Learning Objectives

• Familiarize with the pathogenetic mechanisms of glomerular diseases
• Learn the pathologic landscape and clinical course of C3 glomerulopathies
• Explore the underlying pathogenetic mechanisms of C3 glomerulopathies
• Venture into the unknown and therapy
Percutaneous needle kidney biopsies
Historical context

- Iversen and Brun (1951), Denmark
- Alwall (1952), Sweden
- Kark and Muehrcke (1952), University of Illinois, Chicago
- Pirani (early 50’s), pathology interpretation, University of Illinois, Chicago
Light Microscopy (LM)
Antibody mediated glomerular injury

Deposition of circulating immune complexes

- Subepithelial (at outer surface of glomerular basement membranes)
- Subendothelial (at inner surface of glomerular basement membranes)
- Mesangial

In situ binding of antibodies with or without immune complex formation

- Antibody against antigen on podocytes
- Anti-glomerular basement membrane antibody
- Subepithelial immune complex formation (membranous glomerulonephritis)
- Anti-glomerular basement membrane glomerulonephritis (no immune complexes formed)

Granular or linear immunofluorescence
The hunt for the immune complexes
Mechanisms of glomerular diseases

- Binding of antibodies to cellular or extracellular glomerular constituents
- Glomerular immune complex deposition or in situ immune complex formation
- Binding of antigens to glomerular cells or matrix
- Complement activation via classical pathway
- Complement activation via alternative and mannose binding lectin pathway
- Local complement activation via alternative pathway due to complement dysregulation
- Glomerular recruitment of neutrophils and monocytes
- Glomerular injury via C5b-9 complex
- Glomerular injury via proteases and oxygen-derived free radicals
- Proteinuria, hematuria and structural damage
Mechanisms of glomerular diseases

• Immune mechanisms
  – Circulating Immune Complexes
  – Immune Complexes Formed In Situ
  – Anti-GBM Antibody–Mediated
  – Caused by Abnormal Complement Activation

• Mediators of immune injury
  – Complement and leukocytes

• Podocyte injury

• Nephron loss
Algorithm of Morphologic Interpretation of Glomerular Patterns

The True Glomerulonephritides

A. Is there glomerular hypercellularity (with narrowing of capillary lumens)?

- **GLOMERULAR HYPERCELLULARITY** (usually nephritic)

  - **INTRA-CAPILLARY** or **EXTRA-CAPILLARY** (crescents) Crescentic GN

B. Is the hypercellularity:

- **INTRA-CAPILLARY** or **EXTRA-CAPILLARY** (crescents) Crescentic GN

C. Is the hypercellularity:

1) Acute Postinfectious GN
2) Membranoproliferative GN (Types 1,2,3)

or

- **FOCAL** Global or Segmental

  Focal GN
  (SLE, Collagenvascular diseases infective endocarditis etc.)

The Non-Glomerulonephrititic Glomerulonephrophathies

- **DIFFUSE**
  - **MESANGIUM NORMAL** Minimal Change Nephrotic Syndrome
  - **DIFFUSE MESANGIAL PROMINENCE** Diffuse Mesangial Hypercellularity or **DIABETIC NEPHROPATHY**

- **SEGMENTAL**
  - **MESANGIAL SCLEROSIS** Focal segmental sclerosis

- **MEMBRANOUS**
  - **GLOMERULO-NEPHROPATHY**

- **MINIMAL GLOMERULAR HYPERCELLULARITY** (usually nephrotic)
Diagram showing the classification of MPGN types I, II, and III, including previous and current classification criteria:

**Previous classification**
- **MPGN Type I**
  - Light Microscopy: Mesangial proliferation with GBM duplication (PMGN pattern)
  - Electron Microscopy: Mesangial and subendothelial
  - Immunofluorescence: C3 +/- IgG and/or IgG, C1
  - Complement activation: Classical

**Current classification**
- **MPGN type I**
- **MPGN type II or DDD**
  - Diverse glomerular histology with/without PMGN
  - Electro-dense mesangial and intramembranous
  - Complement activation: Classical
- **MPGN type III**
  - Histological pattern: Mesangials, subendothelial, subepithelial, and/or intramembranous
  - Complement activation: Classical
Membranoproliferative GN

- Types I, II and III
- Children and young adults
- Nephrotic and nephritic
- Rare
- Secondary forms
  - older adults
  - most commonly associated with hepatitis C
MPGN, 1

Endocapillary proliferation

Mesangial matrix accumulation
MPGN, 1

Endocapillary proliferation

Basement membrane reduplication
MPGN, 1

IgG/C3
MPGN, type I, secondary forms

• Infections
  – Viral, bacterial, parasitic
• SLE
• Liver diseases
  – Hepatitis (C & B)
  – Cirrhosis or fibrosis (with or without hepatitis)
• Portosystemic shunt
  – With dominant IgA2 deposits
Classic MPGN type 1 vs Cryoglobulinemic GN
Antibody mediated glomerular injury

Deposition of circulating immune complexes

- Subepithelial (at outer surface of glomerular basement membranes)
- Subendothelial (at inner surface of glomerular basement membranes)

In situ binding of antibodies with or without immune complex formation

- Antibody against antigen on podocytes
- Anti-glomerular basement membrane antibody

Mesangial

- Subepithelial immune complex formation (membranous glomerulonephritis)
- Anti-glomerular basement membrane glomerulonephritis (no immune complexes formed)

Granular or linear immunofluorescence
Dense Deposit Disease (MPGN, Type 2)
Dense Deposit Disease (MPGN, Type II)
Dense deposit disease (DDD) (Type 2, MPGN)

• Recognized by transmission electron microscopy (1962)
• C3 glomerular deposition and low serum C3 levels attributed to the activation of the alternative pathway of complement (1975)
• In the 1980s, several reports in affected families indicated a genetic basis for some cases of DDD

Dense Deposit Disease (Type 2, MPGN)
Clinical presentation

• Nephritic, proteinuria, hematuria, renal insufficiency
• Post tx recurrence common; outcome generally favorable
• Monoclonal gammopathy in older patients
Dense Deposit Disease (MPGN, Type 2)
Etiology

• Associated with complement dysregulation
  – C3NF (anti-C3 convertase)*
  – Genetic
    • Homozygous Factor H mutations
    • Heterozygous Factor H, I, MCP mutations*

• Low serum C3 (but not C4) common, but does not correlate with disease activity
C3 nephritic ‘factor’ (C3NeF)

- Existence inferred from the accelerated C3 breakdown *in vitro* following the addition to normal human serum of serum obtained from patient with persistent hypocomplementecmic GN

DDD, IF & Mass Spec

- 41% C3 only (without Ig)
- 59% dominant C3 with up to 1+ IgM
  - 80% dominant C3 of X2 orders of magnitude of intensity by IF greater than any other immune reactant

- Complement C3, MAC components, CFHR5, vitronectin and apolipoprotein E

- Absence of CFB
  - consistent with AP C3 convertase formation with excessive C3 activation in the fluid phase, with subsequent deposition of C3 breakdown products

Mechanisms of glomerular diseases

- **Immune mechanisms**
  - Circulating Immune Complexes
  - Immune Complexes Formed In Situ
  - Anti-GBM Antibody–Mediated
  - Caused by Abnormal Complement Activation

- **Mediators of immune injury**
  - Complement and leukocytes

- **Podocyte injury**

- **Nephron loss**
MPGN type 3
C3 Glomerulonephritis

MPGN type III

MPGN type

Glomerulopathies

C3

Previous classification

Light Microscopy

Electron Microscopy (location of deposits)

Immunofluorescence

Complement activation

Current classification

MPGN Type I

MPGN Type II or DDD

MPGN Type III

MPGN

Classical

Glomerulopathies C3

Current classification

MPGN type I

MPGN type III
C3 glomerulopathies

• dense deposit disease (DDD) (Formerly MPGN type 2)
• C3 glomerulonephritis (C3GN)
• FHR5 nephropathy
C3 glomerulopathies

• Key histological feature
  – isolated complement C3 glomerular deposits with no or little immunoglobulins

• Etiology
  – dysregulation of the alternative pathway of complement
  – genetic defects and/or autoantibodies are identified in a proportion of patients
Autoantibodies in C3 glomerulopathies

C3NeF
• Common in DDD, less so in C3GN
• Levels do not correlate with the course of nephritis in DDD
• Nonspecific
  — frequent in MPGN Type 1 and rarely in LN or individuals without renal disease

Other autoantibodies
• To CFB (one patient with DDD) - stabilizes AP C3 convertase
• To CFB and C3b (two patients with DDD)
• Anti-CFH monoclonal light chains or (possibly monoclonal) Ig, inhibitory (DDD and C3GN)
• CFH autoantibodies (C3GN)
C3 GN: Genetic abnormalities

• Original French C3GN series
  – heterozygous mutations in the CFH, CFI and MCP genes
• Additional cases of DDD, C3GN, and MPGN Type 1
  – homozygous CFH, heterozygous CFH and CFI mutations
• DDD (50%), C3GN (27%) and MPGN Type 1 (17%)
  – CFH, CFI, CD46 and C3

DDD vs. C3GN IF (C3c)
DDD vs. C3GN EM
DDD vs. C3GN EM
C3GN: Mesangial and subepithelial deposits
C3 GN with conventional mesangial deposits

C3 GN with DDD-like mesangial deposits
C3 glomerulonephritis and dense deposit disease share a similar disease course in a large United States cohort of patients with C3 glomerulopathy

Over an average of 72 months of follow-up, remission occurred in 38% of patients with C3GN and 25% of patients with dense deposit disease.

Progression to late-stage CKD and ESRD was common, with no differences between C3GN (39%) and dense deposit disease (42%).
C3 GN vs PIGN

• Differentiation of PIGN from C3 GN often cannot be made on the basis of morphology and clinical and laboratory data available at the time of biopsy

• Clinical and serologic follow up over several months to determine the course of urinary abnormalities and serum C3 levels

• If these parameters do not follow a typical course of PIGN (i.e., normalization of the decreased peripheral C3 level in 8–12 weeks), a diagnosis of C3 GN should be reconsidered and additional investigations performed

Morphological appearance

- Glomerulonephritis with dominant C3

Disease category

- C3 glomerulopathy
  - DDD
    - Specific genetic forms and/or autoantibodies
  - C3 GN
    - Specific genetic forms for example CFHR5 nephropathy and/or autoantibodies
- Post-infectious GN
- Other

Not otherwise specified
Potential significance/caviats of C3/C5b-9 fragment detection

• Various C3 fragments (C3b, C3c, C3dg) can mediate distinct biological responses through their interactions with complement receptors

• Glomerular and tubular BM deposits of C5b-9 may persist in repeat renal biopsies of C3 GN and DDD 1 year after initiation of Eculizumab therapy despite the normalization of serum C5b-9 levels

• C5b-9 may be present in glomeruli of normal kidneys
Case Challenge

- The patient is a 41-year-old woman with a history of systemic plasmacytosis (On prednisone last year for five months)
- Creatinine 1.3
- Has low grade proteinuria (~1gr/24h) and no hematuria
Audience Response

What would you do next?

A. Do nothing
B. Do a kidney biopsy
C. I’m not sure
Biopsy findings: C3 GN, mesangial variant
C3 GN, mesangial variant

lambda

kappa
Mesangial and subepithelial deposits
C3 GN: Morphologic manifestations

• Mild mesangial proliferative GN
• MPGN
• Crescentic GN
Recurrent C3GN post transplant
CFHR5 nephropathy (Familial C3GN)

- Caused heterozygous internal duplication of the CFHR5 gene in Cypriot families (autosomal dominant)
- Mesangioproliferative or MPGN pattern GN
- Subendothelial and mesangial deposits with occasional subepithelial deposits by EM
- Microscopic haematuria and episodes of synpharyngitic macroscopic haematuria (~50%)
- Serum C3 levels almost invariably normal
- Progression to ESKD common in adults (mostly in males)
- Ten patients with successful transplantation and one other with disease recurrence

Treatment

• BP control and antiproteinuric therapy (ACE inhibitors)
• Steroids, other immunosuppressants
• Long-term plasma infusion
• Administration of CFH (if it becomes available)
• Therapeutic inhibition of complement C3 or C5
  – successful and unsuccessful treatment with Eculizumab in both C3GN and DDD
C3 glomerulonephritis secondary to mutations in factors H and I: rapid recurrence in deceased donor kidney transplant effectively treated with eculizumab.

From: C3 glomerulonephritis secondary to mutations in factors H and I: rapid recurrence in deceased donor kidney transplant effectively treated with eculizumab
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Recurrent allograft C3 glomerulonephritis and unsuccessful eculizumab treatment


- C3Nef positive with C3 activation only
- Analyzing C3Nef-mediated C3 and C5 activation separately could help in choosing the right patients for eculizumab therapy
Thank you!