Transmission electron microscopy in renal transplant pathology

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Transmission electron microscopy in renal transplant pathology

• Current accepted uses of electron microscopy in transplant biopsies – an update
  – Glomerular disease
  – Diagnosis of chronic ABMR

• Electron microscopy in antibody-mediated injury
  – A marker of activity, chronicity or both?
Includes C4d negative AMR

Sellares Am J Transplant 2012;12:388-399
El Zoghby Z et al. 2009 AJT 527-
Recurrent/de novo glomerular disease

• EM important for
  – Early FSGS – foot process effacement
  – **MPGN/C3 GN/DDD**
  – Early/low grade (thrombotic) microangiopathy
  – Early membranous – IF/EM only
  – Paraprotein-related/fibrillary disorders

• Helpful to confirm LM findings
• M69
• 2003: Recent onset of heavy proteinuria; mass found on ultrasound
• Native left nephrectomy 2003: renal cell carcinoma + MPGN (NOS)
• 2004: biopsy right kidney 2004 – MPGN; no EM sample; immunoperoxidase inconclusive (IgG, IgA negative)
• 2008: ESRF “due to IgA nephropathy”, renal transplant
• 2016: new onset proteinuria, DSA negative, slowly increasing creatinine from 120 umol/L to 200.
• Low level IgM kappa paraprotein
• No cryoglobulin detected
• BMT: 4% plasma cell population with aberrant CD56+ and CD117+
• Diagnosis of chronic antibody-mediated rejection
Banff 2013 - chronic, active antibody-mediated rejection

- Morphological evidence of chronic tissue injury
  - Transplant glomerulopathy, if no evidence of chronic TMA*
  - Severe PTCBML (requires EM)**
    - Arterial intimal fibrosis of new onset, excluding other causes
- Evidence of current/recent antibody interaction with vascular endothelium, including at least one of the following:
  - C4d (2/3 by IF; >0 by IP)
  - At least moderate microvascular inflammation (g+ptc = 2 or more)
  - Increased transcripts indicative of endothelial injury
- Serological evidence of donor specific antibodies (HLA or other antigens)

- *includes GBM duplication by EM only (cg1a)
- ** severe PTCBML defined as 1 cortical PTC with 7 or more layers of basement membrane (BM) plus 2 more PTC with 5 or more layers of BM
Banff 2013: recommendations for EM in transplant biopsies

- At centers with EM capability, ultrastructural studies should be performed in biopsies:
  - from patients who are sensitized
  - have documented DSA at any time posttransplantation and/or
  - who have had a prior biopsy showing C4d staining, glomerulitis and/or peritubular capillaritis
  
  - to determine if early changes of cAMR (cg1a/PTCBML) are present
• EM to be considered in
  – all biopsies @ 6 months post-transplantation
  – and in for-cause biopsies @ 3 months post-transplantation

– to determine if early changes of TG (including cg1a) are present, prompting testing for DSA
Banff Working Group for EM (Banff 2015)

- Evaluate current practices
- Standardize definitions and criteria for Cg1a and peritubular capillary multilamination
- Investigate the inter-observer variability on consensus criteria
- Investigate the prognostic significance of cg1a and ptcbml
Banff 2013 - methodology

• Based on 1 carefully examined glomerulus
• Cg1a
  – Ultrastructural double contours
  – Incomplete or circumferential
  – in more than ≥3 capillary loops
  – Associated with endothelial swelling and/or subendothelial electron-lucent widening
Banff 2013 - chronic, active antibody-mediated rejection

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** severe PTCBML defined as 1 cortical PTC with 7 or more layers of basement membrane (BM) plus 2 more PTC with 5 or more layers of BM
How many layers of basement membrane around PTC is diagnostic of cAMR?

- **Banff 2005** - 3 PTC with ≥ 5 layers
  - Ivanyi B (Human Pathology 2000) and Drachenberg C (Ultrastructural Pathology 1997)
- **Banff 2013** - 1 PTC with ≥ 7 + 2 PTC with ≥ 5
  - Liapis G (Transplantation 2012)
• **Current Banff guidelines:**
  – Evaluate 3 worst affected (non-scarred cortical interstitium between tubules)
  – PTCBML is focal – analysis should include at least 20 PTC
– Cg1a – early marker of chronicity
– Ptcbml – early or late marker of chronicity? Both?
Peritubular capillary basement membrane multilayering in early and advanced transplant glomerulopathy: quantitative parameters and diagnostic aspects

Deján Dobi¹ • Zsolt Bodó¹ • Éva Kemény¹ • László Bidiga² • Zoltán Hódi³ • Pál Szenohradszky³ • Edit Szederkényi³ • Anikó Szilvási⁴ • Béla Iványi¹

Cg1  n = 15
Cg2+3  n = 42

Virchows Arch 2016
• in the appropriate clinico-pathologic context (e.g. DSAs/C4d positivity/at least moderate microvascular inflammation)

• permissive threshold (1 PTC with 5 layers, mean PTCCirc ≥3.0) represents the earliest, prognostically relevant morphologic manifestation of chronicity due to antibody
In patients with a *de novo* DSA and a biopsy around 1 year post transplantation:

- $\geq 3$ ptc with $\geq 5$ layers associates with graft loss
- **Mean ptcbml of $>2.5$ or $\geq 10$ ptc with $\geq 3$ layers does not correlate with graft loss but does correlate with future TG**

De Kort et al Transplantation 2015
Detection of “activity” in antibody-mediated rejection
Pathophysiology of antibody-mediated rejection

Poulquen et al., 2015 F1000Prime Reports, 7:51
Zhang and Reed  *Am J Transpl* 2009
Chronic active antibody-mediated rejection

Acute/active antibody-mediated rejection

DSA
HLA or other

Interaction of antibody with endothelium

C4d
and/or
Increased endothelial transcripts
and/or
ptc+g≥2

Histology
ptc, g, v, TMA
Histology
cg, ptcbml, cv

EM

BANFF 2013
Chronic active antibody-mediated rejection

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- C4d
- Increased endothelial transcripts
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Acute/active Antibody-mediated Rejection

Histology

- ptc, g,v, TMA

Histology

- cg, ptcbml, cv

EM?

EM

BANFF 2013
Loss of fenestration

Endothelial cell swelling
Subendothelial rarefaction

Minimal crenellation

Extensive crenellation
• There is evidence of endothelial “activation” in biopsies with features of acute/active AMR.

• These features are associated with future development of features of chronicity (cg).
• Endothelial activation is also seen in biopsies that don’t show AMR, including surveillance biopsies

• The differences noted in patients with AMR are quantitatively
  – More widespread
  – More severe
• Future questions:
  – Which parameters/what scoring system?
  – Glomeruli and/or peritubular capillaries?
  – Does EM add to conventional LM features?
  – How does it compare (cost, PPV/NPV) to transcript analysis?
A Correlation of Ultrastructural Microvascular Features with Endothelial Cell Transcripts in Renal Transplant Biopsies

Linda Moran et al
Tuesday 27th September, 2016
15:05-15:15 Conference Room 5

A Comparison of Ultrastructural Features of Glomeruli and Peritubular Capillaries in Biopsies From Patients With De Novo Donor Specific Antibodies and with Surveillance Biopsies

Linda Moran et al
Wednesday 28th September, 2016
8:40-8:50 Conference Room 4
Tubuloreticular inclusions
Tubulo-reticular inclusions

- phospholipid and glycoprotein which arise from the rough endoplasmic reticulum
- induced in endothelial cells *in-vitro* and *in-vivo* by type I interferon (IFN)
- IFNs are cytokines produced in response to viral infections and autoimmunity
- Native kidney biopsy associations: SLE and HIV
• 316/1164 (27%) transplant biopsies examined by EM 2005-2014
• 41/316 (14%) TRI+
• 82 matched controls
Willicombe et al. JASN 2016
Willicombe et al. JASN 2016

P=0.002
• Multivariate analysis including variables significant [p<0.15] by univariate analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>95% CI</th>
<th>P Value</th>
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<tr>
<td>CI DSAs</td>
<td>3.16</td>
<td>1.20 to 8.36</td>
<td>0.02</td>
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<tr>
<td>Prior rejection</td>
<td>2.90</td>
<td>1.13 to 7.44</td>
<td>0.03</td>
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<td>Viral infection</td>
<td>2.66</td>
<td>1.01 to 7.00</td>
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<td>Microcirculation score ≥1</td>
<td>2.04</td>
<td>0.90 to 4.66</td>
<td>0.08</td>
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Willicombe et al. JASN 2016