Transmission electron microscopy in renal transplant pathology

On behalf of the Banff Working Group for Electron Microscopy

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Transmission electron microscopy in renal transplant pathology

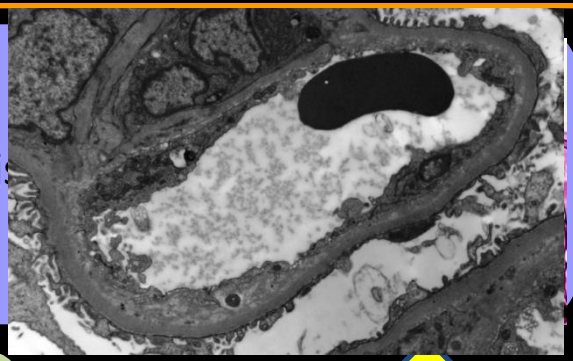
- Current accepted uses of electron microscopy in transplant biopsies
  - Glomerular disease
  - Diagnosis of chronic antibody-mediated rejection

Acute/active antibody-mediated Rejection

Histology { ptc, g,v, TMA

Chronic active antibody-mediated rejection

His



EM

DSA

HLA or other

+

Interaction of antibody with endothelium

C4d

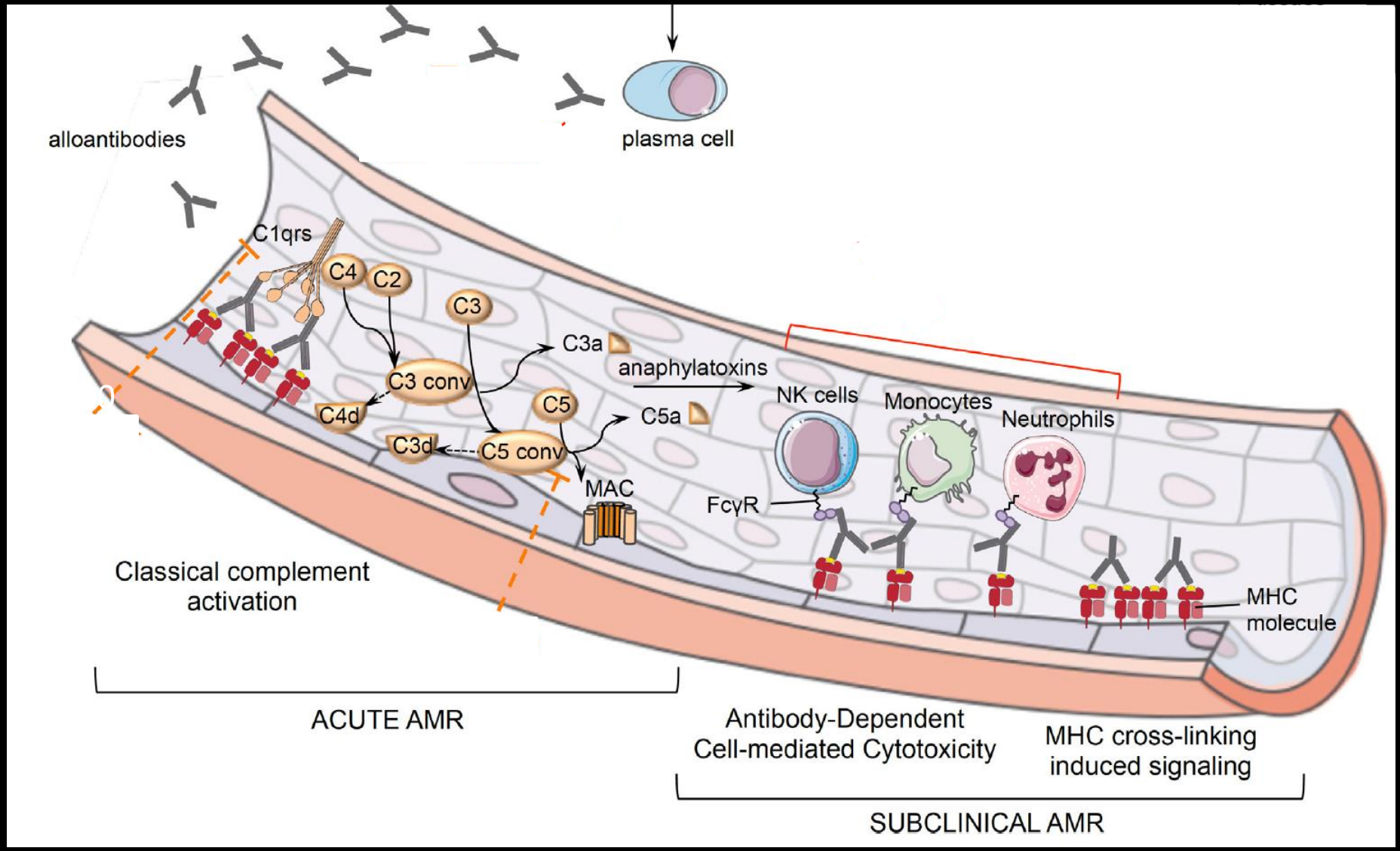
and/or

Increased endothelial transcripts

and/or

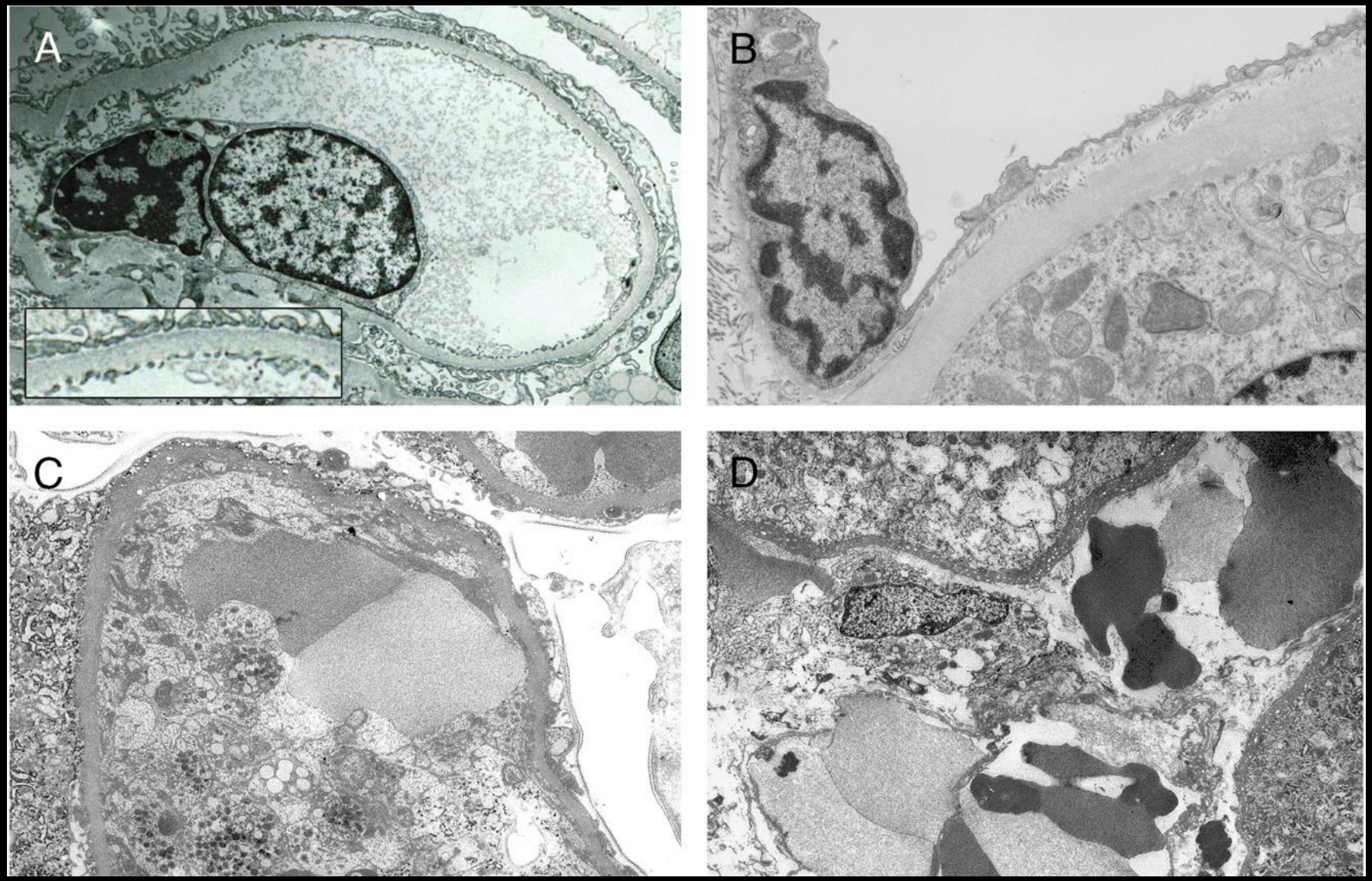
ptc+g≥2

Pathophysiology of antibody-mediated rejection



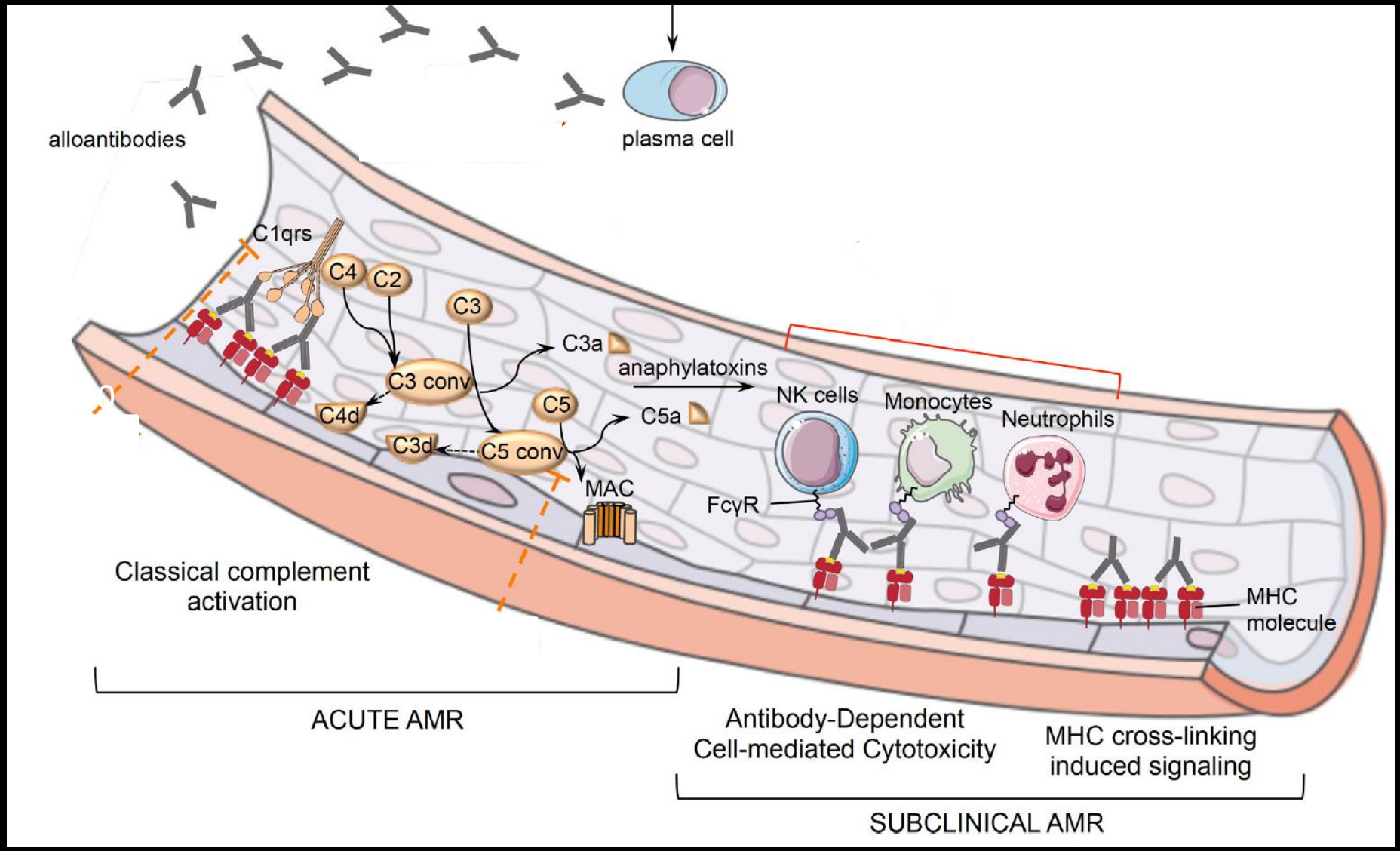
Pouliquen et al *F1000Prime Reports* 2015

Lytic endothelial cell injury

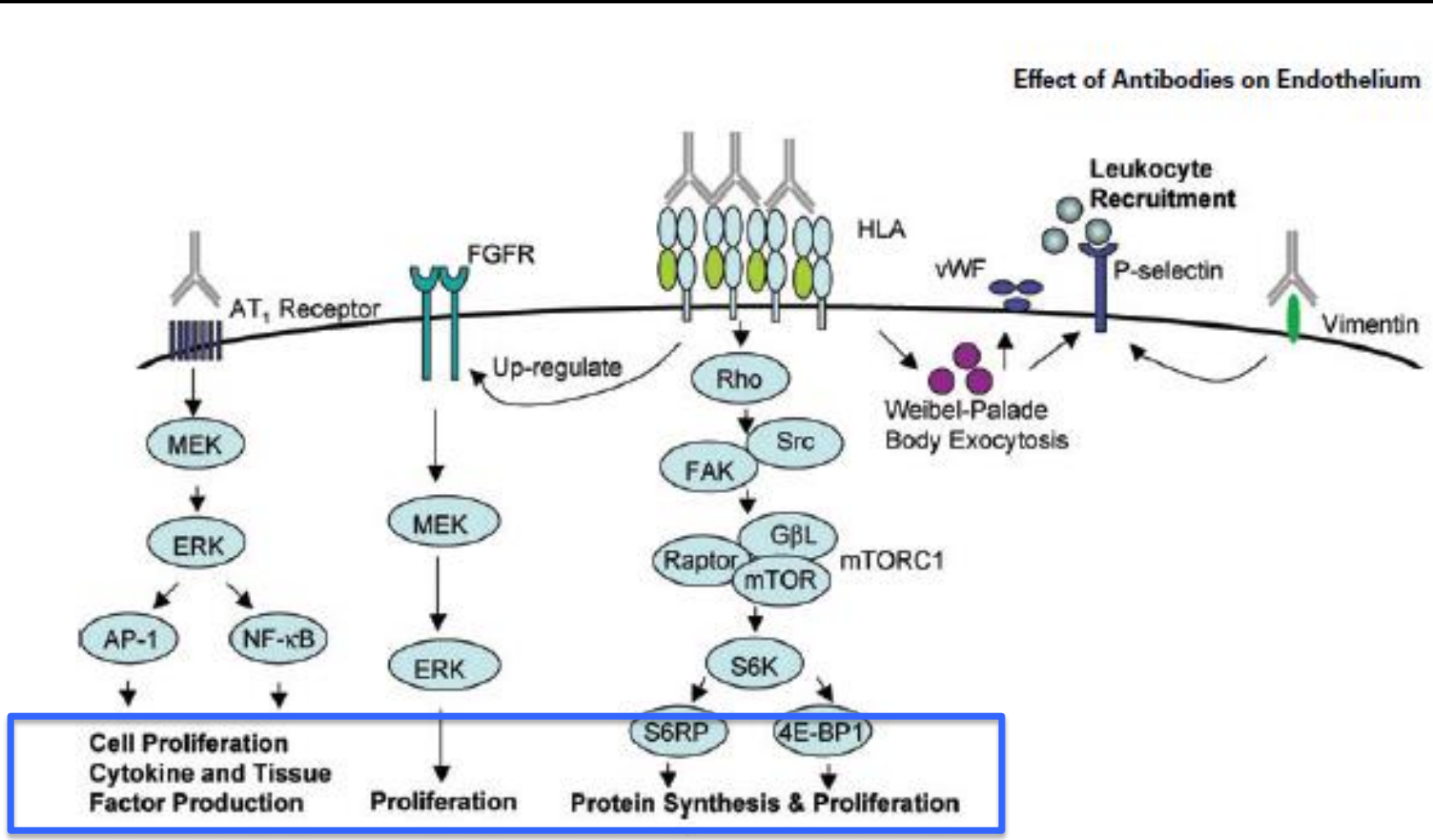


Drachenberg and Papadimitriou *Transplantation* 2013

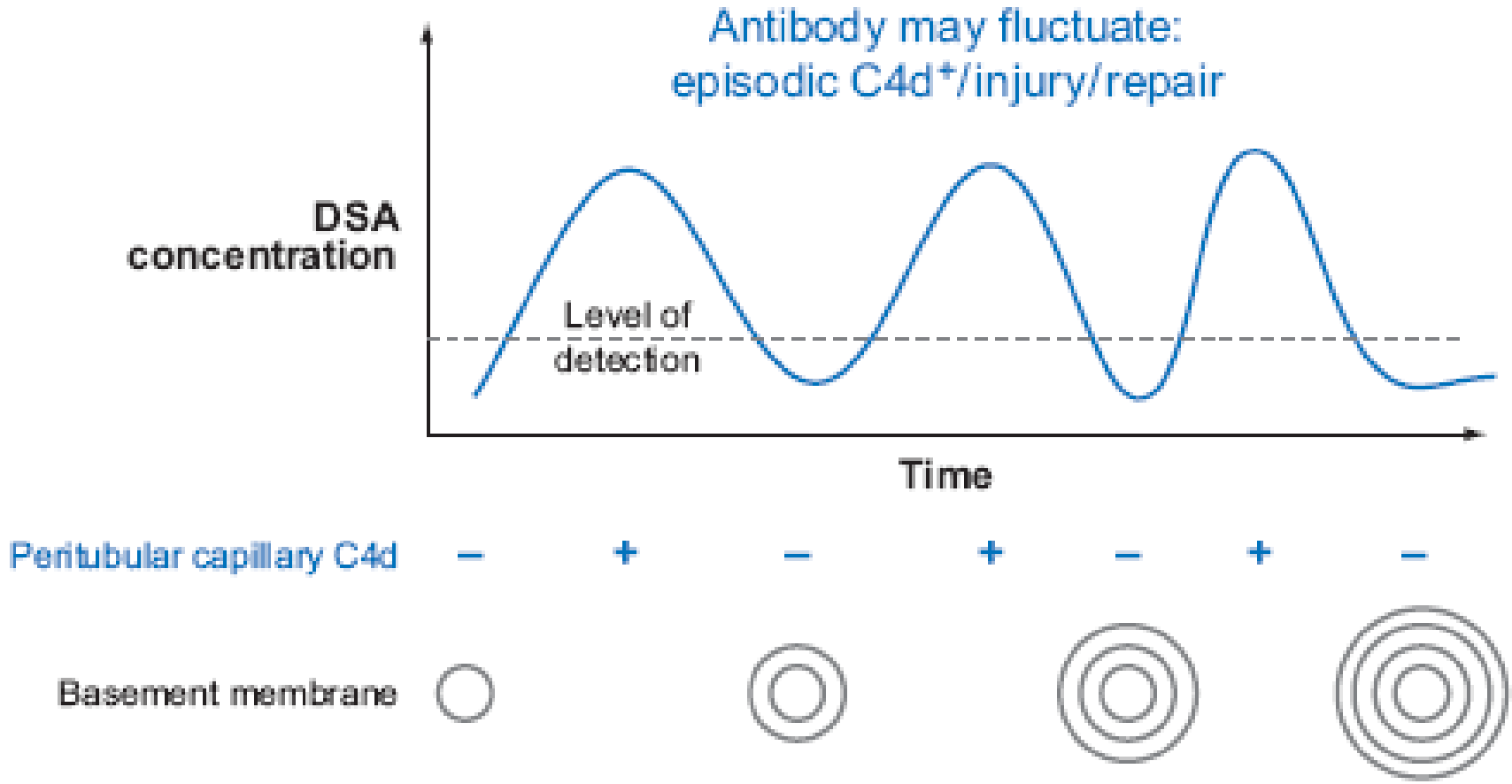
Pathophysiology of antibody-mediated rejection



Pouliquen et al *F1000Prime Reports* 2015



Zhang and Reed *Am J Transpl 2009*



- 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

Cornell L and Colvin RB *Annu Rev Pathol Mech Dis* 2008

Banff Working Group for EM (Banff 2015)

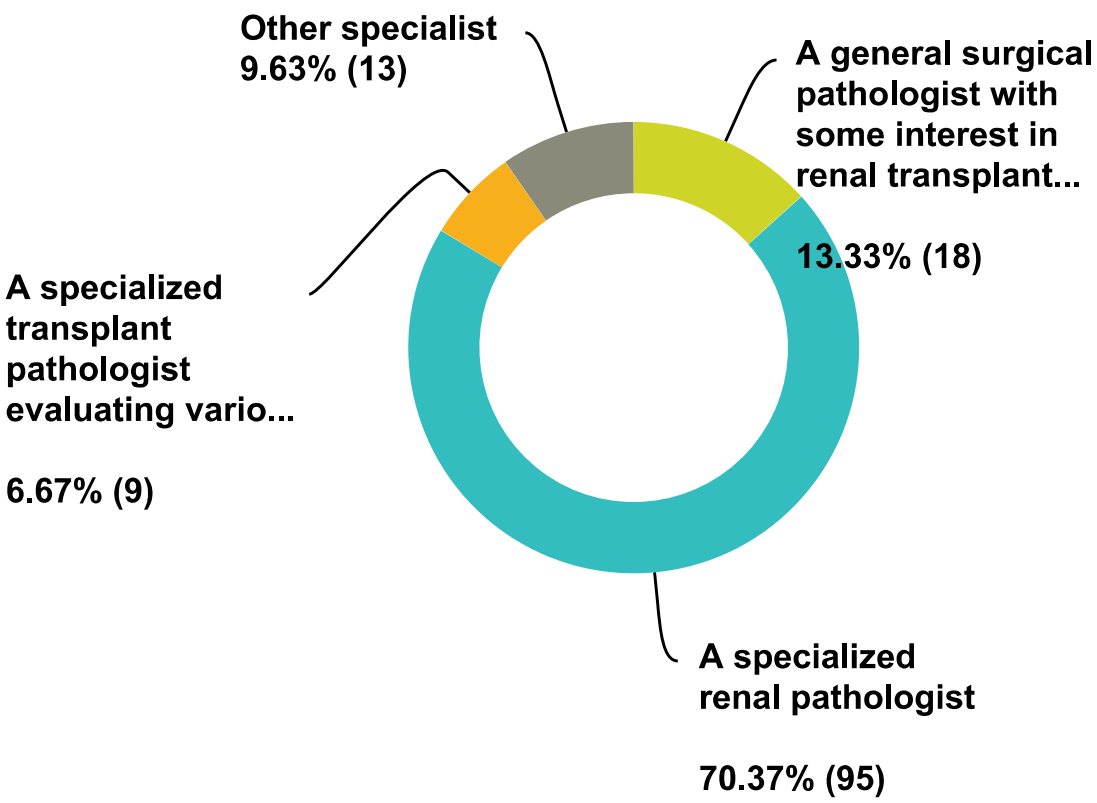
- Cg1a and PTCBML
  - Evaluate current practices
  - Investigate inter-observer variability
  - Standardize definitions and criteria
  - Investigate associations of cg1a and ptcbml with outcomes in a multi-centre study

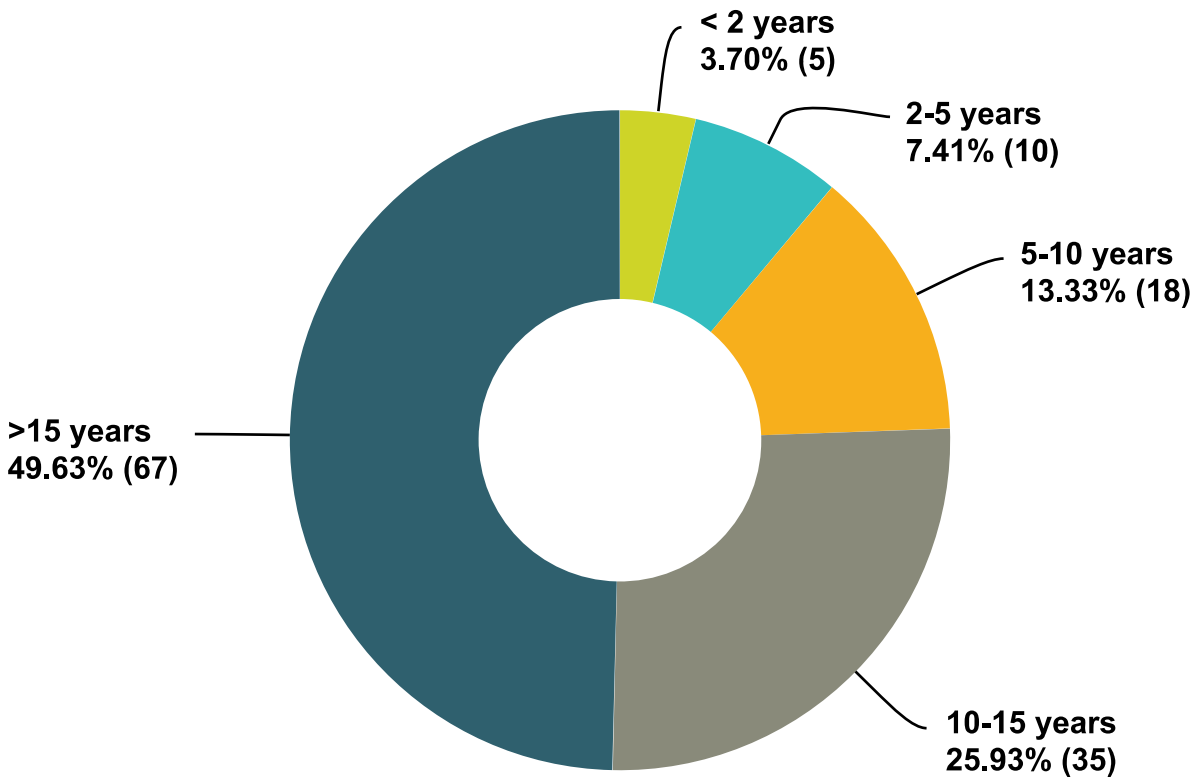
- Part 1:
- Survey of current practice
  - Working Group members
  - Wider renal/transplant pathology community

- 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]


Other specialist
9.63% (13)
A general surgical pathologist with some interest in renal transplant...
13.33% (18)
A specialized transplant pathologist evaluating vario...
6.67% (9)
A specialized renal pathologist
70.37% (95)


< 2 years
3.70% (5)
2-5 years
7.41% (10)
5-10 years
13.33% (18)
10-15 years
25.93% (35)
>15 years
49.63% (67)

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

Haas *et al* Am J Transplant 2014

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

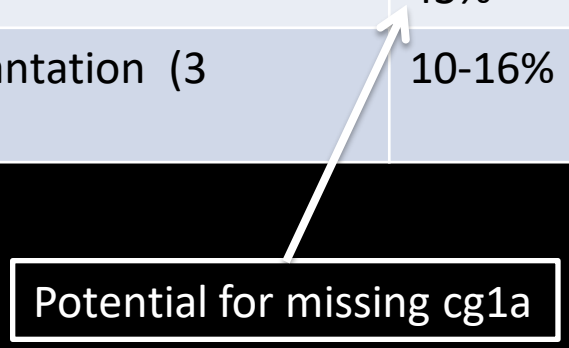
Haas *et al* Am J Transplant 2014

Methodology - glomeruli

- How well are these guidelines followed?

- How many glomeruli do we look at?
- How many capillary loops (CL) do we look at?

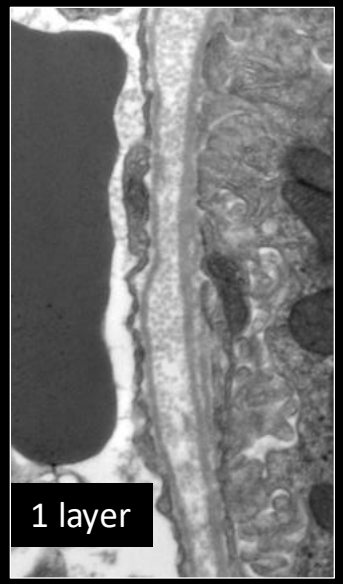
Indication for EM	% respondents
Presence of proteinuria	69%
Clinical suspicion of glomerular/recurrent disease	86%
Abnormal glomeruli on LM and/or positive IHC	71%
Patient clinically at risk for AMR	43%
Indication biopsy after given time-point post transplantation (3 months, 6 months or 1 year)	10-16%

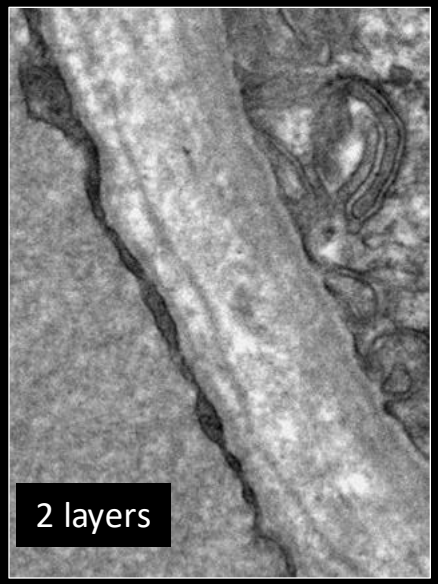


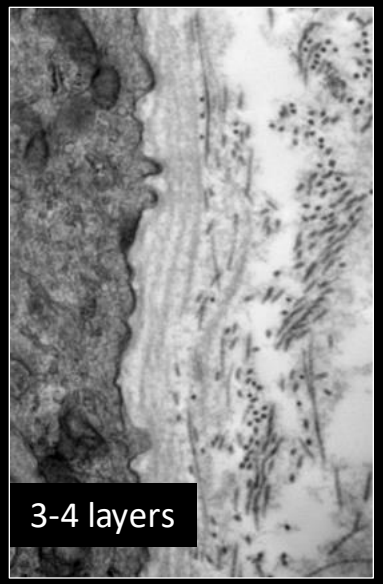
How many glomeruli do you evaluate?	% respondents
1 glomerulus	18%
2 or more glomeruli	28%
All glomeruli on the grid	37%
Depends on specific diagnostic question and based on LM/IF examination	17%

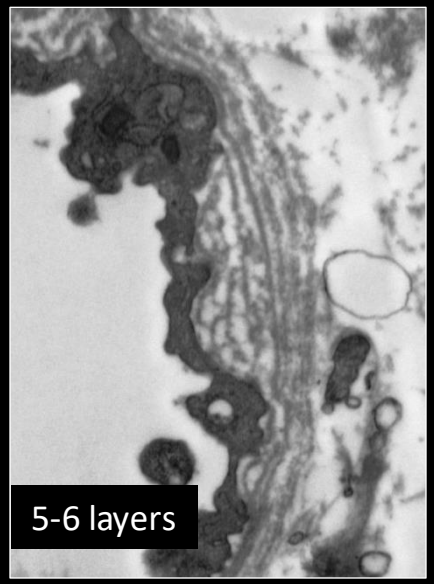
How many capillary loops do you evaluate for double contours?	% respondents
1 loop	2%
2 to 5 loops	13%
All loops in 1 glomerulus	44%
All loops in >1 glomerulus	42%

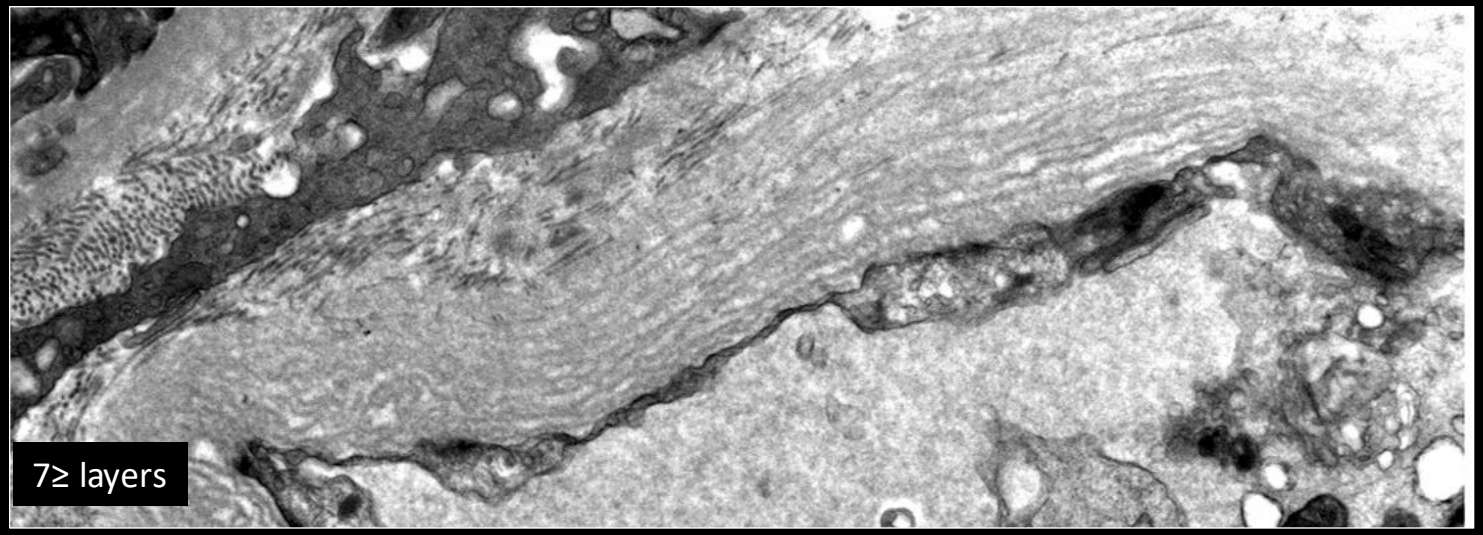
Peritubular capillary basement membrane multilayering











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?

How often do you evaluate PTCBML?	% respondents
Never	17%
Sometimes <50%	21%
Sometimes >50%	3%
Always if the sample is adequate	58%

How many ptc do you look at to count ptcbml?	% respondents
0-3	33%
4-10	50%
10-20	17%
>20	1%

Cortex and/or medulla?	% respondents
Cortex only	48%
Cortex and medulla	12%
Random, including areas of fibrosis	4%

16% specify to exclude areas of fibrosis
39% scan at low power then zoom on affected ptc

What do you record from your PTCBML reading	% respondents
Only average number of layers on all ptc counted	16%
Only number of layers in the 3 worst affected	19%
Only number of PTC with 3 or more layers	7%
Only number of PTC with 5 or more layers	11%
Only number of PTC with 7 or more layers	3%
Combination of several of the above	43%

Most popular combination (16%) = Number of PTC with 5 or more and number with 7 or more

What cut-off do you use as diagnostic of cABMR?	% respondents
1 PTC with ≥ 5 layers	28%
3 PTC with ≥ 5 layers	30%
1 PTC with ≥ 7 layers and 2 more with ≥ 5 layers	30%
Other	12%

Banff 2005

Banff 2013

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%

Do you record segmental or circumferential multilayering	% respondents
Segmental	13%
Circumferential	25%
Both	62%

How do you define circumferential?	% respondants
>50% basement membrane layering of a ptc	44%
>75% basement membrane layering of a ptc	38%
100% basement membrane layering of a ptc	16%

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

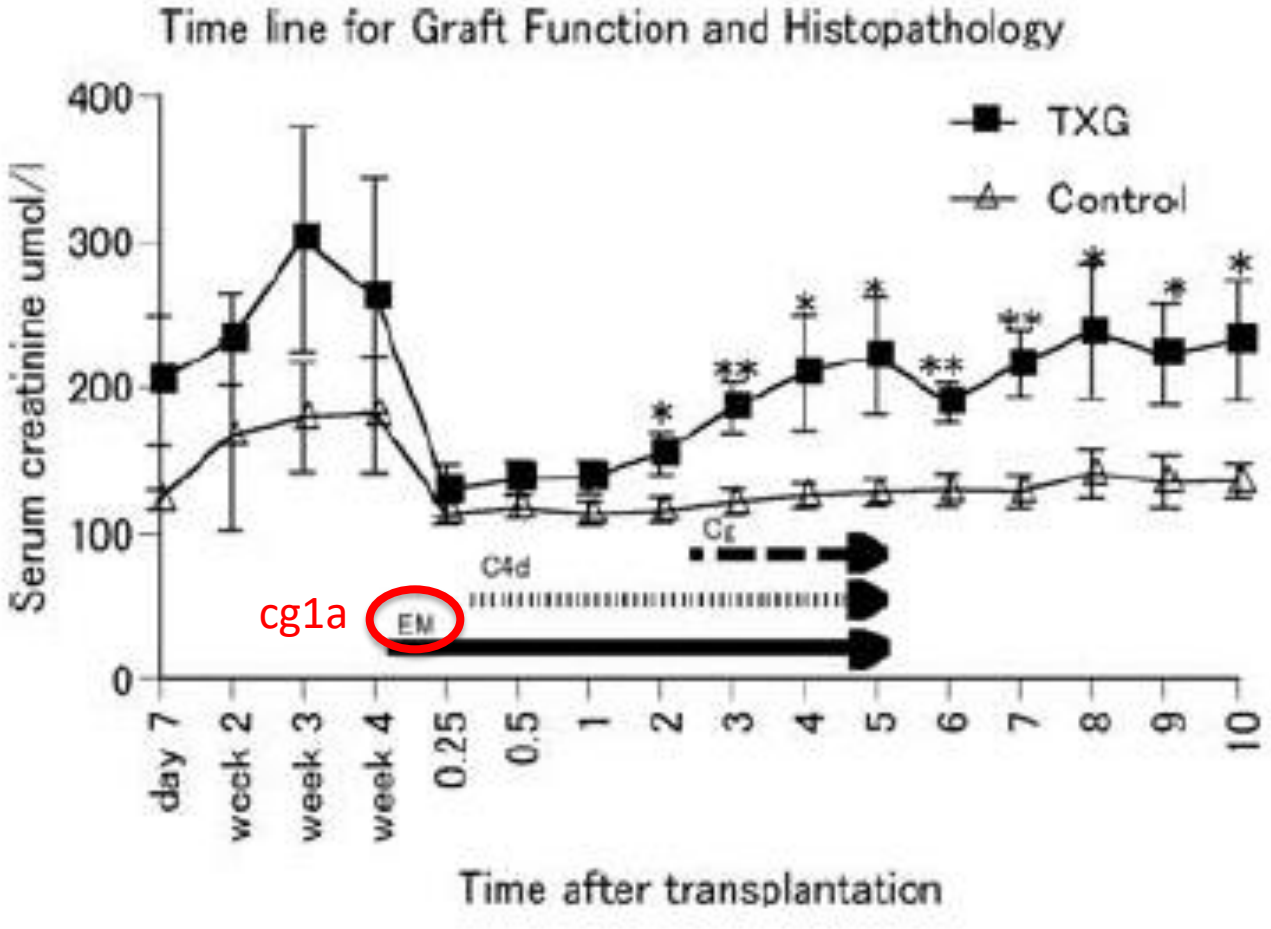
Conclusions

- 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



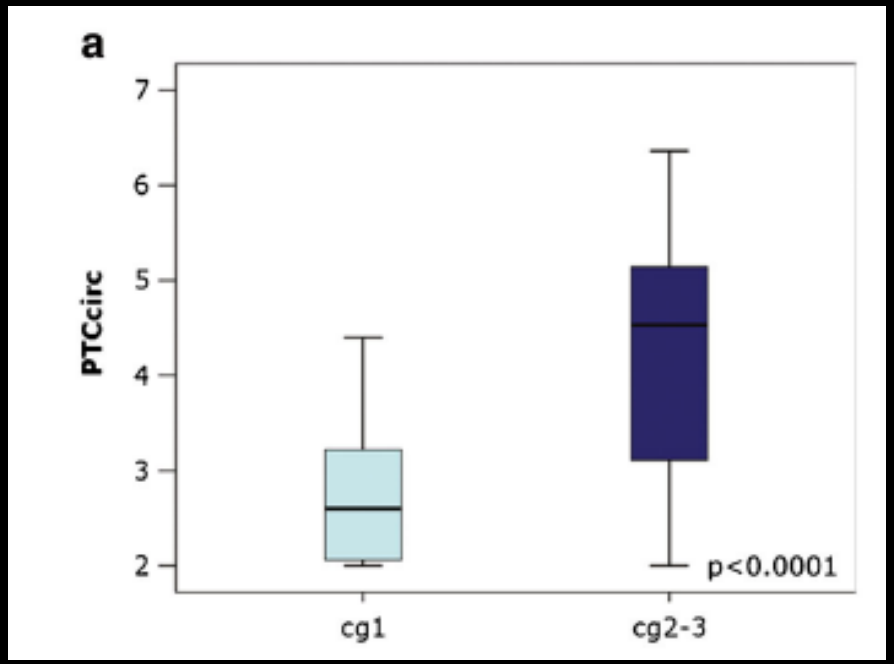
Wavamunno M et al *Am J Transplant* 2007

Dobi et al *Virchows Arch* 2016

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

PTCBML

- Cg1 mean = 2.6 layers
- Cg2/3 mean = 4.5 layers

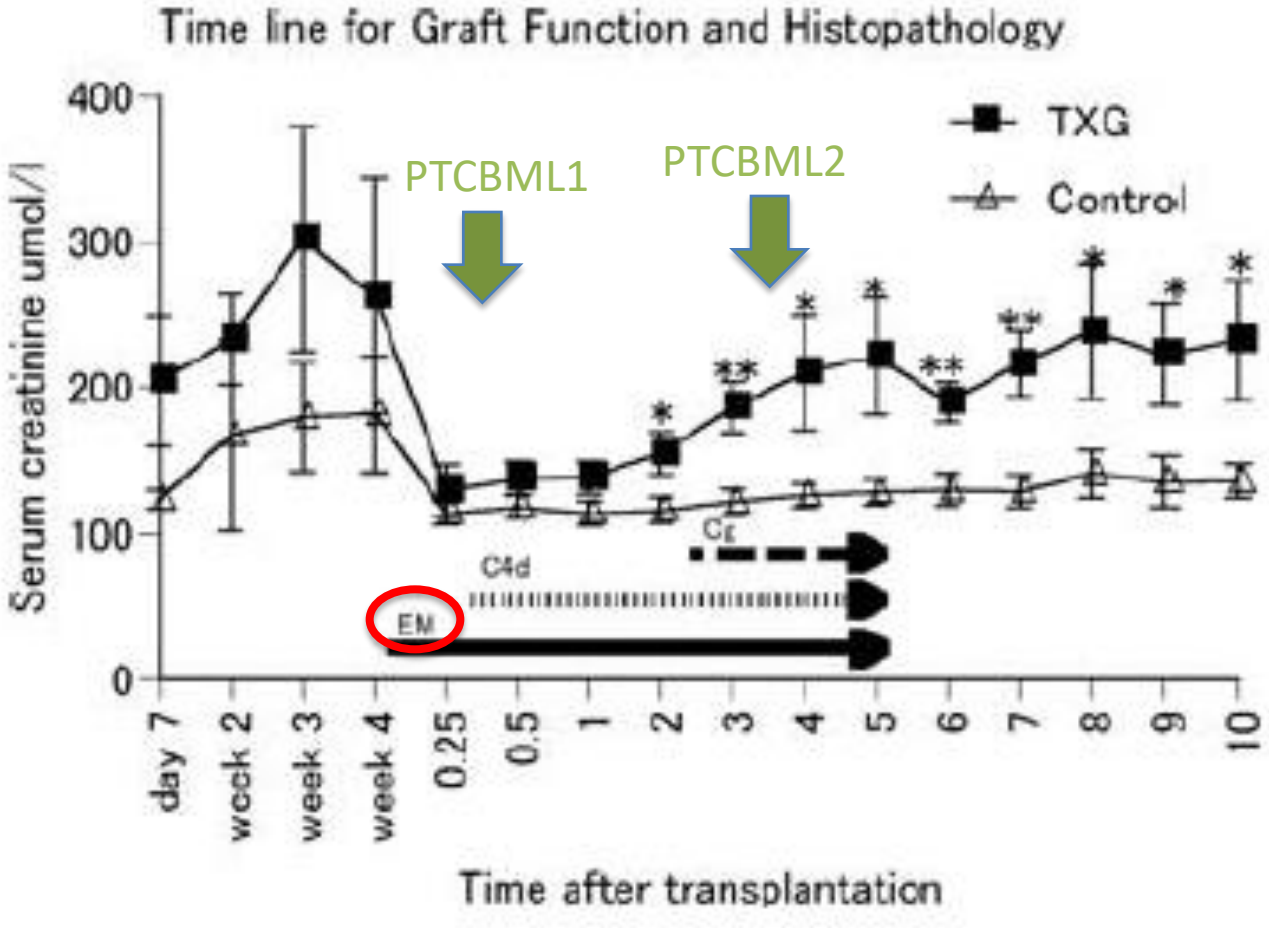


Dobi et al *Virchows Arch* 2016

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 PTCcirc ≥3.0) represents the earliest, prognostically relevant morphologic manifestation of chronicity due to antibody

Diagnostic Prognostic

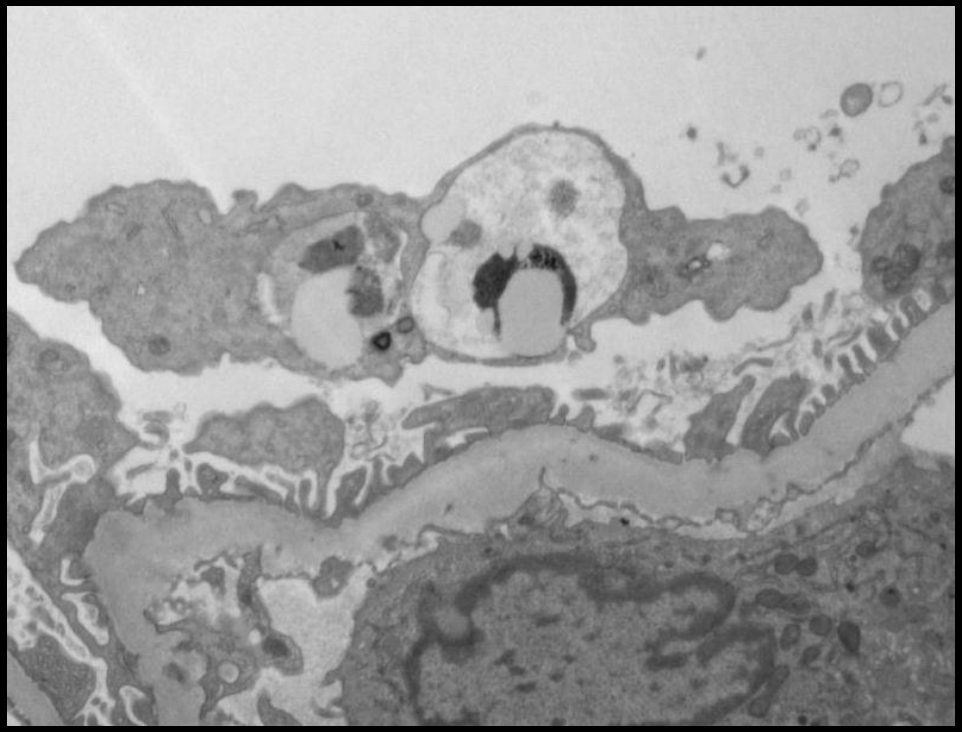


Wavamunno M et al *Am J Transplant* 2007

- 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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Dr Linda Moran
Lavina Bellaramini

Imperial College Healthcare NHS Trust

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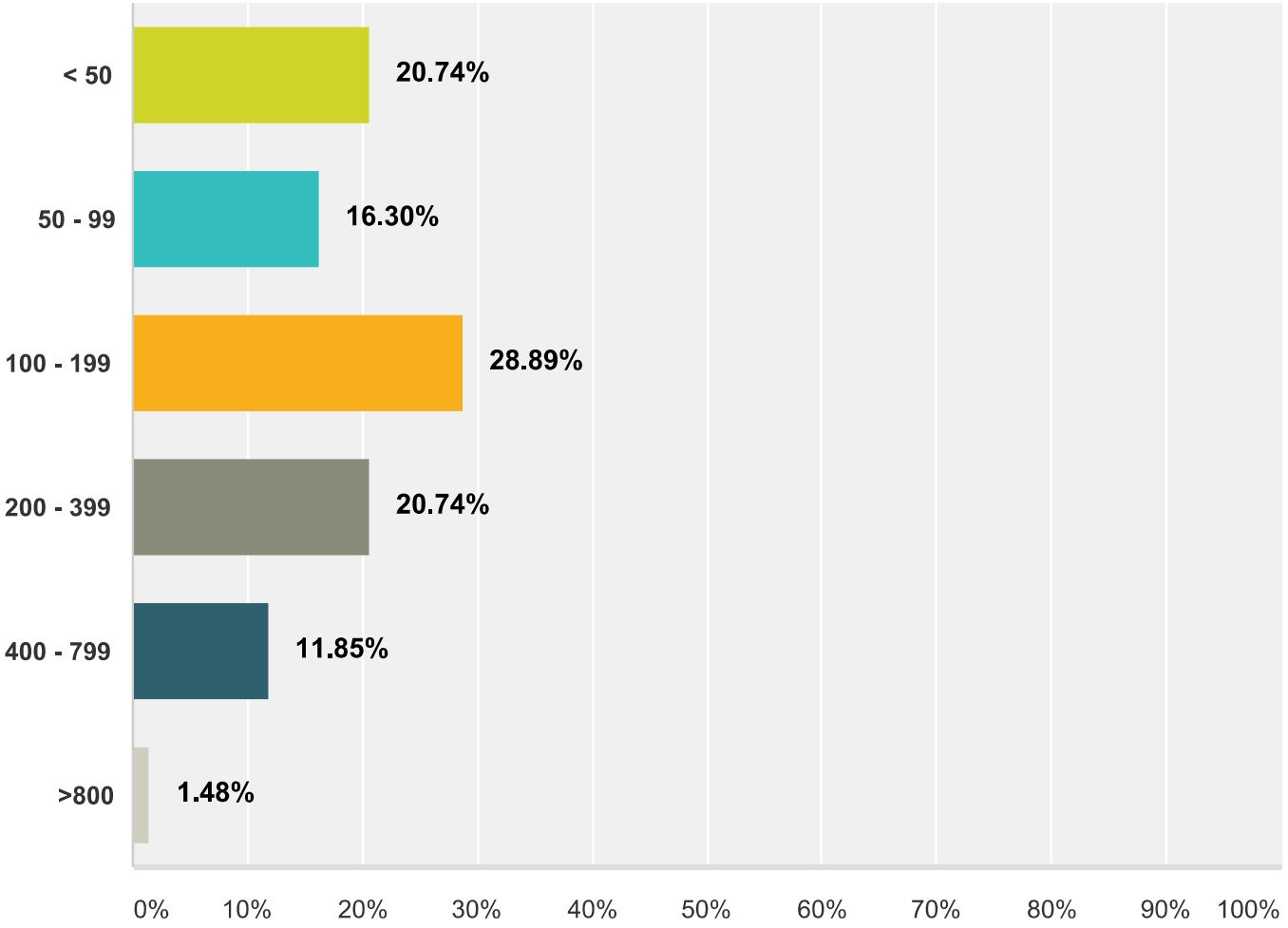
Patricia Revelo
Deb Schady
Surya V. Seshan

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

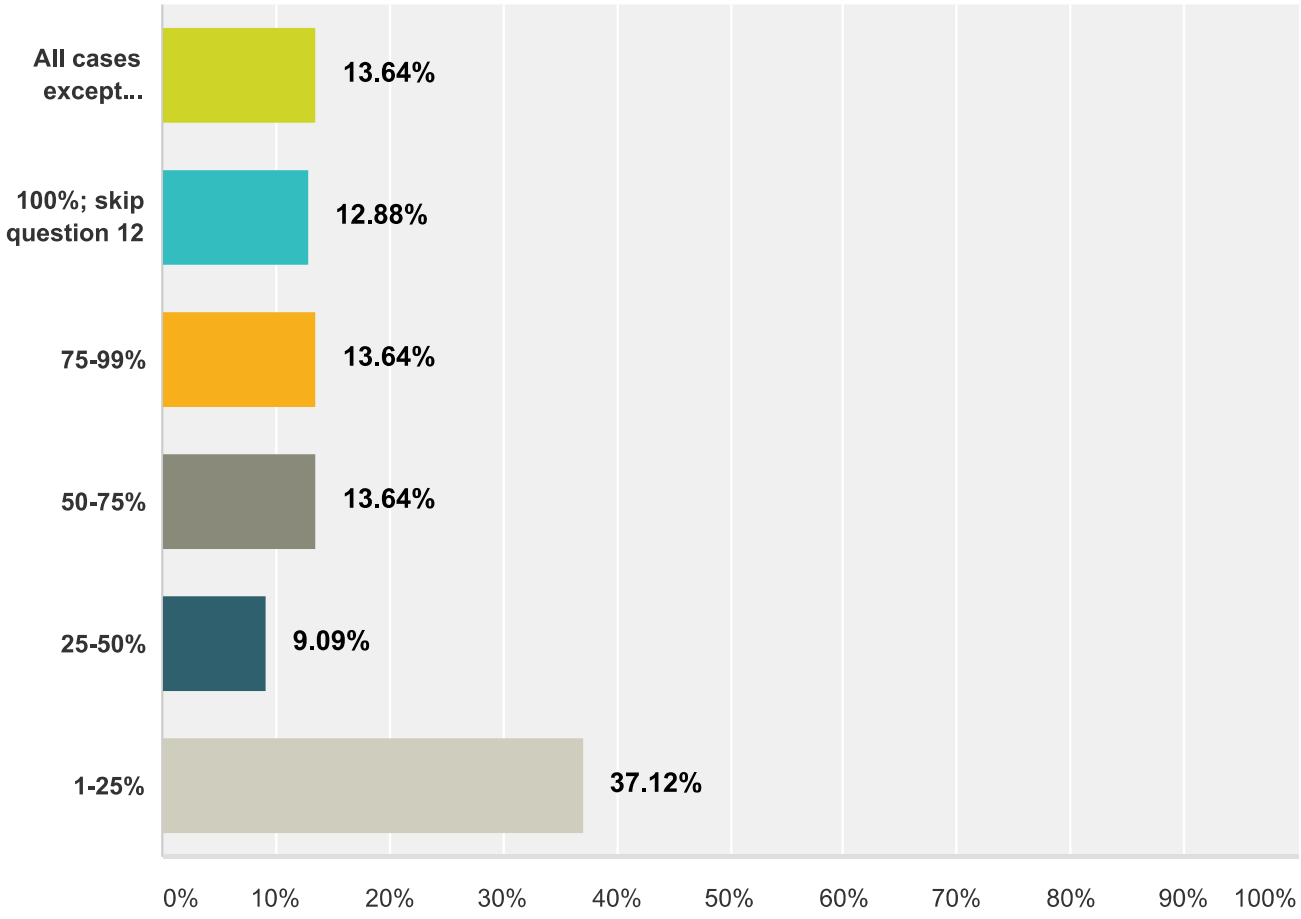
Q5 On average I evaluate per year renal allograft biopsies (kidney transplants only):

Answered: 135 Skipped: 0



Q11 On what approximate % of renal transplant biopsies is EM scoping performed?

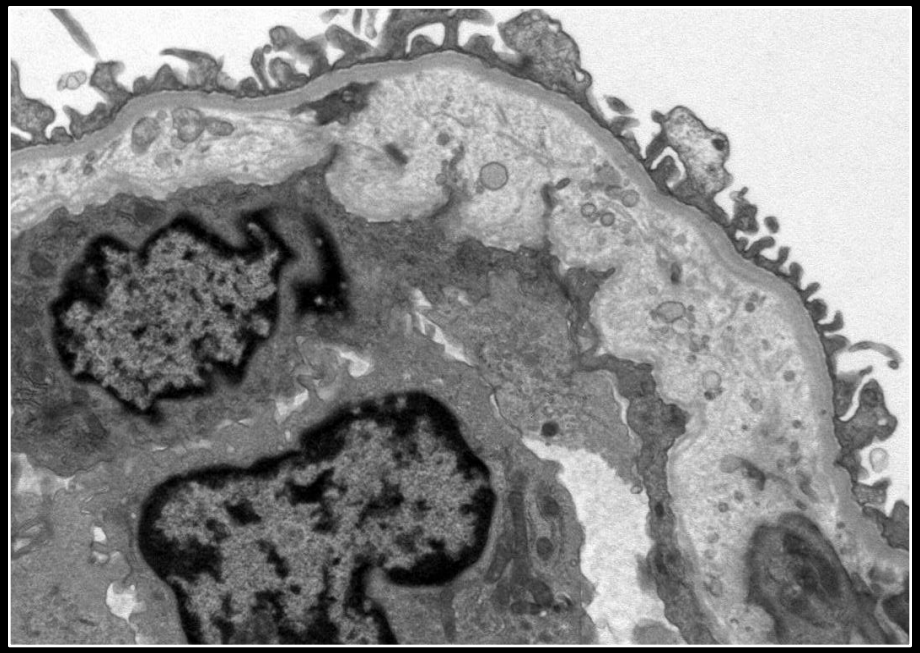
Answered: 132 Skipped: 3



Poster 43: Comparison of Ultrastructural Glomerular Features in Biopsies From Patients With De Novo Donor Specific Antibodies with Surveillance Biopsies

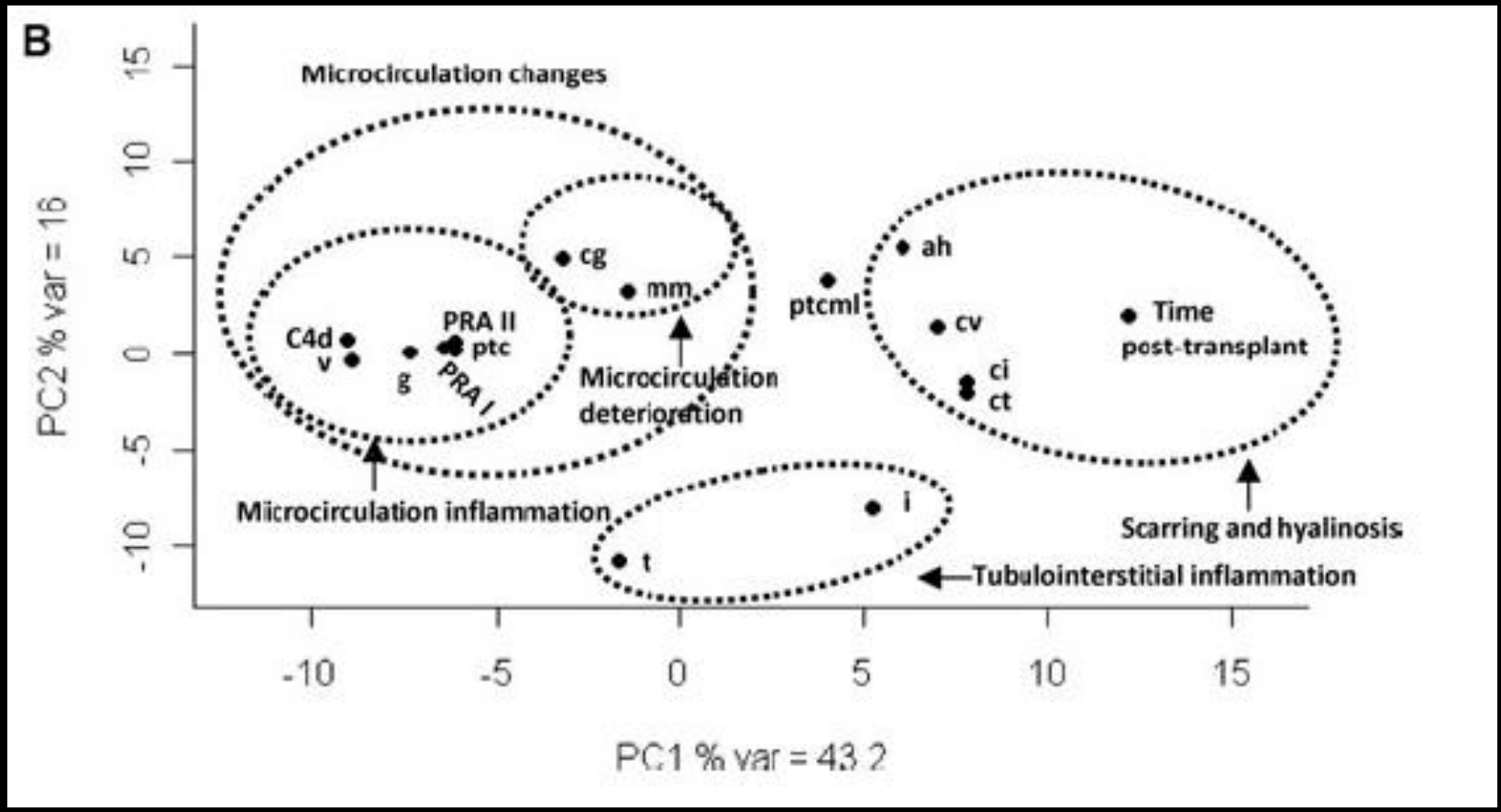
	Surveillance	DSA+ MI 0-1	DSA+ MI 2-6
No cg1a	15	29	9
cg1a	0	2	14

chi-square $p = 9.476 \times 10^{-7}$



Moran L at al Banff 2017

What magnification do you use to evaluate ptcbml?	% respondents
2500x	10%
5000x	29%
8000x	28%
10,000x	29%
20,000x	6%



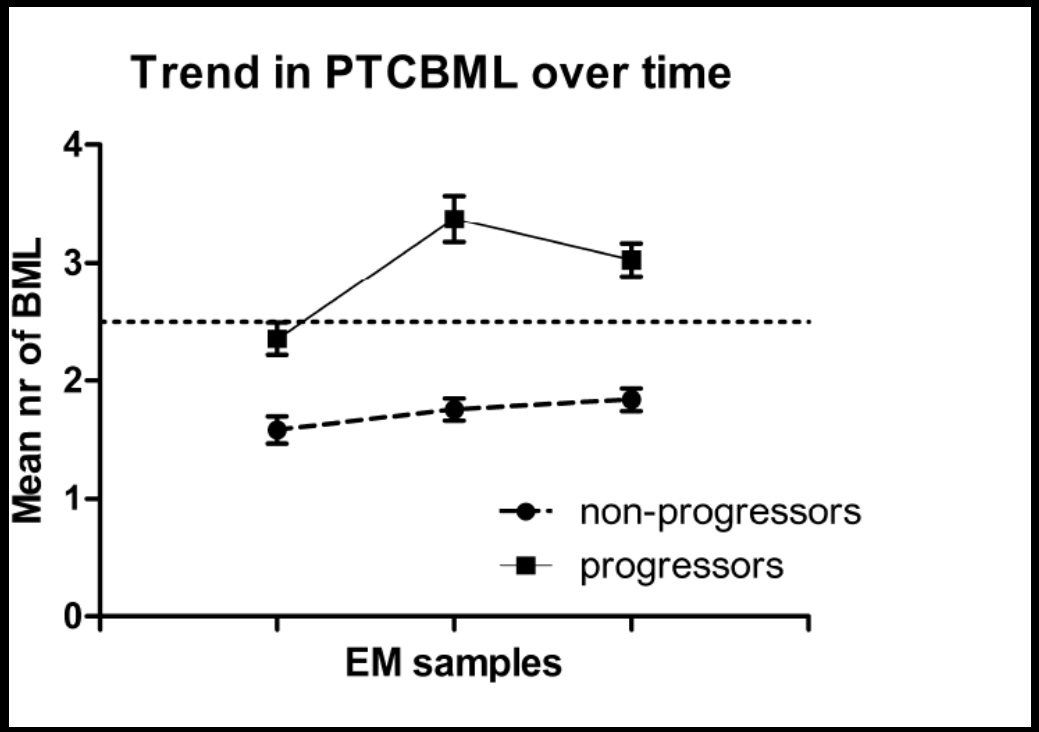
Principal component analysis using Banff lesions, peritubular capillary basement membrane multilayering (ptcml; available in 147 of 234 biopsies), C4d staining, anti-HLA class I or class II panel reactive antibodies, and time posttransplant

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



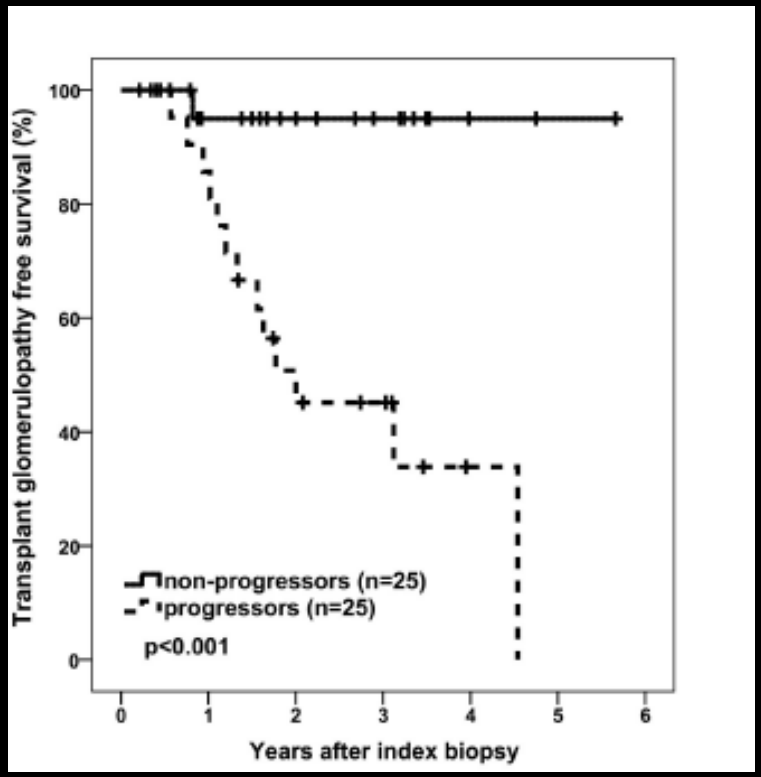


TABLE 2. Mean Number of Circumferential Basement Membrane Layers

	PCcirc ± SD	Range of PCcirc
Normal	0.02 ± 0.06	0-0.21
Cyclosporine-treated psoriatics	0.03 ± 0.14	0-0.5
Acute rejection	0.26 ± 0.3	0-0.89
Native kidney diseases	0.53 ± 0.65	0-2.78
Chronic rejection, biopsy	2.87 ± 1.83*	0-7.36
Chronic rejection, nephrectomy	5.48 ± 2.02	2.28-8.14

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

What do you record from your PTCBML reading	% respondents
Only average number of layers on all ptc counted	19%
Only number of layers in the 3 worst affected	24%
Only number of PTC with 3 or more layers	8%
Only number of PTC with 5 or more layers	11%
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Combination of several of the above	35%

Most popular combination (16%) = Number of PTC with 5 or more and number with 7 or more