Transmission electron microscopy in renal transplant pathology

On behalf of the Banff Working Group for Electron Microscopy
Dr Candice Roufosse
Dept Cellular Pathology
Imperial College Healthcare NHS Trust, London, UK
Transmission electron microscopy in renal transplant pathology

- Current accepted uses of electron microscopy in transplant biopsies
  - Glomerular disease
  - Diagnosis of chronic antibody-mediated rejection
**Acute/active antibody-mediated rejection**

- Histology: ptc, g, v, TMA

**Chronic active antibody-mediated rejection**

- DSA: HLA or other

- Interaction of antibody with endothelium:
  - C4d
  - and/or
  - Increased endothelial transcripts
  - and/or
  - ptc+g≥2

- EM
Pathophysiology of antibody-mediated rejection

Pouliquen et al F1000Prime Reports 2015
Lytic endothelial cell injury

Drachenberg and Papadimitriou Transplantation 2013
Pathophysiology of antibody-mediated rejection

Pouliquen et al. F1000Prime Reports 2015
- Multi-layering occurs as a result of successive bouts or on-going antibody-mediated injury to endothelium
- It increases progressively with time and results in graft fibrosis and dysfunction
Banff Working Group for EM (Banff 2015)

- Cg1a and PTCBML
  - Evaluate current practices
  - Investigate inter-observer variability
  - Standardize definitions and criteria
  - Investigate associations of cg1a and ptcbml with outcomes in a multi-centre study
• **Part 1:**
  • Survey of current practice
    – Working Group members
    – Wider renal/transplant pathology community
• **Part 2:**
• Evaluation of inter-observer reproducibility of current ultrastructural Banff criteria using a photo circulation
  – Thursday Banff Concurrent Kidney 2 (15:00 – 19:00) Sharan Singh
Banff Working Group for EM (Banff 2015)

— Part 1 – Survey of current practices

• **Spring 2016**
• Participants: n = 135 [28 from EM working group; 107 practicing pathologists from around the world]
Banff 2013 - methodology

Cg1a – How to score it:

- No double contours on LM
- ≥3 capillary loops on EM with
  - New basement membrane
    - Incomplete or circumferential
    - Single or multiple
  - Associated with endothelial swelling and/or subendothelial electron-lucent widening

Haas et al  *Am J Transplant* 2014
Loupy et al *Am J Transplant* 2017
Banff 2013 - methodology

Cg1a - When to perform EM?

To determine if early changes of cAMR (cg1a/PTCBML) are present

- 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
Banff 2013 - methodology

Cg1a - When to perform EM?

To determine if early changes of TG (including cg1a) are present, prompting testing for DSA

• EM to be considered in
  – all biopsies @ 6 months post-transplantation
  – and in for-cause biopsies @ 3 months post-transplantation

Haas et al. Am J Transplant 2014
Methodology - glomeruli

- How well are these guidelines followed?
- How many glomeruli do we look at?
- How many capillary loops (CL) do we look at?
<table>
<thead>
<tr>
<th>Indication for EM</th>
<th>% respondents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence of proteinuria</td>
<td>69%</td>
</tr>
<tr>
<td>Clinical suspicion of glomerular/recurrent disease</td>
<td>86%</td>
</tr>
<tr>
<td>Abnormal glomeruli on LM and/or positive IHC</td>
<td>71%</td>
</tr>
<tr>
<td>Patient clinically at risk for AMR</td>
<td>43%</td>
</tr>
<tr>
<td>Indication biopsy after given time-point post transplantation (3 months, 6 months or 1 year)</td>
<td>10-16%</td>
</tr>
</tbody>
</table>

Potential for missing cg1a
<table>
<thead>
<tr>
<th>How many glomeruli do you evaluate?</th>
<th>% respondents</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 glomerulus</td>
<td>18%</td>
</tr>
<tr>
<td>2 or more glomeruli</td>
<td>28%</td>
</tr>
<tr>
<td>All glomeruli on the grid</td>
<td>37%</td>
</tr>
<tr>
<td>Depends on specific diagnostic question and based on LM/IF examination</td>
<td>17%</td>
</tr>
<tr>
<td>How many capillary loops do you evaluate for double contours?</td>
<td>% respondents</td>
</tr>
<tr>
<td>-------------------------------------------------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>1 loop</td>
<td>2%</td>
</tr>
<tr>
<td>2 to 5 loops</td>
<td>13%</td>
</tr>
<tr>
<td>All loops in 1 glomerulus</td>
<td>44%</td>
</tr>
<tr>
<td>All loops in &gt;1 glomerulus</td>
<td>42%</td>
</tr>
</tbody>
</table>
Peritubular capillary basement membrane multilayering

Basal lamina multilayering in peritubular capillaries

Peritubular capillary basement membrane multilayering
Banff 2005 and 2013 - methodology

- Cortical peritubular capillaries
- Number of layers counted in the most affected ptc and at least 2 additional ptc
- Avoid tangentially cut ptc

- Banff 2013
  - PTCBML = 1 PTC with ≥ 7 + 2 PTC with ≥ 5

- Banff 2005
  - no clear definition; “moderate to severe” = ptc with 5-6 or 7 layers

Solez et al Am J Transplant 2007
Loupy et al Am J Transplant 2017
Methodology - PTCBML

- How well are these recommendations followed?
- Should we always examine for PTCBML when doing EM on transplant biopsies?
- How many ptc do we look at?
- What do we record on our report?
- What cut-off do we use for making a diagnosis of cABMR?
- Does the ML have to be circumferential to count?
- What does circumferential mean?
<table>
<thead>
<tr>
<th>How often do you evaluate PTCBML?</th>
<th>% respondents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never</td>
<td>17%</td>
</tr>
<tr>
<td>Sometimes &lt;50%</td>
<td>21%</td>
</tr>
<tr>
<td>Sometimes &gt;50%</td>
<td>3%</td>
</tr>
<tr>
<td>Always if the sample is adequate</td>
<td>58%</td>
</tr>
<tr>
<td>How many ptc do you look at to count ptcbml?</td>
<td>% respondents</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>---------------</td>
</tr>
<tr>
<td>0-3</td>
<td>33%</td>
</tr>
<tr>
<td>4-10</td>
<td>50%</td>
</tr>
<tr>
<td>10-20</td>
<td>17%</td>
</tr>
<tr>
<td>&gt;20</td>
<td>1%</td>
</tr>
<tr>
<td>Cortex and/or medulla?</td>
<td>% respondents</td>
</tr>
<tr>
<td>--------------------------------------------------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Cortex only</td>
<td>48%</td>
</tr>
<tr>
<td>Cortex and medulla</td>
<td>12%</td>
</tr>
<tr>
<td>Random, including areas of fibrosis</td>
<td>4%</td>
</tr>
</tbody>
</table>

16% specify to exclude areas of fibrosis  
39% scan at low power then zoom on affected ptc
<table>
<thead>
<tr>
<th>What do you record from your PTCBML reading</th>
<th>% respondents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Only average number of layers on all ptc counted</td>
<td>16%</td>
</tr>
<tr>
<td>Only number of layers in the 3 worst affected</td>
<td>19%</td>
</tr>
<tr>
<td>Only number of PTC with 3 or more layers</td>
<td>7%</td>
</tr>
<tr>
<td>Only number of PTC with 5 or more layers</td>
<td>11%</td>
</tr>
<tr>
<td>Only number of PTC with 7 or more layers</td>
<td>3%</td>
</tr>
<tr>
<td>Combination of several of the above</td>
<td>43%</td>
</tr>
</tbody>
</table>

Most popular combination (16%) = Number of PTC with 5 or more and number with 7 or more
<table>
<thead>
<tr>
<th>What cut-off do you use as diagnostic of cABMR?</th>
<th>% respondents</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 PTC with ≥ 5 layers</td>
<td>28%</td>
</tr>
<tr>
<td>3 PTC with ≥ 5 layers</td>
<td>30%</td>
</tr>
<tr>
<td>1 PTC with ≥ 7 layers and 2 more with ≥ 5 layers</td>
<td>30%</td>
</tr>
<tr>
<td>Other</td>
<td>12%</td>
</tr>
</tbody>
</table>
How do you record layers of ptc lamination in a given capillary? | % respondents
--- | ---
Count in the segment with the most layers | 75%
Count in the segment with the least layers | 2%
Average the count to get the final number | 18%
Other | 6%
<table>
<thead>
<tr>
<th>Do you record segmental or circumferential multilayering</th>
<th>% respondents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Segmental</td>
<td>13%</td>
</tr>
<tr>
<td>Circumferential</td>
<td>25%</td>
</tr>
<tr>
<td>Both</td>
<td>62%</td>
</tr>
<tr>
<td>How do you define circumferential?</td>
<td>% respondants</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>&gt;50% basement membrane layering of a ptc</td>
<td>44%</td>
</tr>
<tr>
<td>&gt;75% basement membrane layering of a ptc</td>
<td>38%</td>
</tr>
<tr>
<td>100% basement membrane layering of a ptc</td>
<td>16%</td>
</tr>
</tbody>
</table>
Consensus?

• Glomeruli
  – Only a minority look for cg1a in patients at risk of ABMR
  – Not clear how many glomeruli to look at

• Peritubular capillaries:
  – Agree on:
    • Always do ptcbml counting if the sample is adequate
    • Count 4-10 ptc
    • Count in the segments with the most layers
    • Count (and report) both segmental and circumferential multi-layering
  – Disagree on:
    • How to report it
    • Threshold for cABMR
    • What circumferential means
Conclusions

– Some inter-observer variability likely to result from different interpretation of guidelines
  • Current guidelines do not always provide enough detail
  • When guidance is clear, it is not always followed

– Further inter-observer variability may result from visual recognition of the lesions
  • Thursday Banff Concurrent Kidney 2 (15:00 – 19:00) Sharan Singh
Other important considerations

- What are we using EM for?
Cg1a is an **EARLY** lesion
Dobi et al *Virchows Arch* 2016

- PTCBML in early (cg1, n=15) and late (cg2+cg3, n=42) transplant glomerulopathy

PTCBML
- Cg1 mean = 2.6 layers
- Cg2/3 mean = 4.5 layers
Dobi et al *Virchows Arch* 2016

- In AMR or suspicious for AMR (DSA+/C4d+ and/or moderate or severe MI)
  - 1 PTC with 5 layers (mean PTC\textsubscript{circ} ≥3.0) represents the earliest, prognostically relevant morphologic manifestation of chronicity due to antibody
– In cases with DSA/ABMR
  • To establish the presence of chronic (irreversible) features indicative of bad outcomes?
  • To establish the presence of early (potentially reversible) features chronicity?

– In all comers
  • As a diagnostic aide, prompting testing for DSA?
Ultrastructural features of bad prognosis
Acknowledgements

Co-chair Prof Sharan Singh

All those that took the survey and the Banff EM Working Group Members

Prof Terry Cook
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Lavina Bellaramini
Banff EM Working Group Members

Co-chairs: Sharan Singh and Candice Roufosse

Marjan Afrouzian
Ibrahim Batal
Chris Bellamy
Verena Broecker
David Buob
Praveen Chander
Marian Clahsen-Van Groningen
A. Brad Farris
Agnes Fogo
Adil M.H. Gasim
Ian Gibson
Catherine Horsfield
Bela Ivanyi
Amanda Kan

George Liapis
Rafael Maldonado
Johan Molne
Brian Nankivell
Volker Nickeleit
Yasemin Ozluk
Paisit Paueksakon
Marlene Praet
Anne Raisanen-Sokolowski
Paramjeet S. Randhawa
Finn P Reinholt
Patricia Revelo
Deb Schady
Surya V. Seshan
Next steps

• Harmonisation of terminology
  – new lamina densa, new layers of GBM...
  – LRI expansion, subendothelial widening....
  – Endothelial thickening, endothelial hyperplasia...

• Clear definitions

• On-line standard images and test module

• Define reproducible criteria
Q5 On average I evaluate per year renal allograft biopsies (kidney transplants only):

Answered: 135  Skipped: 0

- < 50: 20.74%
- 50 - 99: 16.30%
- 100 - 199: 28.89%
- 200 - 399: 20.74%
- 400 - 799: 11.85%
- >800: 1.48%
Q11 On what approximate % of renal transplant biopsy is EM scoping performed?

Answered: 132  Skipped: 3

- All cases except...: 13.64%
- 100%; skip question 12: 12.88%
- 75-99%: 13.64%
- 50-75%: 13.64%
- 25-50%: 9.09%
- 1-25%: 37.12%
Poster 43: Comparison of Ultrastructural Glomerular Features in Biopsies From Patients With De Novo Donor Specific Antibodies with Surveillance Biopsies

<table>
<thead>
<tr>
<th>Surveillan</th>
<th>DSA+ MI 0-1</th>
<th>DSA+ MI 2-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>No cg1a</td>
<td>15</td>
<td>29</td>
</tr>
<tr>
<td>cg1a</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

chi-square p= 9.476x10^{-7}
<table>
<thead>
<tr>
<th>What magnification do you use to evaluate ptcbml?</th>
<th>% respondents</th>
</tr>
</thead>
<tbody>
<tr>
<td>2500x</td>
<td>10%</td>
</tr>
<tr>
<td>5000x</td>
<td>29%</td>
</tr>
<tr>
<td>8000x</td>
<td>28%</td>
</tr>
<tr>
<td>10,000x</td>
<td>29%</td>
</tr>
<tr>
<td>20,000x</td>
<td>6%</td>
</tr>
</tbody>
</table>
Principal component analysis using Banff lesions, peritubular capillary basement membrane multilayering (ptcml; available in 147 of 234 biopsies), C4d staining, anti-HLA class I or class II panel reactive antibodies, and time posttransplant

Sis et al 2010 AJT
Subset of patients with sequential biopsies:

low level PTCBML on first biopsy OR progression to low level over time correlates with future TG

De Kort et al Transplantation 2015
<table>
<thead>
<tr>
<th>Condition</th>
<th>PC\text{circ} ± SD</th>
<th>Range of PC\text{circ}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.02 ± 0.06</td>
<td>0-0.21</td>
</tr>
<tr>
<td>Cyclosporine-treated psoriatics</td>
<td>0.03 ± 0.14</td>
<td>0-0.5</td>
</tr>
<tr>
<td>Acute rejection</td>
<td>0.26 ± 0.3</td>
<td>0-0.89</td>
</tr>
<tr>
<td>Native kidney diseases</td>
<td>0.53 ± 0.65</td>
<td>0-2.78</td>
</tr>
<tr>
<td>Chronic rejection, biopsy</td>
<td>2.87 ± 1.83*</td>
<td>0-7.36</td>
</tr>
<tr>
<td>Chronic rejection, nephrectomy</td>
<td>5.48 ± 2.02</td>
<td>2.28-8.14</td>
</tr>
</tbody>
</table>

Ivanyi B *Human Pathology* 2000
Filtered for “experts” = renal/transplant specialist, >5 years experience, >200 Tx bx/year, access to EM score
N=37/135

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<td>8%</td>
</tr>
<tr>
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</tr>
<tr>
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</tr>
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