Electron microscopy in renal transplant pathology

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EM in native kidney biopsies

• 21% - crucial for diagnosis
• 21% - important contribution

Mark Haas JASN 1996
<table>
<thead>
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<th>Table 1: Renal Biopsy Diagnoses Usually Requiring Electron Microscopy</th>
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<tbody>
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<td>Alport syndrome</td>
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<tr>
<td>Cryoglobulinemic glomerulonephritis$^a$</td>
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<td>Dense deposit disease</td>
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<td>Diabetic nephropathy—early morphological changes (GBM thickening)</td>
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<tr>
<td>Fabry’s and other lysosomal storage diseases$^b$</td>
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<tr>
<td>Fibrillar glomerulonephritis</td>
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<td>Focal-segmental glomerulosclerosis—early recurrence in renal allograft</td>
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<td>Immunotactoid glomerulopathy</td>
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<tr>
<td>Membranoproliferative glomerulonephritis type III$^c$</td>
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<td>Membranous nephropathy stages I and IV$^d$</td>
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<tr>
<td>Minimal change nephropathy</td>
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<tr>
<td>Post-infectious glomerulonephritis (except acute form with many glomerular neutrophils)</td>
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<td>Thin GBM nephropathy</td>
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EM in renal transplant biopsies

• main indication = Glomerular disease
  – Recurrent and *de novo* glomerulonephritis
  – Alloimmune transplant glomerulopathy
Glomerular disease in transplant

• What glomerular diseases do we see and how often?
• Does it cause graft loss?
• Can we do anything about it?
• Does EM help in the diagnosis?
Glomerular disease in transplant

• Rates of recurrence?

• Chailimpamontree et al. JASN 2009:
  – 5% at 5 years
  – 10% at 10 years
  – 15% at 15 years

  – Higher in patients with biopsy-proven pre-transplant GN (24% versus 10%)
Recurrent GN: FSGS, MPGN, IgA, D-HUS
De novo GN: FSGS, membranous, TMA
• Is it important?

• Glomerular disease important cause of late GL after death with functioning graft and rejection
• **ANZDATA 1988-1997**
• 1505 recipients with ESRD due to biopsy proven GN
• Allograft loss due to recurrent GN = 52 recipients
• **10-year** incidence of graft loss due to recurrence 8.4%
• Third most frequent cause after chronic rejection and death with a functioning graft

Briganti E et al NEJM 2002
Briganti E et al. NEJM 2002
• 153 grafts lost (excluding patient death)
• 56 due to glomerular disease
• 23 recurrent glomerular disease
  – 12 FSGS
  – 4 IgA
  – 3 Mb
  – 4 MPGN
• 10 glomerular disease ?recurrent/de novo (no native biopsy)
  – 7 FSGS
  – 1 MPGN
  – 2 unclassified
• 23 transplant glomerulopathy

El Zoghby Z et al. AJT 2009
• Are there potential therapies that modify the course of the glomerular disease post-transplantation?

• Little hard data on positive effect of prevention and treatment of recurrent glomerular disease and alloimmune transplant glomerulopathy
Recurrent FSGS

- 25-50%
- Early acute or late insidious
- Risk GL – x2.25
- Prevention and/or early diagnosis and treatment may improve outcome
Recurrent FSGS

- FPE can precede proteinuria and segmental scars on LM
- Recommend proteinuria screening; biopsy if increased proteinuria or graft dysfunction
- if normal LM - do EM for FPE

- Foot process effacement - Caution!
  - If FPE extensive (>80%) likely to be podocytopathy
Case

- F35
- Steroid-responsive FSGS
- Several steroid-resistant relapses between ages 42-54 (cyclosporin A, tacrolimus)
- ESRF, few months dialysis; LD Tx aged 54 (May 2012)
- Primary non-function
- Biopsy day 7: ATI, segmental FPE
- Slow function, creatinine down to 120 umol/L
- Increase in proteinuria (uPCR from 100 to 300mg/mmol)
- Biopsy day 40 – ATI, normal glomeruli
PE+ rituximab

Protein:creatinine ratio, urine

Albumin level, blood

Creatinine level, blood
Biopsy 1 year post transplant – no segmental sclerosis
Membranoproliferative pattern

- Double contours
- Differential diagnosis
  - ICGN
  - alloimmune transplant glomerulopathy
  - Other causes of TMA
    - CNI toxicity
    - Recurrent/de novo TMA
• M47
• ESRF due to IgA nephropathy
• Live unrelated transplant May 2007
• Alemtuzumab induction, tacrolimus monotherapy
• Baseline creatinine 115-130 umol/L
• September 2009: new anti-DQ7 DSA, progressive creatinine rise to 300umol/L, low grade proteinuria
Chronic antibody-mediated rejection
Banff Conference on Allograft Pathology 2009

- Histology - Microcirculation damage
  - Transplant glomerulopathy (capillary wall double contours)
  - Peritubular capillary basement membrane multilayering (electron microscopy)
  - Arterial intimal thickening
  - Interstitial fibrosis/tubular injury
- C4d positivity
- Serum donor-specific antibody (DSA)

Sis B et al AJT 2010
Figure 2: Kaplan-Meier plots of death-censored graft survival after conventional transplantation in patients without (---) and with TG (-----). Data generated from patients who received conventional kidney transplants at Mayo Clinic (N = 582, log-rank, p < 0.0001) [5].
Chronic antibody-mediated rejection

• No universally recognised treatment
• Unified criteria for inclusion of patients with cAMR
Case

• SLE – proliferative LN with crescents
• ESRF 1995, few months dialysis
• DD Tx 1996-2002 (0MM) lost to “recurrent disease”
• LRD father 2003 (1:1:1 MM)
• currently 10 years post-transplant
• Tacrolimus/MMF/prednisolone
• Joint pains, gout, NSAIDS
• Lupus serology negative
• Creatinine increase from 200 to 260 umol/L (progressive)
• uPCR 46 mg/mmol
50% TIF
4/15 gloms obsolete
• Thrombotic microangiopathy
  – ABMR
  – CNI toxicity
  – Recurrent HUS/TTP including antiphospholipid syndrome
• ACA briefly positive 2005, currently negative
• hypertensive
Lupus nephritis

- Histological recurrence – up to 50% with protocol bx (Norby et al.), mostly class I and II
- Clinical recurrence – 5%
- Effect on outcome debated - GL uncommon
- Contreras JASN 2010
  - Severe LN more common if black, female, young
- APS (Canaud et al AJT 2010)
  - Poor patient outcomes (thrombotic/haemorrhagic events)
  - Poor graft function
• **UTILITY OF IMMUNOFLUORESCENCE AND ELECTRON MICROSCOPY IN RENAL TRANSPLANT BIOPSIES**

• G Giannico, A Fogo et al. USCAP 2013 Abstract (unpublished data)

• 267 consecutive Tx bx

• Compared pre-biopsy clinical suspicion with post-biopsy diagnosis
  – 57% accurate clinical prediction
  – 23% final pathological diagnosis un-predicted
  – 19% partial correspondence; at least 1 new finding on biopsy
• Most common unpredicted diagnoses
  – TMA (C4d+ and negative)
  – de novo/recurrent GN and transplant glomerulopathy
    • FSGS, ; Mesangial IgG/IgM (?infection), IgA, LN, MPGN (cryo/infection), HUS, fibrillary GN
  – BKNP

• Most glomerular disease several years post tx (except FSGS)
• EM particularly useful for early FSGS recurrence
• Also useful for NODAT diabetic NP
- M52
- Type II DM/atherosclerosis
- LR (cousin) Tx 2006
- 6-month surveillance bx – normal
- Fluctuating creatinine 100-130 umol/L
- Low level de novo DSA antiDQ7
- Tacrolimus/MMF/pred
- HbA1c 46 mmol/mol
- Low level proteinuria (uPCR ~60 mg/mmol) now increasing to 200; albumin 38 g/L
Post transplant glomerular disease

- Alloimmune transplant glomerulopathy
- Recurrent/de novo glomerular disease
  - FSGS
  - MPGN
  - IgA
  - Membranous
  - SLE
  - Etc.
IgA nephropathy

- Histological recurrence 50-60% (including surveillance bx) (Fairhead Curr Op Nephrol Hypert 2010)
- Clinical recurrence 15-30%
- If crescentic 50% GL
- probably does lead to increased risk of graft loss – 15 to 20 years post transplant (Yu World J Surg 2012, Moroni NDT 2013)

- Early detection could provide basis for RCT
- IP usually sufficient for diagnosis
Membranous glomerulonephritis

- Recurrence up to 40% (protocol biopsies) (Dabade et al. AJT 2008)
- Early recurrence subclinical, normal LM, sometimes normal EM! - IF (Rodriguez et al. AJT 2012)
- Subclinical recurrence can progress to progressive proteinuric disease
- Increased GL
- High rates of PR/CR and resorption of deposits on EM after rituximab (El Zoghby et al. AJT 2009)
Recurrent membranous can be:

IF only
Clinically silent

Rodriguez 2012 AJT
EM in transplant biopsies

• Research applications
  – Better define incidence and impact of recurrent/de novo glomerular disease
  – Provide histological criteria for guiding RCT of post-transplant GN treatment
  – Natural history of glomerular disease
  – Pathogenesis of antibody-mediated damage
    • early glomerular and peritubular capillary changes
Imperial College Kidney and Transplant centre

• EM sample taken on most transplant biopsies
• 2012: 554 EM samples received, 146 analysed (26%)

• Indication for EM examination:
  
  – Mainly for abnormal LM histology (alone or with proteinuria or DSA)
    • Abnormal histology = g,cg,ptc, increased CW, MM or MC, FSGS
  
  – Few others for proteinuria alone, DSA alone or proteinuria+DSA
Conclusion

• Request a biopsy sample for EM in transplant biopsies
  – Known ESRF due to GN
  – Clinical suspicion of glomerular disease (proteinuria/haematuria)
  – Late post transplant biopsies
Thanks!

Imperial College Healthcare
NHS Trust
EM unit at Charing Cross Hospital
Prof T Cook
Dr Galliford
Dr Loucaidou
Dr Hill

Vanderbilt University
Prof Agnes Fogo
Giovanna Giannico
Graft survival in the first year post transplantation has improved (solitary renal allograft 1989-2009)

Lamb et al. AJT 2011; 11: 450
Graft half life (solitary renal allograft 1989-2009)
Includes C4d negative AMR
Understanding the Causes of Kidney Transplant Failure: The Dominant Role of Antibody-Mediated Rejection and Nonadherence

Sellares et al. AJT 2012;12:388-399
### Table 1. Value of electron microscopy in renal biopsy diagnosis of individual diseases*

<table>
<thead>
<tr>
<th>Primary final diagnosis</th>
<th>No. of cases</th>
<th>No. (%) of cases in each category</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Not required</td>
</tr>
<tr>
<td>Focal Segmental Glomerulosclerosis (includes collapsing glomerulopathy)</td>
<td>34</td>
<td>25 (74)</td>
</tr>
<tr>
<td>IgA Nephropathy and Henoch-Schönlein Nephritis</td>
<td>28</td>
<td>25 (89)</td>
</tr>
<tr>
<td>Diabetic Nephropathy</td>
<td>20</td>
<td>10 (50)</td>
</tr>
<tr>
<td>Pauci-Immune Crescentic GN (with or without vasculitis)</td>
<td>20</td>
<td>18 (90)</td>
</tr>
<tr>
<td>Membranous Nephropathy</td>
<td>18</td>
<td>11 (61)</td>
</tr>
<tr>
<td>Lupus Nephritis, WHO Class V (includes $V_e$ and $V_d$)</td>
<td>16</td>
<td>4 (25)</td>
</tr>
<tr>
<td>Lupus Nephritis, WHO Class IV</td>
<td>16</td>
<td>12 (75)</td>
</tr>
<tr>
<td>Lupus Nephritis, WHO Class III</td>
<td>7</td>
<td>2 (29)</td>
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<tr>
<td>Lupus Nephritis, WHO Class II</td>
<td>5</td>
<td>1 (20)</td>
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<tr>
<td>Minimal Change Nephropathy</td>
<td>12</td>
<td>2 (17)</td>
</tr>
<tr>
<td>Hypertensive Nephrosclerosis</td>
<td>10</td>
<td>5 (50)</td>
</tr>
<tr>
<td>Acute Interstitial Nephritis and Acute Pyelonephritis</td>
<td>9</td>
<td>9 (100)</td>
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<tr>
<td>Membranoproliferative GN (includes types I, II, and III)</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Amyloid Nephropathy (types AA and AL)</td>
<td>5</td>
<td>5 (100)</td>
</tr>
<tr>
<td>Post-Infectious GN</td>
<td>4</td>
<td>2 (100)</td>
</tr>
<tr>
<td>CIq Nephropathy</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Thin Basement Membrane Nephropathy</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Acute Tubular Necrosis</td>
<td>3</td>
<td>3 (100)</td>
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<tr>
<td>Anti-GBM Nephritis</td>
<td>2</td>
<td>2 (100)</td>
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<tr>
<td>HIV-Associated Nephropathy</td>
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<td>2 (100)</td>
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<tr>
<td>Light Chain Cast Nephropathy</td>
<td>2</td>
<td>1 (50)</td>
</tr>
<tr>
<td>No Pathologic Diagnosis</td>
<td>2</td>
<td>2 (100)</td>
</tr>
<tr>
<td>All Other Diagnoses (see Notes)</td>
<td>6</td>
<td>2 (33)</td>
</tr>
<tr>
<td><strong>All Cases</strong></td>
<td><strong>233</strong></td>
<td><strong>135 (58)</strong></td>
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*All data are from Mark Haas, JASN 1996.