NEXT GENERATION LEARNING

2016 ANNUAL MEETING

March 12-18 Seattle, Washington

USCAP
Creating a Better Pathologist
ACCME/Disclosures

The USCAP requires that anyone in a position to influence or control the content of CME disclose any relevant financial relationship WITH COMMERCIAL INTERESTS which they or their spouse/partner have, or have had, within the past 12 months, which relates to the content of this educational activity and creates a conflict of interest.

Dr. Christopher Larsen declares he has no conflict(s) of interest to disclose.
Membranous-like Glomerulopathy with Masked IgG Kappa Deposits

Chris Larsen, MD

Renal Pathology Society Companion meeting, USCAP
March 2016
Paraffin Immunofluorescence

• Originally described by Fogazzi et al (Pathol Res Pract 1989)
• Introduced to the practice of renal pathology by Dr. Vivette D’Agati (Columbia University, NY)
• Salvage technique in renal pathology when frozen tissue for routine IF is inadequate
• Protease digestion is used for antigen retrieval
**Immunofluorescence on pronase-digested paraffin sections: A valuable salvage technique for renal biopsies**

SH Nasr, SJ Galgano, GS Markowitz, MB Stokes, and VD D'Agati

Department of Pathology, Columbia University, College of Physicians and Surgeons, New York, New York, USA

---

**Table 2| Percentage of cases in which diagnostic IF-P findings**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>% of cases with diagnostic findings on IF-P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membranous glomerulopathy</td>
<td>4/8 (50%)</td>
</tr>
<tr>
<td>Membranoproliferative glomerulonephritis</td>
<td>3/5 (60%)</td>
</tr>
<tr>
<td>Lupus nephritis</td>
<td>5/5 (100%)</td>
</tr>
<tr>
<td>Acute post-infectious glomerulonephritis</td>
<td>5/5 (100%)</td>
</tr>
<tr>
<td>IgA nephropathy</td>
<td>7/8 (88%)</td>
</tr>
<tr>
<td>Cryoglobulinemic glomerulonephritis</td>
<td>5/5 (100%)</td>
</tr>
<tr>
<td>Fibrillary glomerulonephritis</td>
<td>5/5 (100%)</td>
</tr>
<tr>
<td>Anti-GBM disease</td>
<td>1/5 (20%)</td>
</tr>
<tr>
<td>Myeloma cast nephropathy</td>
<td>5/5 (100%)</td>
</tr>
<tr>
<td>Primary amyloid</td>
<td>5/5 (100%)</td>
</tr>
<tr>
<td>Light-chain deposition disease</td>
<td>4/5 (80%)</td>
</tr>
<tr>
<td>Light-chain Fanconi syndrome</td>
<td>10/10 (100%)</td>
</tr>
<tr>
<td>All cases</td>
<td>59/71 (83%)</td>
</tr>
</tbody>
</table>
Fourteen cases over two year period
Mean age 26 years
12 female/2 male
62% with “vague” autoimmune disease

Membranous-like glomerulopathy with masked IgG kappa deposits

Christopher P. Larsen¹, Josephine M. Ambruze¹, Stephen M. Bonsib¹, Christie L. Boils¹, Larry N. Cossey¹, Nidia C. Messias¹, Fred G. Silva¹, Yihan H. Wang¹, Neriman Gokden² and Patrick D. Walker¹

¹Nephropath, Little Rock, Arkansas, USA and ²University of Arkansas for Medical Sciences, Little Rock, Arkansas, USA
Comparison with proliferative glomerulonephritis with monoclonal IgG deposits and monoclonal membranous glomerulopathy

<table>
<thead>
<tr>
<th>Dx (n)</th>
<th>Clinical</th>
<th>LM</th>
<th>IF</th>
<th>Deposit location</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean age (range)</td>
<td>&lt;40 (n; %)</td>
<td>Autoim (n; %)</td>
<td>Endo (n; %)</td>
</tr>
<tr>
<td>PGNMID (19)</td>
<td>67.3 (29-84)</td>
<td>2 (11)*</td>
<td>1 (5)*</td>
<td>18 (95)</td>
</tr>
<tr>
<td>MonoMG (9)</td>
<td>69.2 (48-87)</td>
<td>0 (0)*</td>
<td>0 (0)*</td>
<td>0 (0)</td>
</tr>
<tr>
<td>MGMT (14)</td>
<td>25.7 (15-49)</td>
<td>13 (93)</td>
<td>8 (62)*</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>
Membranous-like Glomerulopathy with Masked IgG Kappa Deposits

Update:
41 patients
Mean age (range)= 27.6 (10-73)
32 females and 9 males
90% Proteinuria was indication for biopsy
Membranous-like Glomerulopathy with Masked IgG Kappa Deposits

<table>
<thead>
<tr>
<th>LM Pattern</th>
<th># (%) of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>20 (49%)</td>
</tr>
<tr>
<td>Mesangial proliferative</td>
<td>8 (20%)</td>
</tr>
<tr>
<td>Crescentic/Focal Crescentic</td>
<td>7 (17%)</td>
</tr>
<tr>
<td>FSGS</td>
<td>6 (15%)</td>
</tr>
</tbody>
</table>
Membranous-like Glomerulopathy with Masked IgG Kappa Deposits

<table>
<thead>
<tr>
<th>Antibody</th>
<th>IF-F % positive (mean intensity among positive)</th>
<th>IF-P % positive (mean intensity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA</td>
<td>5% (1+)</td>
<td>5% (1+)</td>
</tr>
<tr>
<td>IgM</td>
<td>17% (0.9+)</td>
<td>22% (0.9+)</td>
</tr>
<tr>
<td>IgG</td>
<td>15% (0.8+)</td>
<td>100% (2.9+)</td>
</tr>
<tr>
<td>C3</td>
<td>78% (2+)</td>
<td>NA</td>
</tr>
<tr>
<td>Kappa</td>
<td>17% (1+)</td>
<td>100% (2.9+)</td>
</tr>
<tr>
<td>Lambda</td>
<td>10% (1+)</td>
<td>12% (1+)</td>
</tr>
<tr>
<td>PLA2R (n=29)</td>
<td>NA</td>
<td>0</td>
</tr>
<tr>
<td>THSD7A (n=5)</td>
<td>NA</td>
<td>0</td>
</tr>
</tbody>
</table>
Membranous-like Glomerulopathy with Masked IgG Kappa Deposits

- 18/41 cases fulfilled criteria for C3 glomerulopathy prior to paraffin IF
- Among cases with IgG staining by IF-F 6/6 were IgG1 only
Membranous-like Glomerulopathy with Masked IgG Kappa Deposits

<table>
<thead>
<tr>
<th>Electron microscopy finding</th>
<th>% of cases (n=41)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subepithelial deposits</td>
<td>98%</td>
</tr>
<tr>
<td>Mesangial deposits</td>
<td>95%</td>
</tr>
<tr>
<td>Subendothelial deposits</td>
<td>10%</td>
</tr>
<tr>
<td>Subepithelial ‘humps’</td>
<td>59%</td>
</tr>
<tr>
<td>Hinge-region deposits</td>
<td>59%</td>
</tr>
</tbody>
</table>
Membranous-like Glomerulopathy with Masked IgG Kappa Deposits

Clinical
• Normal SPEP
• Typically <40 years
• Frequent evidence of autoimmune disease

Pathologic
• Monotypic deposits
• Significantly brighter staining with paraffin IF
• Mesangial and/or subepithelial deposits without subendothelial
• No endocapillary proliferation
Paraffin immunofluorescence in the renal pathology laboratory: more than a salvage technique

Nidia C Messias, Patrick D Walker and Christopher P Larsen

Nephropath, Little Rock, AR, USA

304 (6.1%) cases

207 (68%) salvage

87 (42%) necessary or significantly contributed to diagnosis

120 (58%) no change in diagnosis

97 (32%) unmasking

22 (23%) necessary or significantly contributed to diagnosis

75 (77%) no change in diagnosis
Among 20 cases with masked glomerular Ig:

- 9 cases of membranous-like glomerulopathy with masked IgG kappa deposits
- 7 cases with mixed essential cryoglobulinemia
- 4 cases of MPGN with monoclonal Ig
Membranoproliferative glomerulonephritis with masked monotypic immunoglobulin deposits

Christopher P. Larsen¹, Nidia C. Messias¹, Patrick D. Walker¹, Mary E. Fidler², Lynn D. Cornell², Loren H. Hernandez², Mariam P. Alexander², Sanjeev Sethi² and Samih H. Nasr²

¹Nephropath, Little Rock, Arkansas, USA and ²Division of Anatomic Pathology, Mayo Clinic, Rochester, Minnesota, USA

• 16 cases of MPGN pattern with masked monoclonal Ig deposits
  - 9 from Nephropath
  - 7 from Mayo Clinic
Renal Characteristics

- 9 F and 7 M, mean age 62 yrs.
- Renal presentation: renal insufficiency (88%, mean Scr 2.7 mg/dl), proteinuria (100%, mean 7.1 g/day), nephrotic syndrome (81%), and hematuria (100%)
- Hypocomplementemia in 67%
- Neg testing for hep B&C in all, pos cryo in 2 (13%), weakly pos ANA in 1 (6%)
MPGN with Masked Monotypic Ig Deposits

Hematologic Characteristics

• SPEP
  – 14 (88%) had M-Ig on SPEP/SIF that matched the glomerular paraprotein detected by IF-P
  – 2 (12%) had neg SPEP/SIF but had a clonal B-cell population on bone marrow biopsy

• Bone marrow biopsy abnormal in 13 (81%)
  – 9 with plasma cell dyscrasia (including 6 with <10% monoclonal plasmacytosis)
  – 4 with clonal B-cell populations
    2 lymphoplasmacytic lymphoma
    1 CLL
    1 small clonal low grade B-cell population
MPGN with Masked Monotypic Ig Deposits

Pathology

• Light Microscopy- All 16 cases had an MPGN pattern (focal crescents in 25%)

• Electron Microscopy
  – Texture of deposits: granular (non-organized) in 10, microtubular in 3, vague fibrillar in 3, crystalloidal in 2
  – Location of deposits: subendo (100%), mes (75%), subepi (38%)
MPGN with Masked Monotypic Ig Deposits

Pathology

• Routine Immunofluorescence
  10 (63%) consistent with “C3 glomerulonephritis”
  (sole staining for C3 by routine IF-F)

• Paraffin Immunofluorescence
  12 IgGκ
  2 IgGλ
  1 IgMλ
  1 IgMκ
MPGN with Masked Monotypic Ig Deposits

Mayo Clinic: 36% of C3 GN with monoclonal gammopathy cases had underlying masked deposits (6/6 with DDD were negative for masked deposits)

Nephropath: 50% of MPGN cases with C3-only in adults proved to have masked deposits

C3 GN in adults is a diagnosis of exclusion only after masked deposits have been excluded by paraffin IF
MPGN with Masked Monotypic Ig Deposits

**Treatment and Outcome**

- F/U available in 10 pts
- Mean F/U 12.2 mos (range 2-27)
- Most pts treated with chemotherapy directed against the underlying hematologic neoplasia, if present
- Proteinuria improved in 62%, stabilized in 25%, worsened in 12%
- Serum Cr. improved in 50%, stabilized in 20%, and worsened in 30% (1 progressed to ESRD)
Why do some monotypic deposits mask?

• Antigenic epitopes sequestered in the tertiary or quaternary structure of paraprotein?
• Proteins washed off of the slide during IF-F while they are held in place during IF-P due to formalin-induced cross linking?
• Charge-charge interaction with antibody and/or slide?
When is paraffin IF indicated?

Salvage technique
- Inadequate tissue for IF

Unmasking
- Light chain proximal tubulopathy with negative crystal staining for $\kappa$ and $\lambda$ by IF-F
- Numerous subepithelial deposits with little to no Ig staining by routine IF
- MPGN with little to no staining for immunoglobulins by routine IF
- Discrepancy between deposits seen by LM or EM and staining pattern by IF
IF-P compared to standard IF-F for the diagnosis of paraprotein-related lesions

<table>
<thead>
<tr>
<th>Less sensitive</th>
<th>Comparable</th>
<th>More sensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-GBM disease</td>
<td>Membranous</td>
<td>Light chain proximal tubulopathy</td>
</tr>
<tr>
<td>MIDD</td>
<td>Fibrillary GN</td>
<td>Membranous-like glomerulopathy with masked IgGκ deposits</td>
</tr>
<tr>
<td>Ig-related amyloidosis</td>
<td>IgA nephropathy</td>
<td>MPGN with masked monoclonal deposits</td>
</tr>
<tr>
<td></td>
<td>Myeloma cast nephropathy</td>
<td>Cryoglobulinemic GN</td>
</tr>
</tbody>
</table>
References