C4d positivity

- Poor prognostic factor
- Reversal to C4d negativity did not change prognosis, *with current therapy*
- Prognostic factor for CAV
- Variable time line for CAV/death
- No correlation with cellular rejection
- No evidence of clinical dysfunction at time of first C4d positivity
Deposition of C4d and C3d in cardiac transplants: A factor in the development of coronary artery vasculopathy

Ellen L. Moseley, BSc, a Carl Atkinson, PhD, b Linda D. Sharples, PhD, c John Wallwork, FRCS, d and Martin J. Goddard, FRCPath a

- No evidence of C4d association with AMR
- However, C3d and AMR correlated well
Antibody-mediated rejection of the cardiac allograft: where do we stand in 2012?
Gerald J. Berry

Purpose of review: The review will discuss the current pathological criteria for the diagnosis and classification of antibody-mediated rejection (AMR) in the cardiac allograft.

Recent findings: Until recently, the diagnosis of AMR required clinical dysfunction, presence of donor specific antibodies and pathological alterations. The concept of asymptomatic AMR and its adverse long-term outcomes created, in part the need to reevaluate diagnostic criteria. The results of a recent consensus meeting sponsored by International Society For Heart And Lung Transplantation are discussed.

Summary: The diagnosis of AMR rests on histopathological and immunophenotypic findings. These provide the basis for a new grading scheme.
(1) pAMR 0: Negative for pathologic AMR: both morphological and immunophenotypic evaluations are negative.
(2) pAMR 1(hþ): morphological findings are found but negative immunostaining.
(3) pAMR 1(ip): morphological findings are absent but positive immunophenotyping.
(4) pAMR 2: both classic histological and immunophenotypic features are present.
(5) pAMR 3: Currently uncommon pattern with histological findings including interstitial hemorrhage, microvascular injury, karyorrhectic debris, mixed interstitial inflammation, and marked edema.
Take home points

- C4d IHC is very useful in heart Tx
- Performed routinely in every post-tx biopsy
- Only strong diffuse endothelial staining is positive
- Even one episode of C4d positivity correlates with poor outcome
Lung AMR
Does AMR in the Lung Exist?

- Are there histological changes that correlate to acute or chronic antibody mediated rejection?
  
  - Bronchiolitis obliterans syndrome (BOS) – fibroproliferative process of the lamina propria and lumen resulting in scarring of small airways, decline in pulmonary function, and eventual graft failure
  - FEV1 <80% of post-op baseline
  - Activation of epithelial cells and production of growth factors
  - Donor specific antibodies predispose for development of BOS

- Is C4d a marker of complement activation in the lung?

- Does the presence of circulating donor specific antibodies correlate with the above findings?
C4d Staining of Pulmonary Allograft Biopsies: An Immunoperoxidase Study

W. Dean Wallace, MD, Elaine F. Reed, PhD, David Ross, MD, Charles R. Lassman, MD, PhD, and Michael C. Fishbein, MD

The Journal of Heart and Lung Transplantation
October 2005

- 68 biopsies from 63 lung transplant patients from 2002-2004
- Allografts ranged from 1 day to 15 years
- 20 with acute cellular rejection
- 28 with bronchial/bronchiolar inflammation
- 11 with BOS consistent with chronic rejection
- 5 with diffuse alveolar damage
- 21 no rejection or inflammation
- C4d stained on FFPE tissue
C4d IF in renal allograft

C4d IHC in cardiac allograft

Cardiac negative control

C4d in pulmonary venous endothelial cells

C4d in pulmonary arteries

C4d in alveolar septae

C4d in alveolar capillaries

C4d in DAD hyaline membrane
• 33 lung transplant patients

• Transbronchial biopsies during the first 3 months post transplant
  • Blinded pathologist assessed histological evidence of AMR
  • C3d and C4d IHC staining

• Correlated staining with acute rejection, airway infection, CMV infection, BOS and survival
C4d and AMR

A. C4d staining in capillary endothelium

C. AMR with septal fibrin and admixed hemosiderin
C4d Staining Common Early in Transplant

- Positive staining showed a continuous linear pattern in the capillary walls and/or endothelial cells.

- Grading related to number of positive staining vessels in 5 400x fields:
  - Grade 0: none
  - Grade 1: <10 (mild)
  - Grade 2: 10-15 (moderate)
  - Grade 3: >15 (severe)

- 13 of 33 patients with severe graft dysfunction and positive C4d staining had developed airway infections during the first 3 months.

- Conclude complement deposition does not always equate AMR.
• 74 lung transplant patients at the time of study

• Selected all patients with a minimum of 1 year follow up who had undergone antibody testing, resulting in 23 patients

• The PRA/DSA status and C4d IHC biopsy staining of these 23 patients was prospectively gathered

• Patients were retrospectively divided into 3 groups
  • Any positive PRA
  • Any positive DSA
  • Always antibody negative

• Representative biopsies were examined for histologic and IHC evidence of AMR
23 Patients

PRA+/DSA-:
- 13 patients
- Class I and II
- Range 4-47%
- Time 9.76 months (0-41)

DSA+
- 3 patients
- Class II
- Range 3-63%
- Time 8.67 months (4-13)

PRA-/DSA-:
- 7 patients

- Cd4 staining was previously evaluated for each biopsy at time of sign out
- Biopsies were evaluated for histologic evidence of AMR
  - Capillary injury, septal fibrin, hemorrhage
- Biopsies stained for CD3, CD20, and CD138
Acute rejection
## 23 Patients

<table>
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<td>14 (7-21)</td>
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p=0.425
Discussion

• No histological differences between the PRA+, DSA+, and antibody negative groups suggestive of AMR

• C4d was negative in all biopsies (133)

• No differences in staining of CD3, CD20, or CD138 between antibody groups

• Antibody status did not affect development of BOS or time to BOS
  • 7/13 PRA+ group
  • 5/7 PRA-/DSA- group
  • Time to BOS not statistically significant
  • 2 DSA+ deceased, 1 without BOS
• In our study, we were unable to diagnose AMR on biopsy

• C4d negative in all biopsies (133 in our study)

• No histological, B-cell, or plasma cell differences suggestive of AMR between the antibody positive or negative groups

• Presence of antibodies did not correlate to presence of BOS or time to BOS
ISHLT summary statement


1. Clinical allograft dysfunction
2. Circulating DSA and
3. Pathologic findings (capillaritis, C4d + in >50% of capillaries)
Witt et al. Acute antibody-mediated rejection after lung transplantation. JHLT 2013;32:1034
Take home points

• AMR in lung Tx occurs rarely
• Literature and U of C experience is variable
• Diagnosis: Clinical dysfunction, presence of antibodies (DSA/PRA), C4d staining
Antibody-Mediated Rejection in Liver and Intestinal Transplantation
AMR in Liver Transplantation: Definitions

• Primary AMR: Recipient with preformed antibodies to donor antigens
  – Major ABO antigens*
  – MHC class I antigens
  – Endothelial cell antigens

• Secondary AMR: de novo antidonor antibodies develop after transplantation