0</td>
<td>5 (9.4%)</td>
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</table>

Abbreviations: C3NeF, C3 nephritic factor; DDD, dense deposit disease; GNC3, glomerulonephritis with isolated C3 deposits; MPGN, membranoproliferative glomerulonephritis.

*Normal values are indicated in brackets.
*C3NeF determination was not available in four patients.

Patients under immunosuppressive therapy at the time of complement assessment were excluded from this analysis (N=19). Mean ± s.d., number (percentage).
C3 Glomerulopathy

Clinical course not typical for post-infectious glomerulonephritis

Screen for a paraproteinemia in patients over 50 years old

Test for autoantibodies and protein deficiencies
- C3 nephritic factors
- FHAA
- FBAA
- FH level
- FB level
- Properdin level
- CD46 expression on leukocytes
- Western blot for FHR proteins

Assess serum complement activity
- Alternative pathway functional assay
- Hemolytic assay
- C3 breakdown products
- C5 breakdown products
- Terminal complement cascade activity

Screen for genetic mutations and copy number variation
- CFH
- CFI
- MCP
- C3
- CFB
- CFHR1-5
- Copy number variation across CFH-CFHR region

Current therapeutic options are based on limited case reports and a single unblinded, uncontrolled trial

Remove or replace abnormal proteins
- Plasma exchange
- Plasma infusion
- Prednisone
- Anti-cellular therapy
  - Rituximab
  - Mycophenolate mofetil

Reinstate complement control
- Anti-complement therapy
  - Anti-C5
  - C3 convertase inhibitors

Replace defective or absent gene product
- Plasma infusion
- Protein-specific targeting
  - FH replacement

Smith et al 2014
<table>
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<th><strong>aHUS</strong></th>
<th><strong>C3 glomerulopathy</strong></th>
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<td><strong>Functional assays</strong></td>
<td>CH50, AP50, FH function</td>
<td>CH50, AP50, FH function</td>
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<td><strong>Quantification of</strong></td>
<td>C3, C4, FI, FH, FB, MCP</td>
<td>C3, C4, FI, FH, FB, Properdin</td>
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<td><strong>complement components</strong></td>
<td>C3d, Bb, sMAC</td>
<td>C3d, Bb, sMAC</td>
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<td><strong>and regulators</strong></td>
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<td>Antigen (anti-FH)</td>
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<td><strong>Measurement of</strong></td>
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<td>C3Nef, anti-FH, anti-FB</td>
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<td><strong>complement activation</strong></td>
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<td><strong>markers</strong></td>
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<td><strong>Autoantibodies</strong></td>
<td>anti-FH</td>
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<td><strong>Genetic testing</strong></td>
<td>C3, CFH, CFI, CFB, MCP, CFHR1-5, THBD, DGKE</td>
<td>C3, CFH, CFI, CFB, CFHR1-5</td>
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<td><strong>Plasma cell disorder</strong></td>
<td>Serum free light chains, serum and urine electrophoresis and immunofixation</td>
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<td><strong>Immunofluorescence</strong></td>
<td>IgA, IgG, IgM, C1q, C3, fibrinogen, kappa, lambda (usually all negative, with thrombi positive for fibrinogen)</td>
<td>IgA, IgG, IgM, C1q, C3, fibrinogen, kappa, lambda, C4d (usually bright C3, negative or minimal Ig, negative C4d)</td>
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<td><strong>studies on kidney biopsy specimen</strong></td>
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Angioi, Smith…De Vriese - Kidney Int (in press)
**DO THE SAME MUTATION TRANSLATES TO THE SAME PHENOTYPE?**

**THE R1210C CFH MUTATION: ONSET AND OUTCOME**

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<th>DD*</th>
<th>DG*</th>
<th>D9*</th>
<th>LU</th>
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<th>GS</th>
<th>ZM</th>
<th>BV</th>
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<td>8</td>
<td>3</td>
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<td><strong>Outcome (1st episode)</strong></td>
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<td><strong>Recurrences</strong></td>
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<td><strong>Long term outcome</strong></td>
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*these patients also carry mutations in MCP*

Caprioli J. et al., 2006
Clinical Phenotype & Assessment

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Clinical Phenotype & Assessment

Will depend on:

a. Renal function
b. Proteinuria
c. Hypertension
d. Laboratory markers?
e. Others?
Making sense of inflammation: with rapid quantification of complement split products

Complement activation pathways converge on C3/iC3b
Complement is a major mediator of the body’s inflammatory response to infection, tissue injury, or autoimmune disease\(^1\). There are three complement activation pathways, the classical, lectin, and alternative pathways which are activated by a variety of substances including antigen-antibody complexes, subcellular and nuclear components of damaged cells and pathogen associated macromolecules \(^2\). All three complement pathways converge to cleave C3, the central component of the system. C3 is cleaved into several proteolytic activation fragments including iC3b. As a reliable and time-sensitive marker of complement activation, iC3b\(^3,4\) represents a valuable biomarker for complement-mediated inflammation. Furthermore, iC3b is a potent inflammatory modulator itself\(^5\). C3 is an acute phase protein whose production increases during inflammation, but its measurement in blood may remain low depending on the relative levels of production and consumption. Monitoring both C3 and iC3b simultaneously provides an iC3b/C3 ratio that may help differentiate changes in C3 metabolism due to complement activation.

Complement autoactivation in vitro
Complement split product measurements have been plagued by inconsistency due to in vitro autoactivation of complement\(^6,7\). Complement can also be activated by common materials used in sample collection and storage\(^8,9\) making it difficult to obtain reliable data. As a result, the clinical utility of complement-based diagnostics has not been fully realized.

Kypha’s lateral flow assay (LFA)*
Kypha’s C3 and iC3b tests solve this problem by bringing complement diagnostics to a point-of-care platform\(^10\). Almost any biofluid sample (blood, plasma, serum, cerebrospinal fluid, etc.) is first diluted in a proprietary buffer that prevents complement activation during the assay**. The sample is then transferred to the test cartridge and placed into the reader for analysis. The results are available just minutes after sample preparation and can be exported in a variety of easily usable formats.
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Serum Creatinine at Presentation and Follow-up (in months) of All Patients

Sethi et al. Kidney Int 2012
Clinical Phenotype & Assessment

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Spectrum of proliferative GN

Diffuse proliferative GN

Acute

Chronic

MPGN
Questions & Discussion