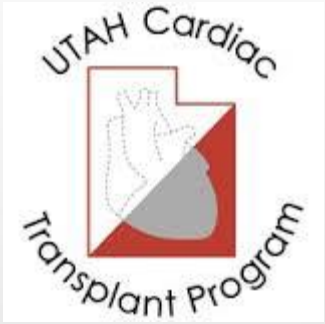
Chronic injury to the microcirculation in EMB

Dylan V Miller MD M Patricia Revelo MD



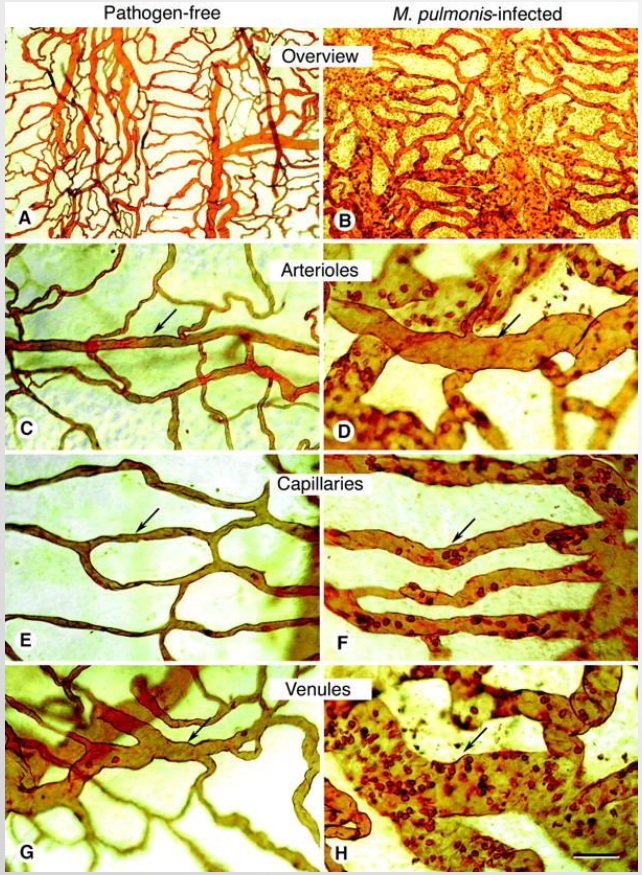
Disclosures

- Miller: None
- Revelo: None

Goals & Objectives

- Characterize the tissue-level pathologic changes occurring in the microvasculature of failing cardiac allografts.
- Describe possible pathogenetic mechanisms accounting for microcirculation pathology in failing cardiac allografts.
- Review causal links between antibody mediated rejection (ABMR) and microcirculation pathology in failing cardiac allografts.
- Recognize tissue level histopathologic alterations in the myocardial microvasculature of failing cardiac allografts.
- Discuss possible mechanisms whereby microcirculation injury may lead to cardiac allograft failure and the reported histopathologic changes in this setting.
- Assess routine monitoring of microcirculation injury features in cardiac allograft biopsies to monitor long term effectiveness of anti-rejection therapy.

Ischemia/Wound Healing Paradigm



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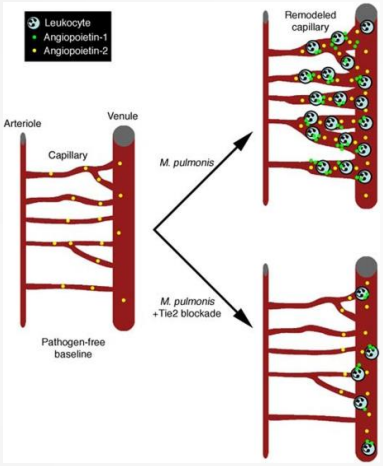
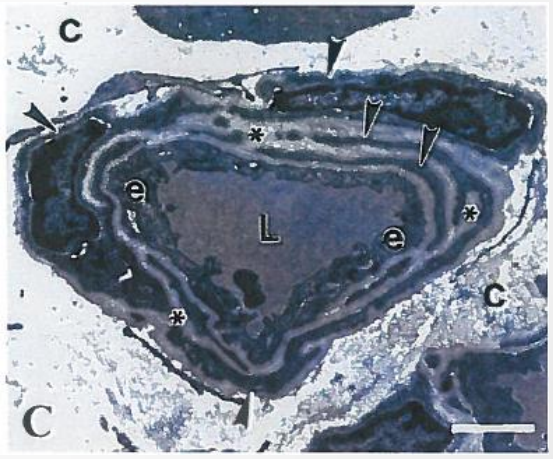


Am J Pathol. 2010 Apr;176(4):2009-18



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Dedkov EI, et al. Resistance vessel remodeling and reparative angiogenesis in the microcirculatory bed of long-term denervated skeletal muscles. Microvasc Res. 2002 Jan;63(1):96-114.

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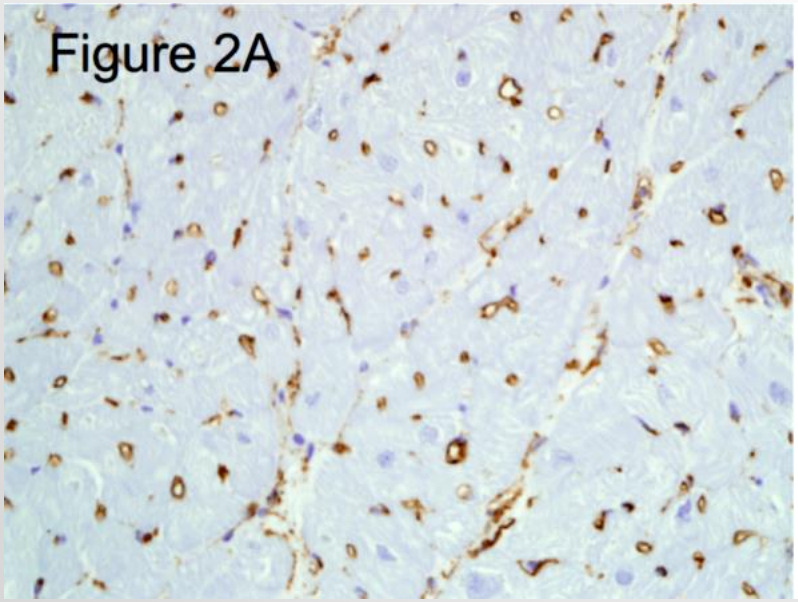
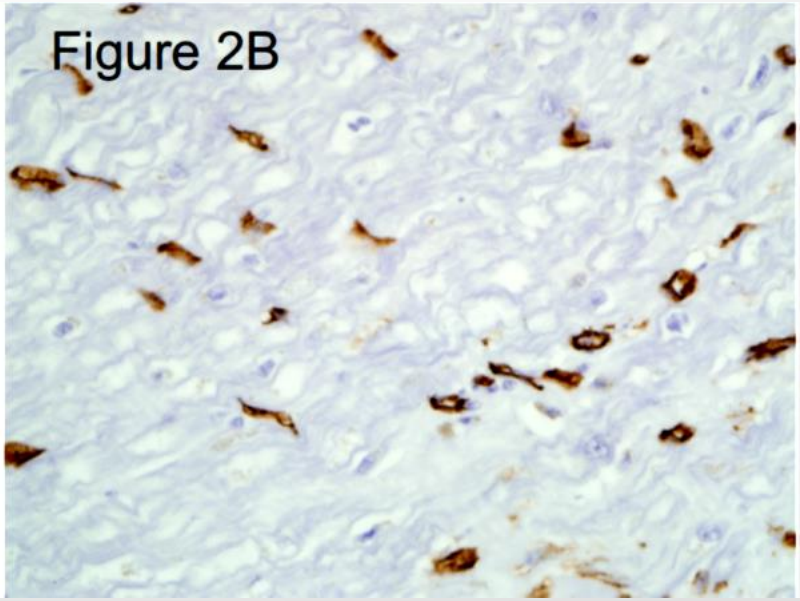
“Microvessel Density” at



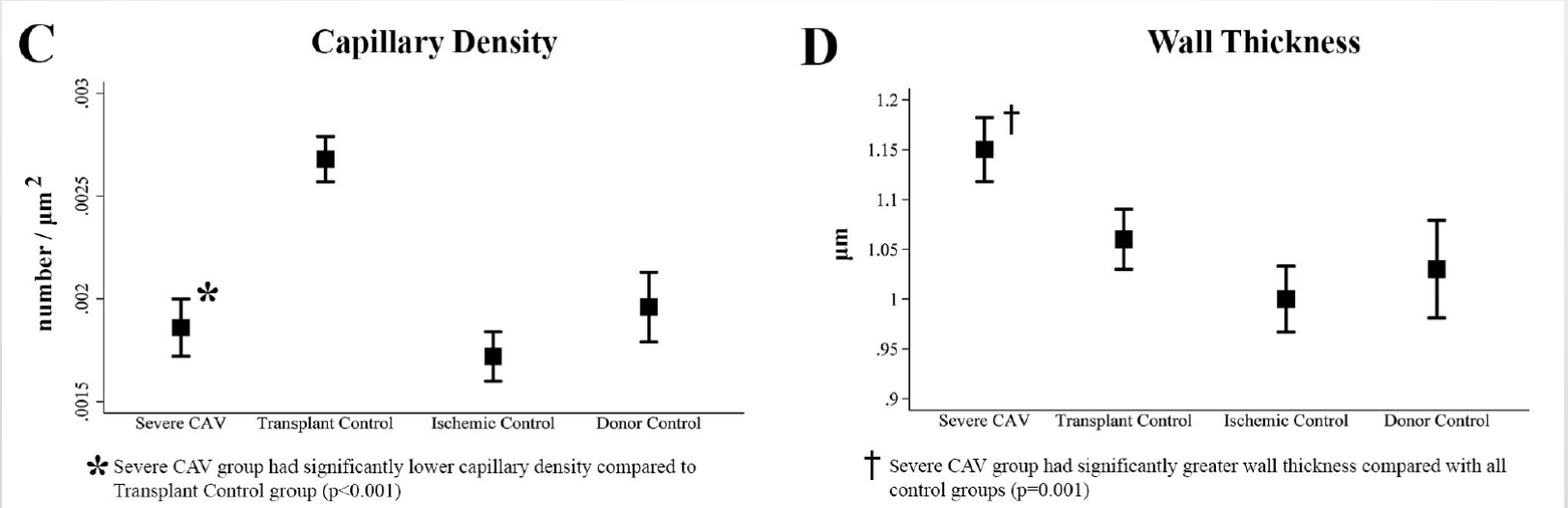


Unique characteristics of allograft microvasculature in patients with severe symptomatic cardiac allograft vasculopathy

Anees Daud, MD; David Xu, MD; Monica P. Revelo, MD, PhD; Stavros G. Drakos, MD, PhD; Elizabeth Dranow, PhD; Gregory Stoddard, MPH, MBA; Abdallah Kfoury, MD; Elizabeth Hammond, MD; Jose Nativi-Nicolau, MD; Rami Alharethi, MD; Dylan V. Miller, MD; Edward M. Gilbert, MD; Omar Wever-Pinzon, MD; Deborah Budge, MD; Stephen H. McKellar, MD, MSc; Kia Afshar, MD; James C. Fang, MD; Craig H. Selzman, MD; Josef Stehlik, MD, MPH

Utah Transplant Affiliated Hospitals (U.T.A.H.) Cardiac Transplant Program

(ISHLT 2017)

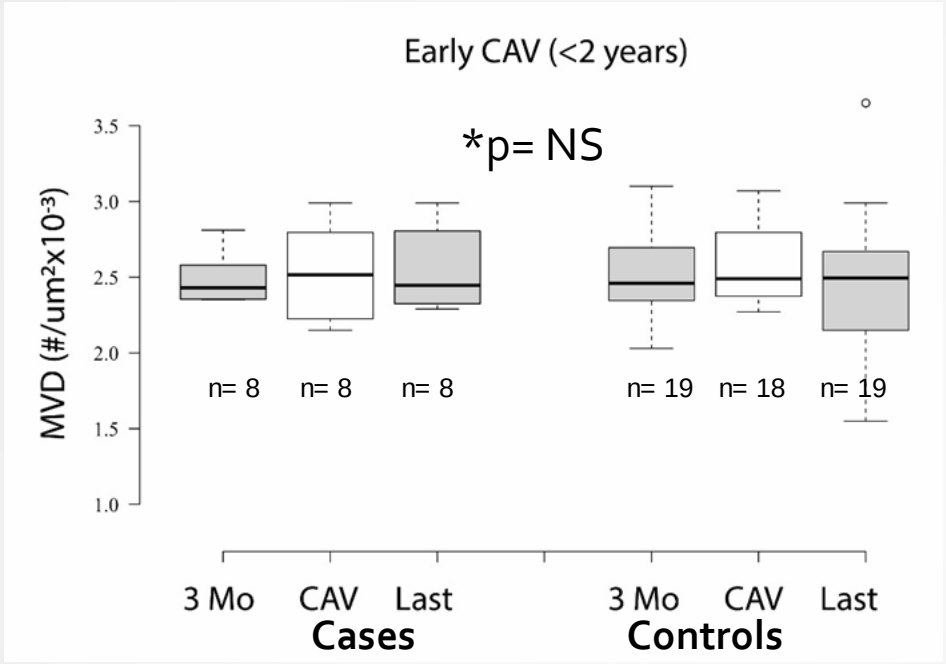


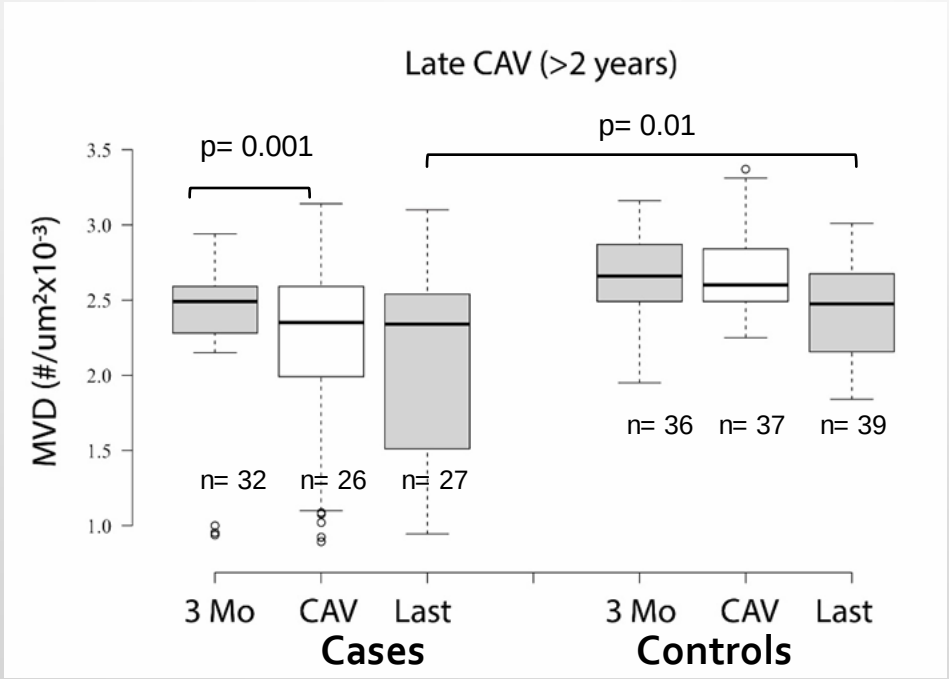
CAV Case-Control Cohort

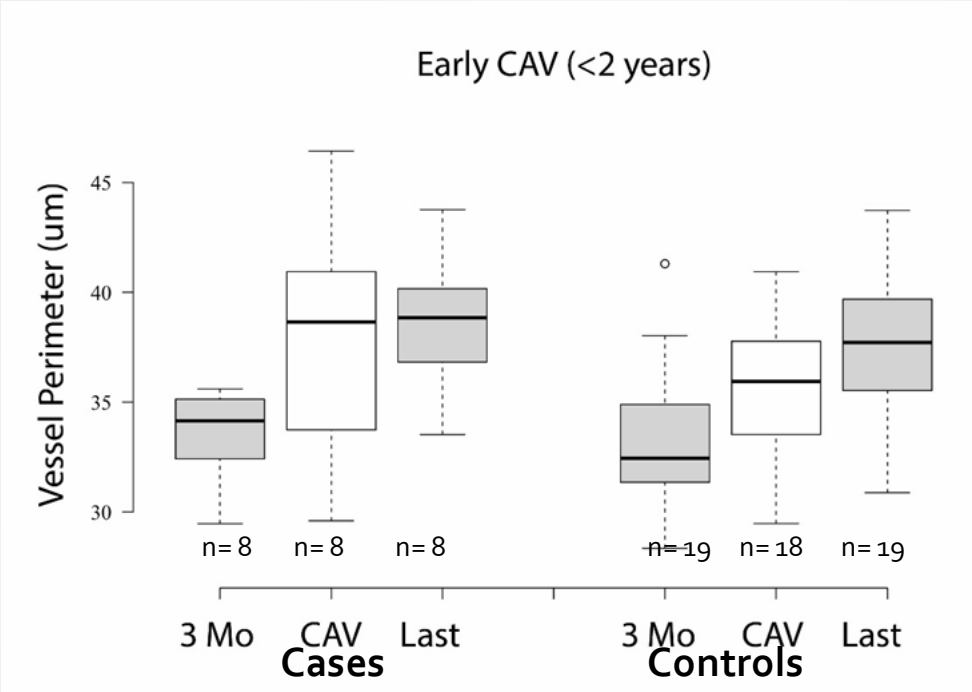
- Adult Heart Transplant Patients
  - Mean age 51
  - 68 Men, 32 women
  - Cases: 41 patients with CAV
  - Controls: 59 patients without CAV or any treated AMR
    matched for age / gender / "era" (1:2 ratio)
  - 3 time points:
    - 3 Months post transplant
    - @ time of CAV diagnosis by angiography (mean 3.6 years)
    - @ time of most recent biopsy (mean 7 years)

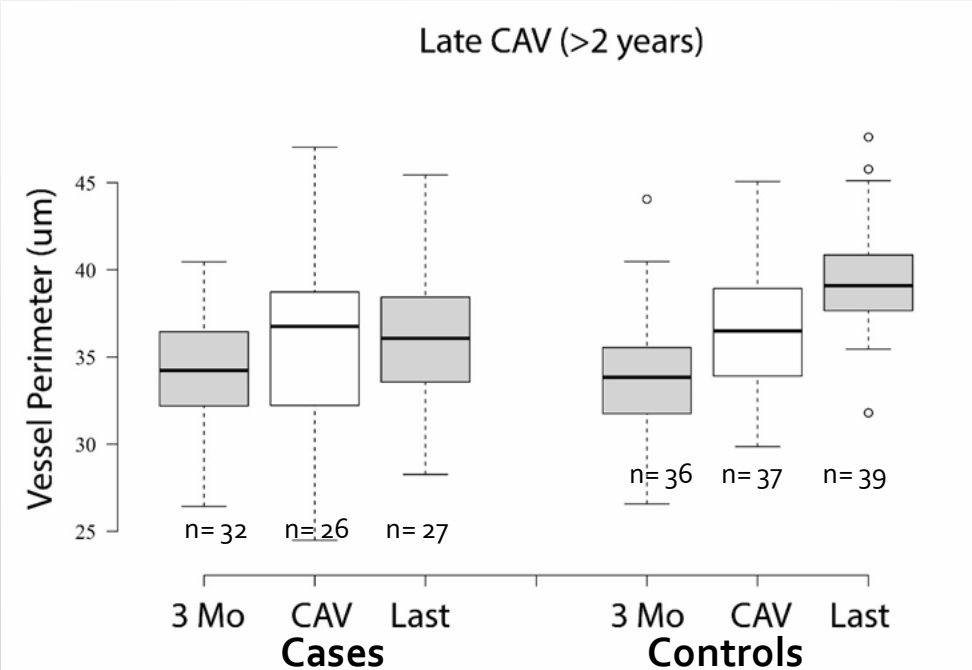
Microvessel Density Analysis

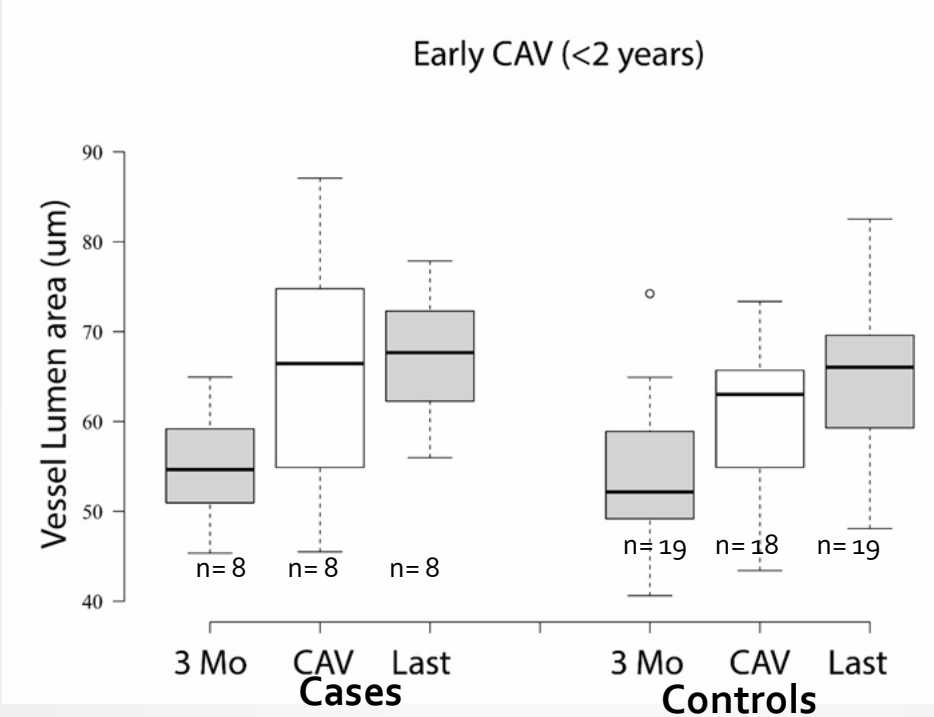
- Endomyocardial biopsies stained for CD34
- Whole slide images captured
- Leica Biosystems Aperio® Microvessel Analysis Algorithm
  - Microvessel density (# vessels/unit area)
  - Vascular perimeter (um)
  - Vascular area (um^2)
  - Vessel wall thickness (um)
- Hypothesis:
  - CAV patients ↓ MVD, ↑ perimeter, area, thickness

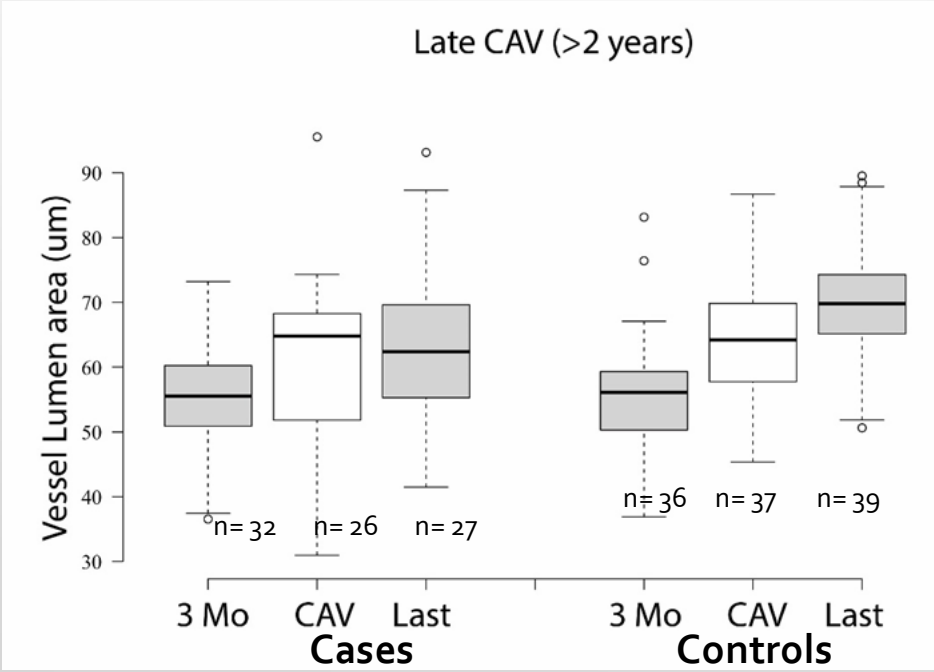

Early CAV (<2 years)
*p= NS
MVD (#/um²x10⁻³)
3.5
3.0
2.5
2.0
1.5
1.0
n= 8
n= 8
n= 8
n= 19
n= 18
n= 19
3 Mo
CAV
Last
3 Mo
CAV
Last
Cases
Controls

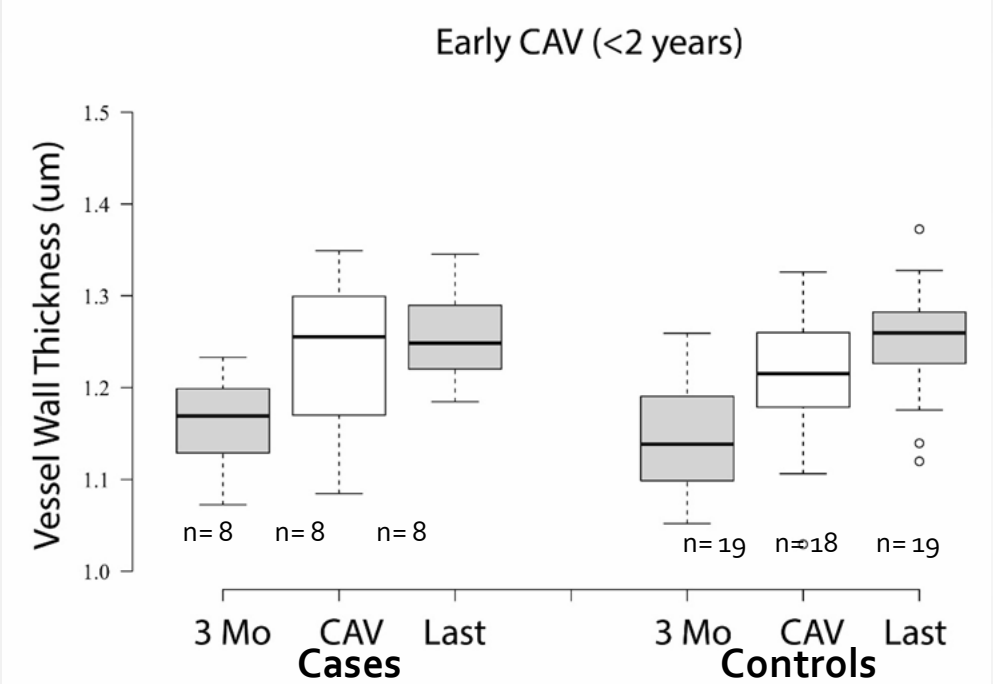

Late CAV (>2 years)
p= 0.001
p= 0.01
MVD (#/um²x10⁻³)
3.5
3.0
2.5
2.0
1.5
1.0
n= 32
n= 26
n= 27
n= 36
n= 37
n= 39
3 Mo
CAV
Last
3 Mo
CAV
Last
Cases
Controls

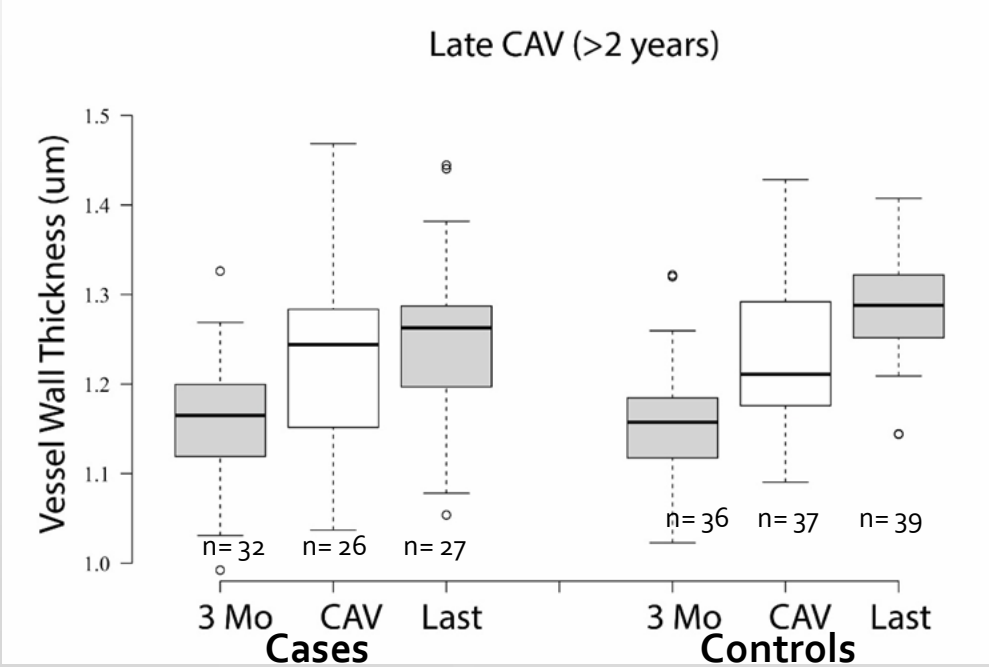

Early CAV (<2 years)
Vessel Perimeter (um)
45
40
35
30
n= 8
n= 8
n= 8
n= 19
n= 18
n= 19
3 Mo
CAV
Last
3 Mo
CAV
Last
Cases
Controls


Late CAV (>2 years)
Vessel Perimeter (um)
45
40
35
30
25
n= 32
n= 26
n= 27
n= 36
n= 37
n= 39
3 Mo
CAV
Last
3 Mo
CAV
Last
Cases
Controls


Early CAV (<2 years)
Vessel Lumen area (um)
90
80
70
60
50
40
n= 8
n= 8
n= 8
n= 19
n= 18
n= 19
3 Mo
CAV
Last
3 Mo
CAV
Last
Cases
Controls


Late CAV (>2 years)
Vessel Lumen area (um)
90
80
70
60
50
40
30
n= 32
n= 26
n= 27
n= 36
n= 37
n= 39
3 Mo
CAV
Last
3 Mo
CAV
Last
Cases
Controls


Early CAV (<2 years)
Vessel Wall Thickness (um)
1.5
1.4
1.3
1.2
1.1
1.0
n= 8
n= 8
n= 8
n= 19
n= 18
n= 19
3 Mo
CAV
Last
3 Mo
CAV
Last
Cases
Controls


Late CAV (>2 years)
Vessel Wall Thickness (um)
1.5
1.4
1.3
1.2
1.1
1.0
n= 32
n= 26
n= 27
n= 36
n= 37
n= 39
3 Mo
CAV
Last
3 Mo
CAV
Last
Cases
Controls


Intermountain
Medical Center
EMERGENCY
Receiving

Ultrastructural Alterations in Explanted Failed Cardiac Allografts: Insights into "Chronic Rejection"

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Background

- The long term effects of multiple repetitive antibody-mediated rejection episodes on the cardiac allograft (i.e. “chronic rejection”) are subject of much speculation, but sparse pathologic characterization
- Ultrastructural changes, including endothelial swelling and capillary basement membrane multilayering, have been described in this setting, but systematic studies of the prevalence, severity, and morphologic spectrum of these changes are lacking
- In contrast, in kidney literature it is well stablished that transplant glomerulopathy and peritubular capillary multilayering are hallmarks of chronic AMR

Hammond, E, Ultrastructural Path 1994;18:213
Sis, B et al, Am J Transplantation 2007; 7:1743

Background

Type of rejection	Endothelial swelling	Basement membrane multilayering	Fibrosis
AMR (2)	2	1	1
ACR (1)	0	0	1
Mixed (3)	3	2	2
None (4)	2	0	2

Revelo MP, et al 2016

Background

American Journal of Transplantation 2017; 17: 496–505
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Molecular Assessment of Microcirculation Injury in Formalin-Fixed Human Cardiac Allograft Biopsies With Antibody-Mediated Rejection

B. Afzali[1,2], E. Chapman[1], M. Racapé[3], B. Adam[1], P. Bruneval[3], F. Gil[4], D. Kim[4], L. Hidalgo[1], P. Campbell[1], B. Sis[1], J. P. Duong Van Huyen[3] and M. Mengel[1,*]

Abbreviations: ACR, acute cellular rejection; AMR, antibody-mediated rejection; AUC, area under curve; DSA, donor-specific antibodies; EMB, endomyocardial biopsies; EM, electron microscopy; FDA, Food

Aim

- Systematic study of perimyocyte capillaries using electron microscopy in failed explants due to cardiac allograft vasculopathy
- Correlate findings with prior episodes of cellular and/or antibody-mediated rejection
- Correlate findings with time post-transplant

Methods

- We identified 16 heart transplant recipients with advanced CAV requiring re-transplantation
- Ultrastructural examination was performed with special attention paid to endothelial swelling and capillary basement membrane multilayering
- 25 capillaries were examined in each case
- Correlations were made between these changes and the post-transplant interval, as well number of rejection episodes during that time, using Student's t-test for paired samples

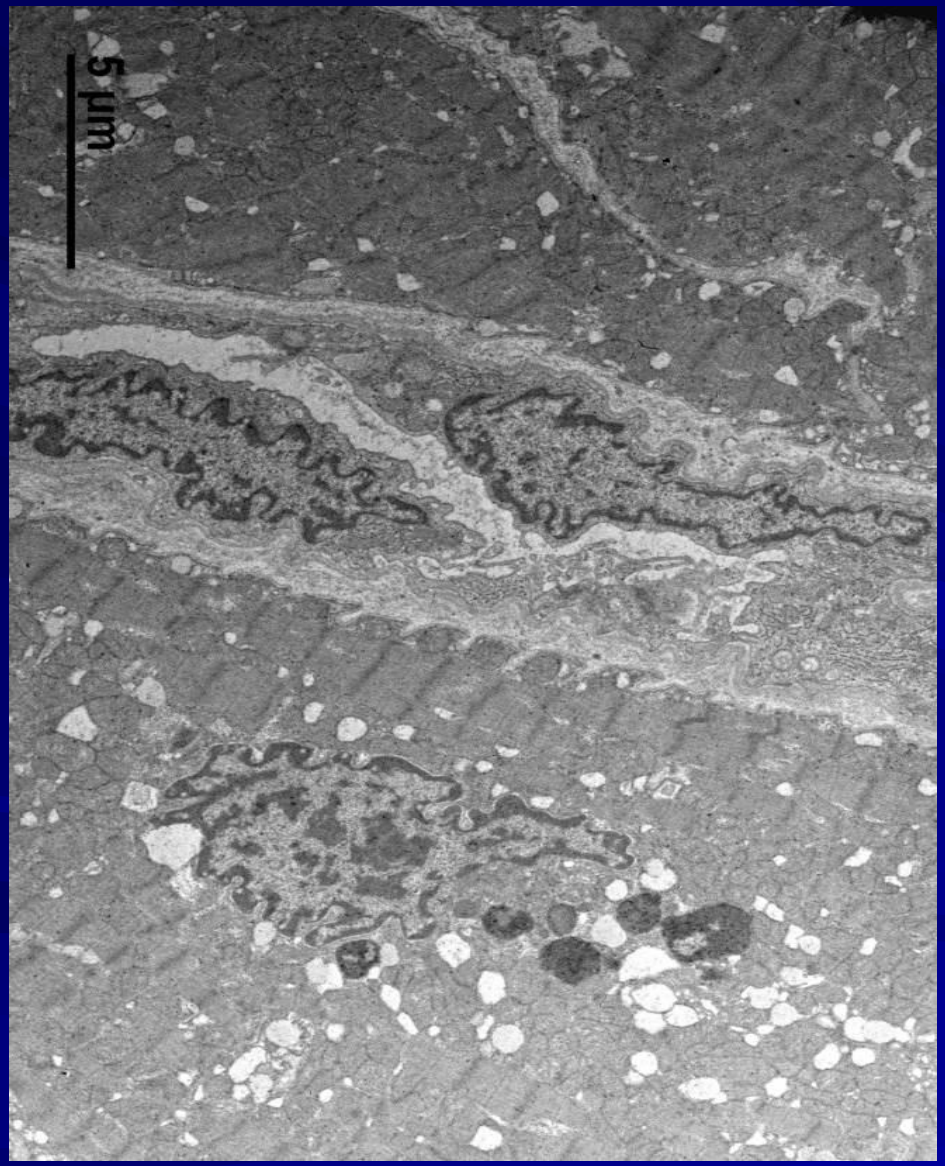
Results

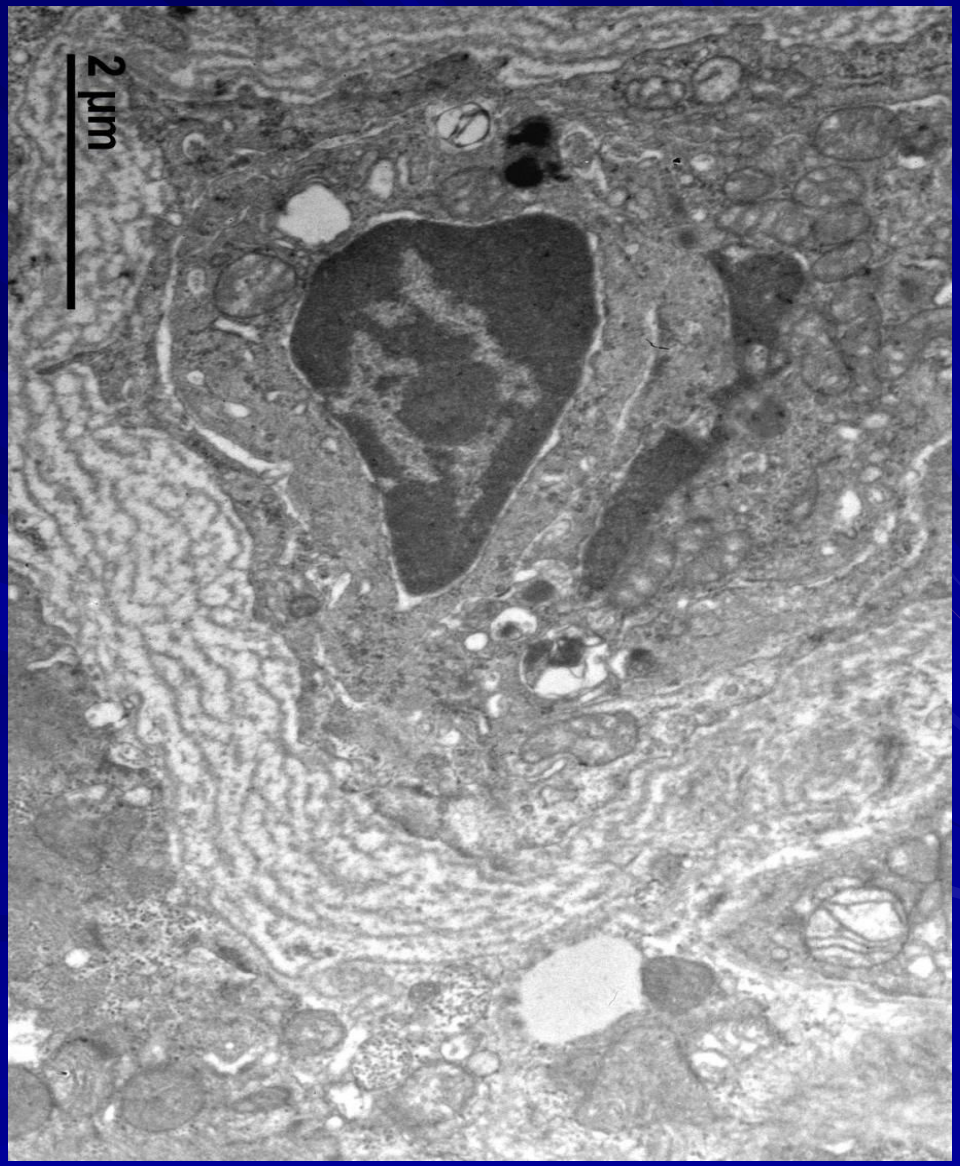
Age at re-transplant	**34 (range 24-67)**
Sex	**10 male (66.6%)**
Time post transplant	**7.7 years (range 4-16)**
Primary heart disease: **Ischemic** **Non-ischemic** **Congenital**	 **7** **6** **3**

Results

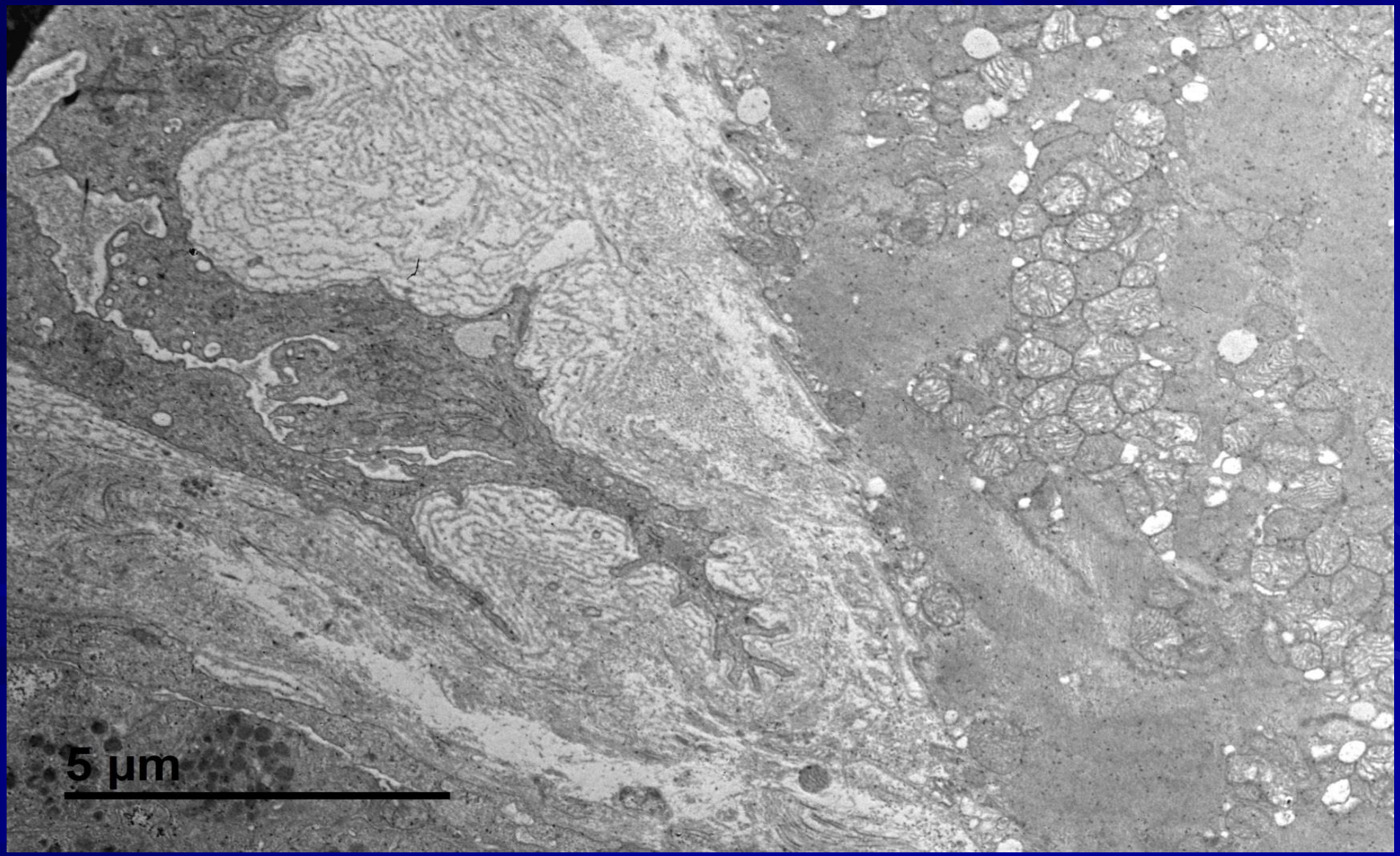
- The mean number of prior biopsies per patient showing acute cellular rejection was 12 (range: 0 to 26)
- The mean number of prior biopsies showing antibody-mediated rejection was 7 (range: 0 to 25)
- 4 cases showed definite structural capillary changes (basement membrane multilayering and endothelial swelling)
- 6 cases showed only endothelial swelling

Results cont..





Results cont..



Results

- The likelihood of showing multilayering correlated with time post-transplant ($p<0.01$), the number of cellular rejection biopsies per post-transplant year ($p<0.01$), as well as the number of antibody-mediated rejection biopsies per post-transplant year ($p=0.05$)

Conclusions

- Specific ultrastructural changes in explanted failed allografts were seen only in a small number of the hearts examined
- Although they were more likely after a longer engraftment interval and with greater numbers of episodes of either cellular or antibody mediated rejection
- Since the anticipated tissue level changes were not more universally seen in this series, additional mechanisms of late graft failure deserve further exploration

Gràcies !