

# 2017 BANFF - SCT Congress

Monday 27<sup>th</sup> to Friday 31<sup>st</sup> March 2017

BARCELONA



## Histopathology of AMR in the lung

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*Department of Cardiac Thoracic and Vascular Sciences*

*University of Padova, Medical School*





# Lung AMR Key Questions



- AMR surrogates: **Why** and **What** ?
- **Which** strategy to improve our knowledge ?



# Lung AMR Surrogate

## Why ?

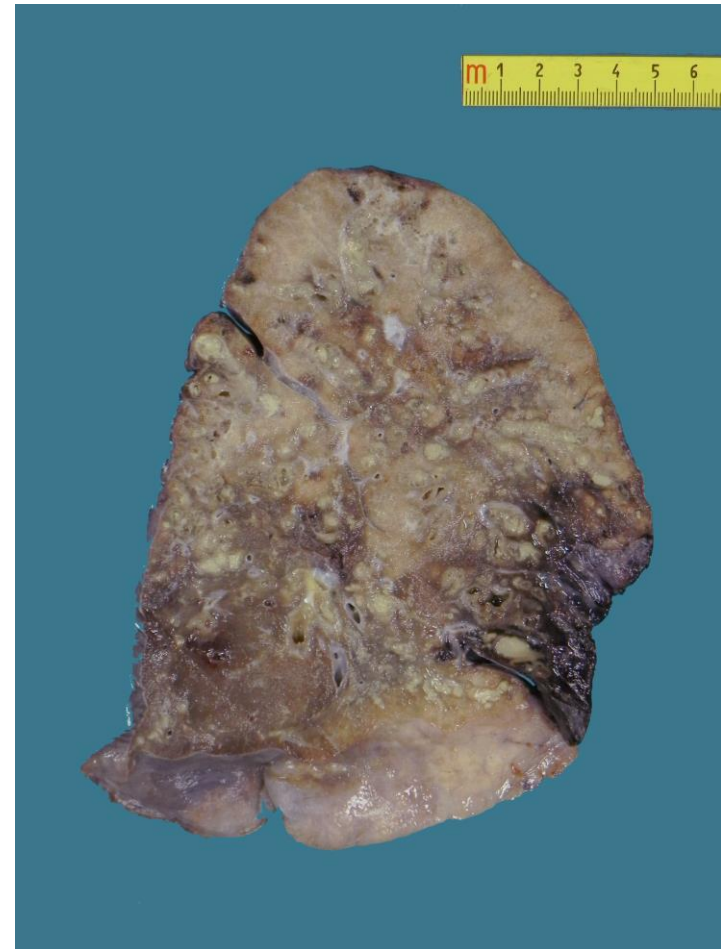
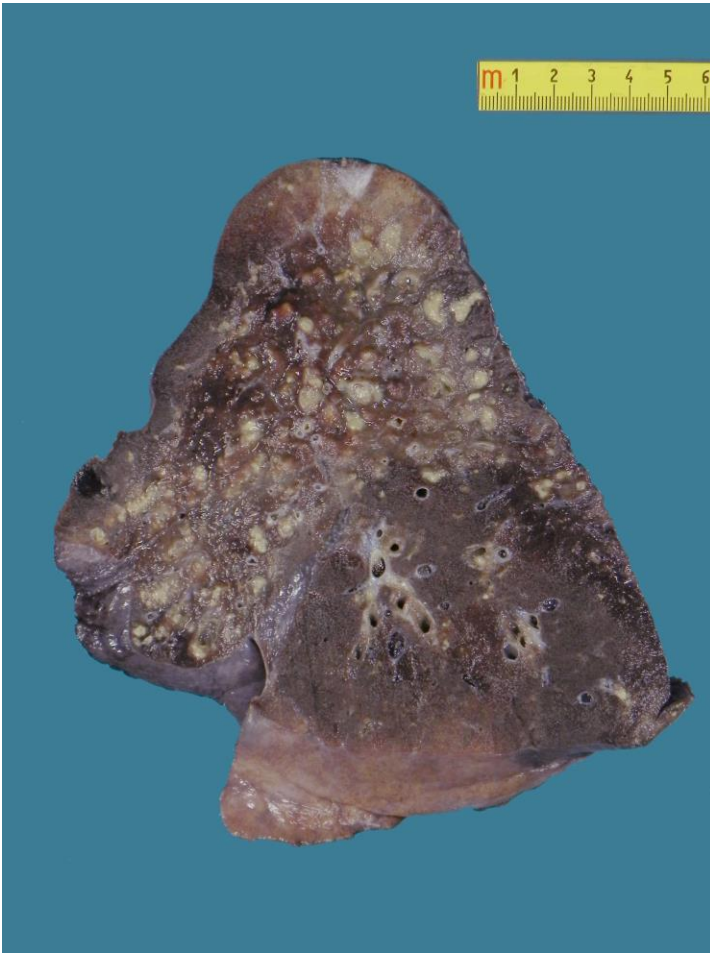
Capillary inflammation, acute lung injury and endothelitis significantly correlated with DSAs  
(*Banff study; J Heart Lung Transplant 2016;35:40-48*)



Slight interobserver agreement (also among expert pathologists)  
Low specificity (found in any injury or insult)

# CASE 1

Case: A.I; Female, 16 yrs; BLTX for CF.



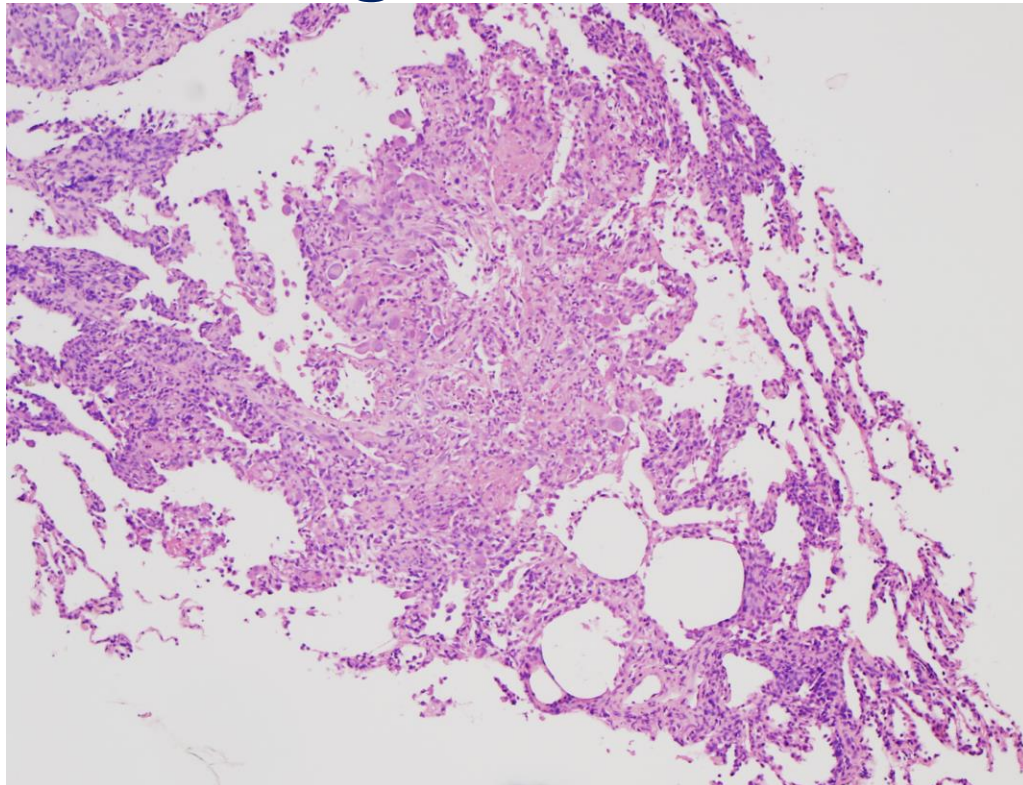
*Good early outcome after transplantation*

## Case: A.I; Female, 16 yrs; BLTX for CF.

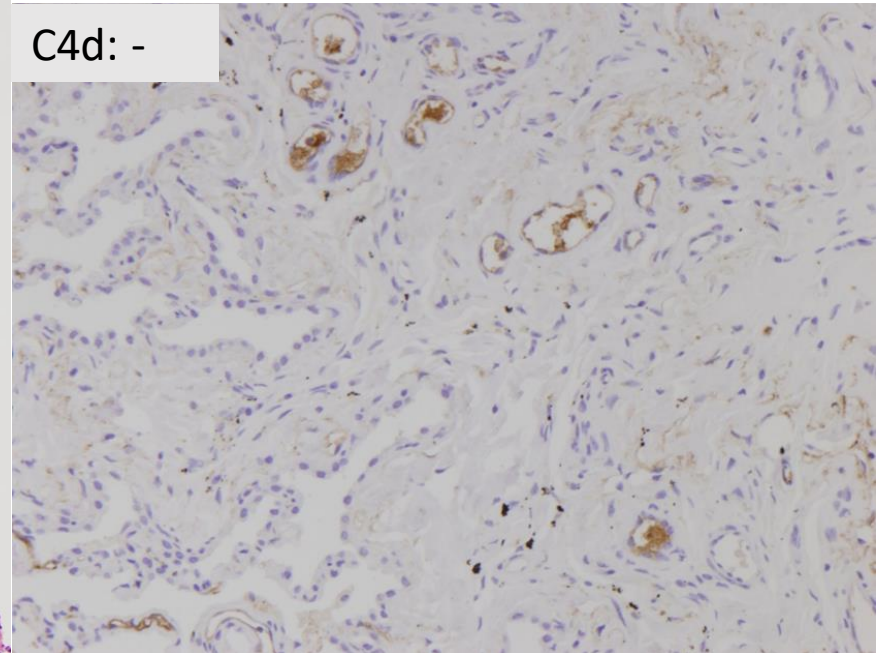
Severe graft dysfunction 1 month after TX:

II TBB (34 days after LT): **ALI**

**Sign of humoral damage ?**



C4d: -





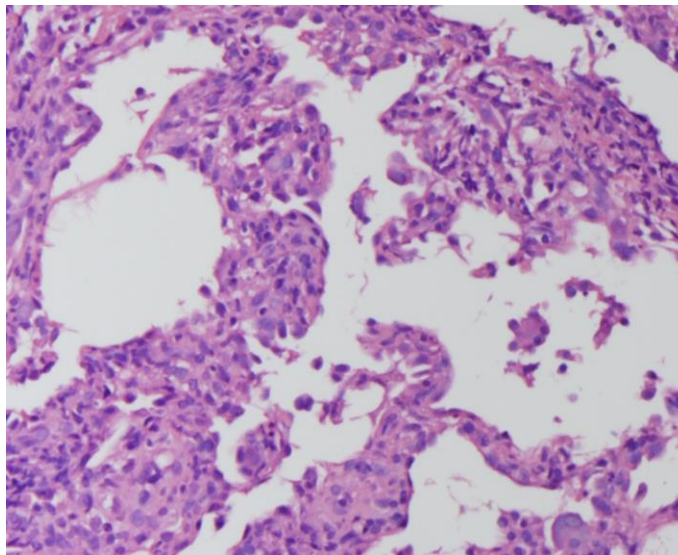
# Acute Lung Injury: mandatory MDT



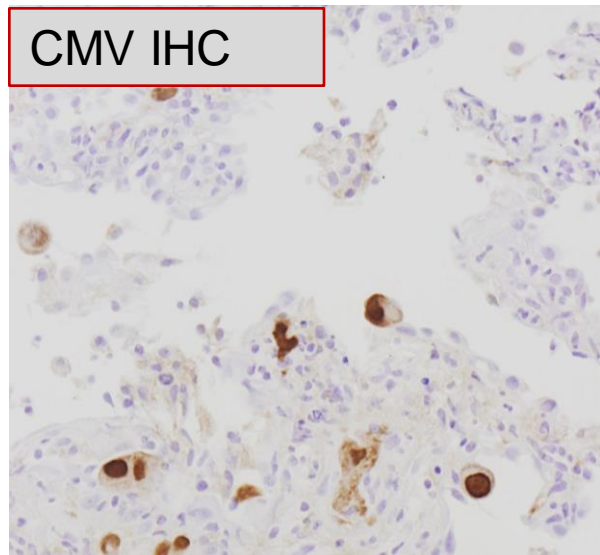
**DSA:** negative

**Microbiology:** (high viral load BAL:28,616 copies/ml and blood: 84,740 copies/ml )

Final Diagnosis: severe CMV pneumonitis

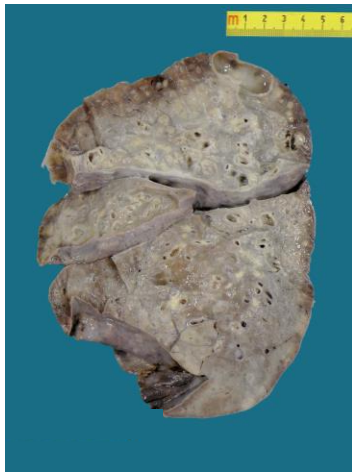
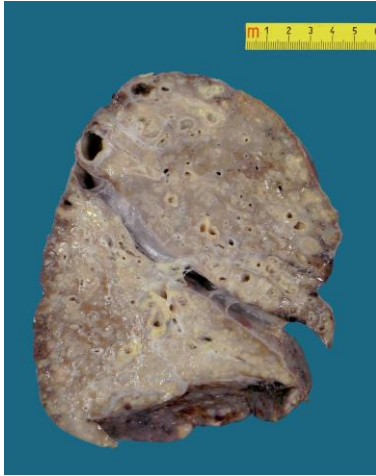


CMV IHC

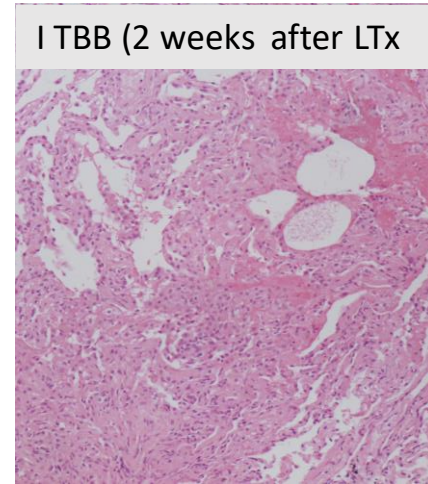
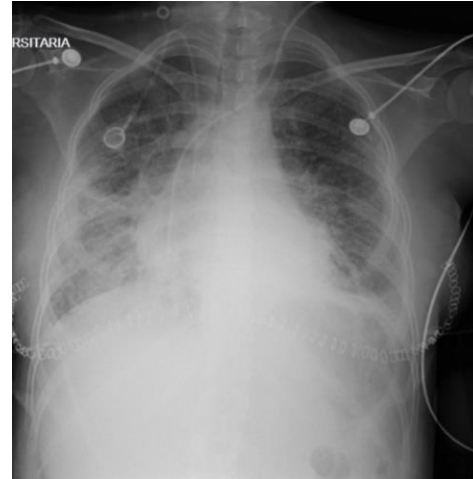


# CASE 2

**Case: G.M; Female 20 yrs; BLTX for CF**



During implantation of the second lung: edema, systemic hypotension, hypoxemia and low cardiac output



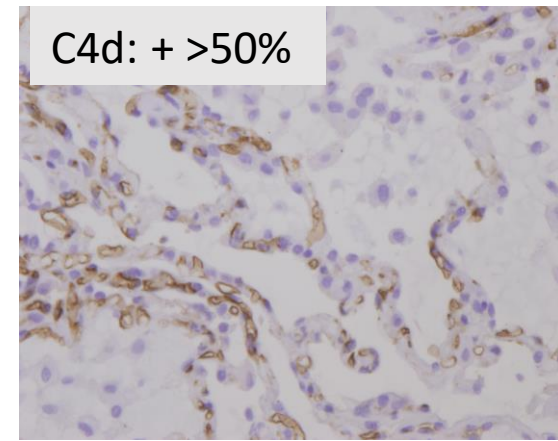
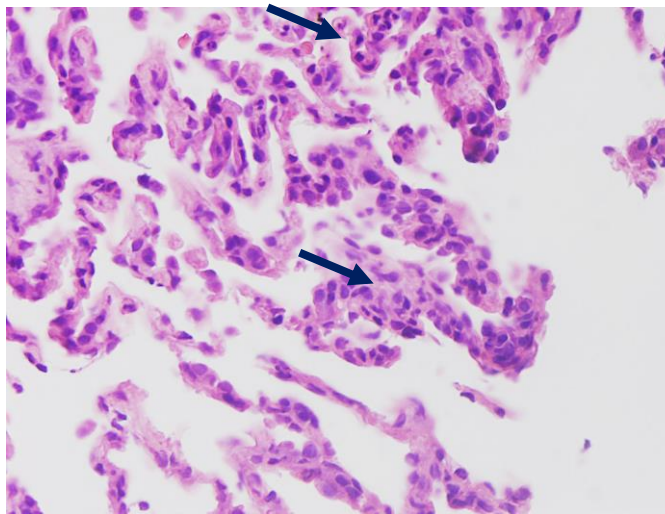
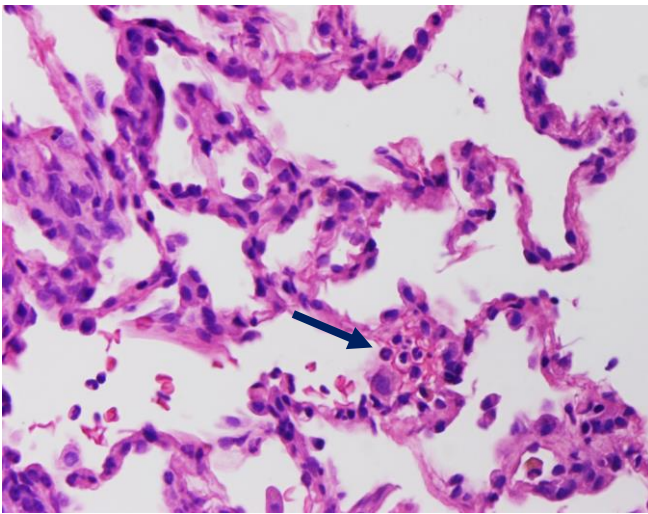
After cardiovascular drugs and pulmonary vasodilators.



# CASE 2

Case: G.M; Female 20 yrs; BLTX for CF

1 month after TX: II TBB (32 days after LT):  
Capillaritis







DSA: negative

Microbiology: negative

Final diagnosis: A0 B0 + **capillaritis**



## **Persistent/ongoing I/R injury ?**

The I/R injury can persist for up to 6 months after transplantation. However, in most lung transplant recipients, it will have resolved completely by postoperative month 1 (Krishnam MS. Radiographics. 2007;27:957-74)



DSA: negative  
Microbiology: negative

Final diagnosis: A0 B0 + **capillaritis**

**Sign of humoral damage ?**

**Table 1** Definition and Diagnostic Certainty of Clinical Pulmonary Antibody-mediated Rejection

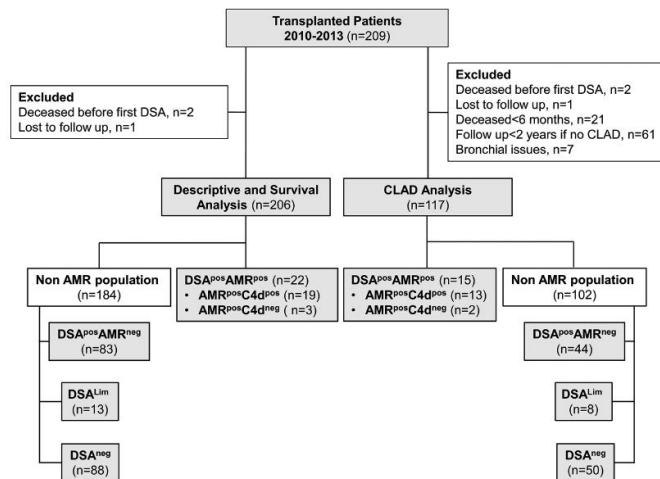
	Allograft dysfunction	Other causes excluded	Lung histology	Lung biopsy C4d	DSA
Definite	+	+	+	+	+
Probable <sup>a</sup>	+	+	+	-	+
Probable	+	+	+	+	-
Probable	+	+	-	+	+
Probable	+	-	+	+	+
Possible	+	+	+	-	-
Possible	+	+	-	-	+
Possible	+	+	-	+	-
Possible	+	-	+	+	-
Possible	+	-	+	-	+
Possible	+	-	-	+	+



## Antibody-Mediated Rejection in Lung Transplantation: Clinical Outcomes and Donor-Specific Antibody Characteristics

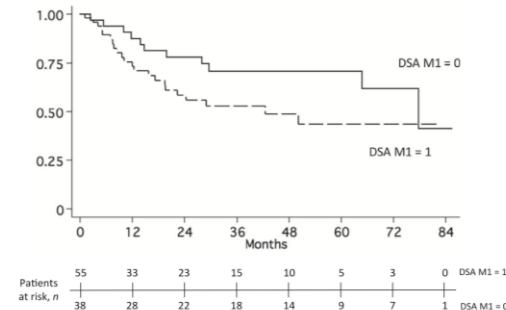
A. Roux<sup>1,2,3,\*</sup>, I. Bendib Le Lan<sup>1</sup>,  
S. Holifanjaniaina<sup>4</sup>, K. A. Thomas<sup>2</sup>,  
A. M. Hamid<sup>1</sup>, C. Picard<sup>1</sup>, D. Grenet<sup>1</sup>,  
S. De Miranda<sup>1</sup>, B. Douvry<sup>1</sup>, L. Beaumont-  
Azuar<sup>1</sup>, E. Sage<sup>3,5</sup>, J. Devaquet<sup>6</sup>,  
E. Cuquemelle<sup>7</sup>, M. Le Guen<sup>8</sup>, R. Spreafico<sup>9,11</sup>,  
C. Suberbielle-Boissel<sup>11</sup>, M. Stern<sup>1</sup> and  
F. Parquin<sup>7</sup> on behalf of the Foch Lung  
Transplantation Group

roux et al



## De-novo donor-specific anti-HLA antibodies 30 days after lung transplantation are associated with a worse outcome

Jérôme Le Pavec, MD, PhD,<sup>a,b,c</sup> Caroline Suberbielle, MD,<sup>d</sup> Lilia Lamrani,<sup>a,c</sup>  
Séverine Feuillet, MD,<sup>a,b,c</sup> Laurent Savale, MD, PhD,<sup>a,c,e</sup>  
Peter Dorfmueller, MD, PhD,<sup>a,c,f</sup> François Stephan, MD,<sup>a,c,g</sup>  
Sacha Mussot, MD,<sup>a,b,c</sup> Olaf Mercier, MD, PhD,<sup>a,b,c</sup> and Elie Fadel, MD, PhD<sup>a,b,c</sup>




Early de-novo DSA may significantly impact long-term outcomes after lung transplantation and should therefore prompt regular screening.

# But also in absence of capillaritis.....

## several morphological patterns reported in patients with graft dysfunction and de novo DSA

**Table 2** Histopathologic Indications for Immunopathologic Evaluation

---

- 
1. Neutrophilic capillaritis
  2. Neutrophilic septal margination
  3. High-grade acute cellular rejection ( $\geq$  A3)
  4. Persistent/recurrent acute cellular rejection (any A Grade)
  5. Acute lung injury pattern/diffuse alveolar damage
  6. High-grade lymphocytic bronchiolitis (Grade B2R)
  7. Persistent low-grade lymphocytic bronchiolitis (Grade B1R)
  8. Obliterative bronchiolitis (Grade C1)
  9. Arteritis in the absence of infection or cellular rejection
  10. Graft dysfunction without morphologic explanation
  11. Any histologic findings in setting of de novo DSA positivity
-





# Lung AMR Surrogates

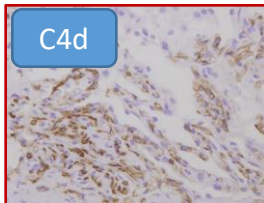
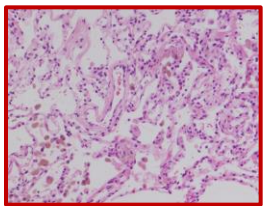
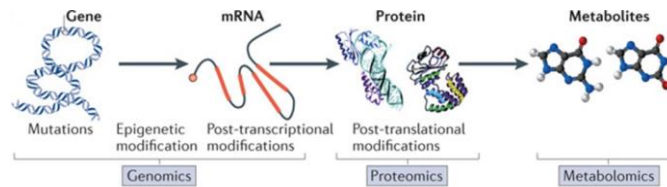
## What ?

Before focusing on some



**WARNING**

: “not ready for (not tissue) biomarkers”



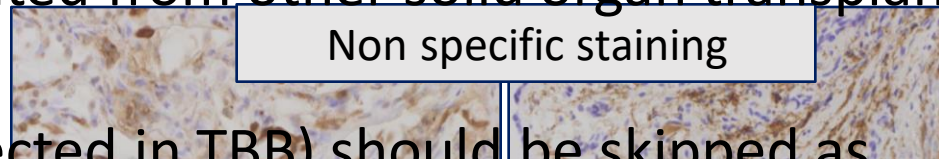
➤ The right biomarkers discovery processing: morphology first of all

# Lung AMR Surrogates:

## *C4d immunostaining*

Several weak points, we have to learn more.....

- Inter-reader reliability is poor (also among experts)
- ✓ Difficult interpretation: non specific serum and septal staining
- Staining score is extrapolated from other solid organ transplants (is it right ??)
- Small airways (rarely detected in TBR) should be skipped as the large one ?
- Dubious specificity in presence of infection (bacteria): complement system can also be activated by surface components of gram-positive bacteria, as well as by C-reactive phase protein
- Low sensitivity
- ✓ C4d-negative cases of AMR are distinct from C4d-positive cases or if the difference is due to technical staining and interpretation limitations.



Non specific staining

*Pediatr Transplantation* 2005; 9: 84-93. DOI: 10.1111/j.1399-3046.2004.00270.x  
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Pediatric Transplantation

Immune mechanisms in the pathogenesis of bronchiolitis obliterans syndrome after lung transplantation

*American Journal of Transplantation* 2005; 5: 2126-2134  
doi: 10.1111/j.1600-6143.2005.00999.x

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doi: 10.1111/j.1600-6143.2005.00999.x

Andrés Jaramillo<sup>1</sup>,  
Félix G. Fernández<sup>1</sup>, Elbert Y. Kuo<sup>1</sup>,  
Elbert P. Trulock<sup>2</sup>, G. A. Patterson<sup>1</sup> and  
T. Mohanakumar<sup>1,3</sup>

Induction of Obliterative Airway Disease by Anti-HLA Class I Antibodies

Takkihiro Maizumi<sup>1</sup>, Andrés Jaramillo<sup>1</sup>,  
Kishore Narayanan<sup>1</sup>, Toru Higuchi<sup>1</sup>  
and T. Mohanakumar<sup>a,b,\*</sup>

Induction of  
The main cause of long-term morbidity and mortality after lung transplantation is the development of bronchiolitis

# Lung AMR Surrogates

## What ?

Histopathology is the bedrock and cornerstone for the diagnosis of immunological disorders

**Aim: are any other morphological AMR stigmata ?**

### Multi Task

Fiorella Calabrese (IT)

Marie-Pierrette Chenard (FR)

Emanuele Cozzi (IT)

Martin Goddard (UK)

Deborah Levine (USA)

Desley Neil (UK)

Angeles Montero Fernandez (UK)

Sandrine Hirschi (FR)

Wim Timens (NL)

Eric Verbeken (BE)

Several cases with clinical/path suspicious of AMR reviewed at multi-head LM (4 meetings)

MD discussion with clinician and immunologist

- ✓ AMR concomitant to ACR and CLAD (OB)
- ✓ C4d: low reproducibility among different labs; difficult interpretation
- ✓ Histological signs: capillaritis, arteritis, ALI, **widening of interstitial spaces**

# Widening of Interstitial Spaces

Study population: (updated February 2017; additional cases from 2 other centres: Iowa City and Paris )

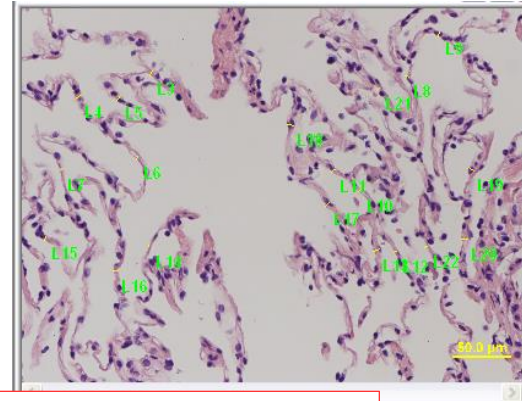
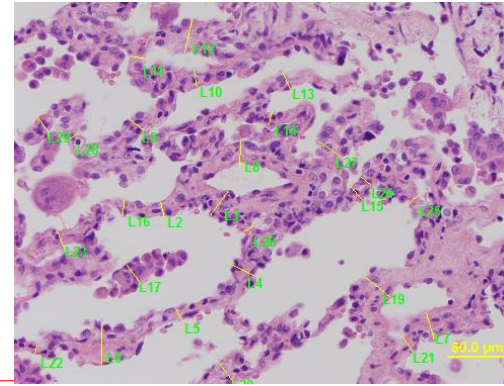
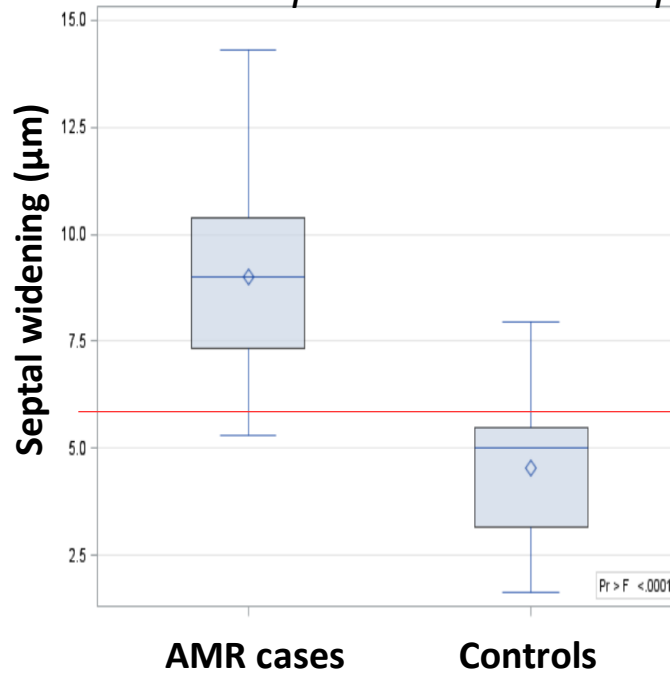
50 AMR cases and 22 control cases (absent any kind of immunological insult; no infections; negative DSA)

	AMR cases (50)	Controls (22)
Age (mean $\pm$ SD)	42.7 $\pm$ 18.2	38.1 $\pm$ 15.9
Sex (M:F)	25 : 25	15 : 7
Native disease		
- CF	11	13
- IPF	16	6
- Other	23	3
Clinical : Subclinical	42 : 8	-
Donor age (mean $\pm$ SD)	36.1 $\pm$ 17	32.9 $\pm$ 15
Donor sex (M:F)	30:20	13:9
Donr smoking status (smoker:non smoker)	16:34	5:17
AMR cathegory		
- Definite	9	
- Probable	25	-
- Possible	16	
DSA		
- Negative	2	
- MFI <3000 (% anti-HLA II)	14 (10/14=71,4%)	-
- MFI $\geq$ 3000 (% anti-HLA II)	34 (30/34=88,2%)	



# Widening of Interstitial Spaces

Computer-assisted morphometry (Image ProPlus software 6.1)



Widening cut-off: **6  $\mu\text{m}$**  (1st quartile)

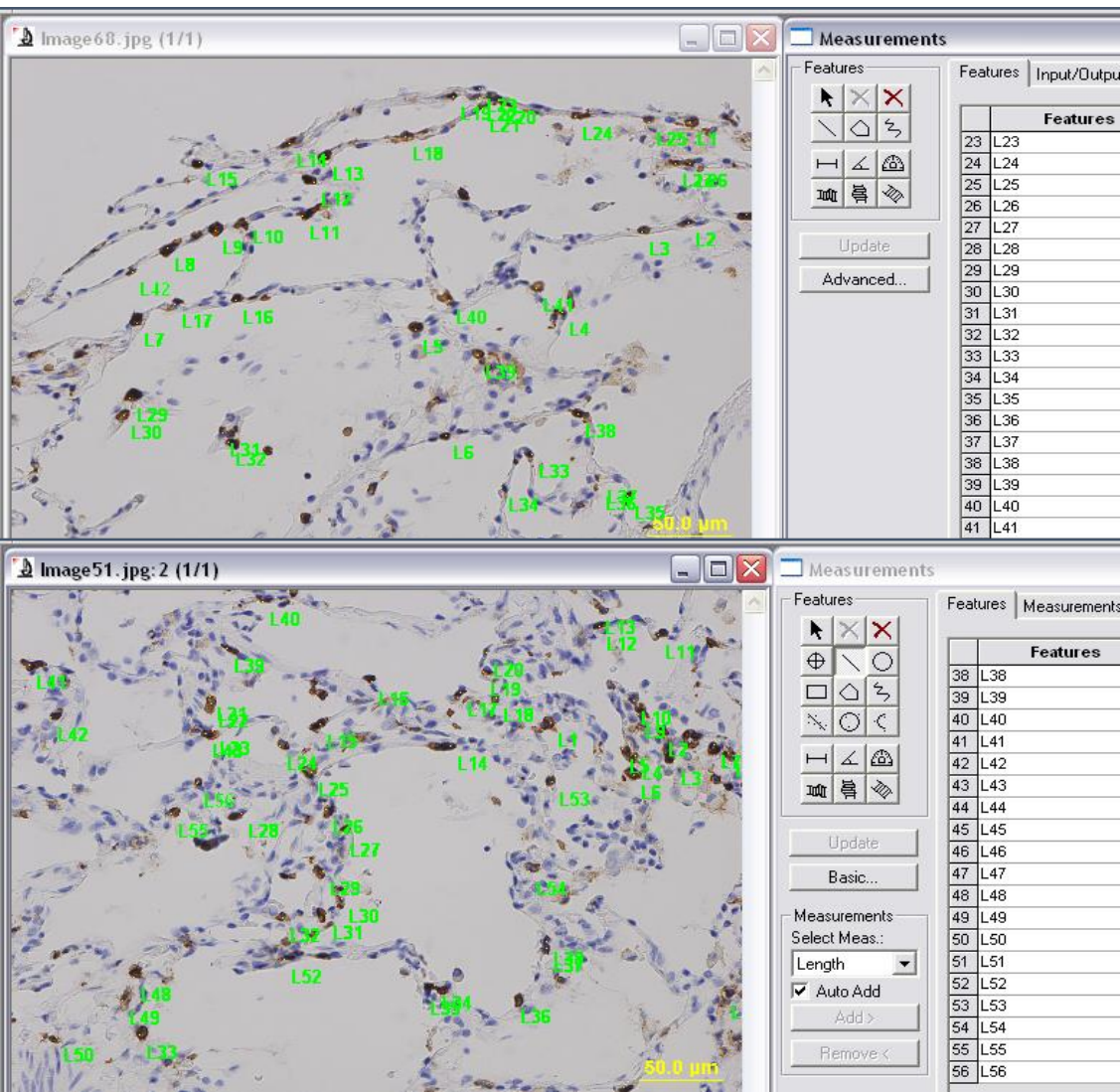


Feature	auc	Sensitivity	Specificity	P-value	OR	95%CI	PPV %	NPV %
Widening>6 $\mu\text{m}$	0.935	96%	91%	<0.0001	159.11	24.8-999.9	96	91
Capillaritis	0.780	56%	100%	0.006	57.03	3.1-999.9	100	50
ALI	0.680	36%	100%	0.03	25.61	1.4-482.8	100	41
C4d	0.653	31%	100%	0.05	20.21	1.05-389.0	100	39

# Widening of Interstitial Spaces

## Inflammatory cell burden

*Computer-assisted morphometry (Image ProPlus software 6.1)*



***CD45 positive cell number was evaluated in 15 cases vs 7 controls.***

***No linear relation was found between widening and CD45 positive cell number.***

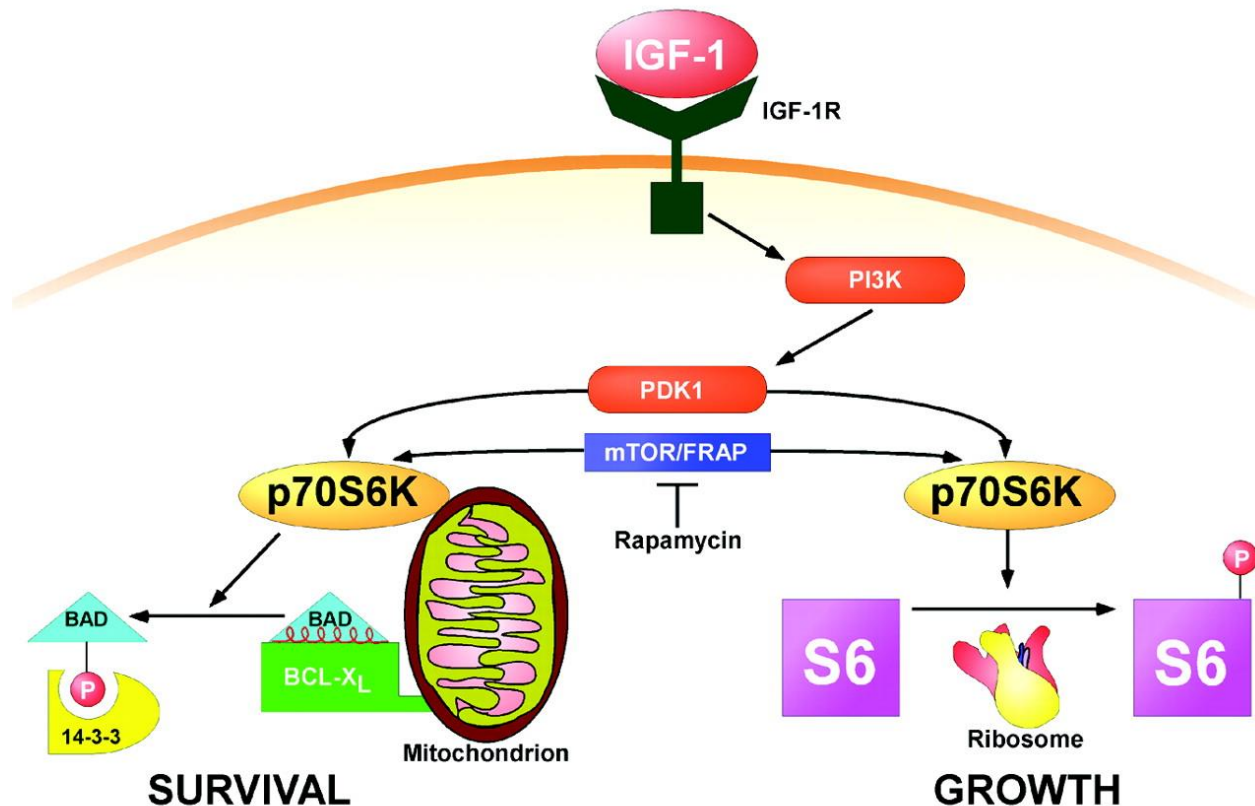
# Widening of Interstitial Spaces

## *Conclusion and Future Steps*

- Widening of alveolar septa may represent another AMR stigmata
  - A larger prospective case series is mandatory
    - ✓ to confirm the data
    - ✓ to better understand widening substrate (inflammation?;endothelial swelling?;edema ?)
  - Association of several histological parameters in order to obtain a combined score (capillaritis + widening +C4d/other marker)
    - ✓ More sensitive diagnosis
    - ✓ Prognosis

# Other lung AMR surrogates under current evaluation.....

## Phospho-70-S6K and S6RP associated AMR



Hisashi Harada et al. PNAS 2001;98:9666-9670



# Phospho-S6K and S6RP associated AMR

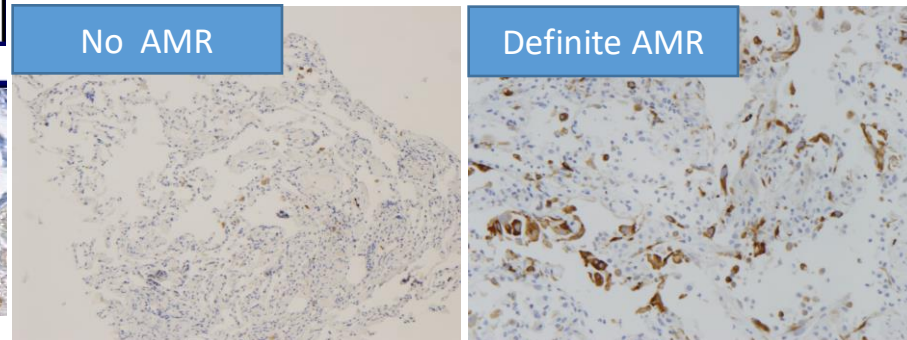
- Increasing levels of phosphorylation of S6K and S6RP exhibited strongest association with pAMR
- A level of 2+ or greater significantly augments the risk of AMR
- More sensitive than C4d

	Odds Ratio	p-value	95% CI
pS6K, grade 1+	18	0.001	3 – 100
pS6K, grade 2+	52	<0.001	6 – 425
pS6K, grade 3+	49	0.001	5 – 521
pS6RP, grade 1+	4	0.06	1 – 13
pS6RP, grades 2+/3+	10	0.008	2 – 52

In the lung: our preliminary data showed :  
More specific staining of S6RP more than S6K  
Good sensitivity also for non HLA DSA

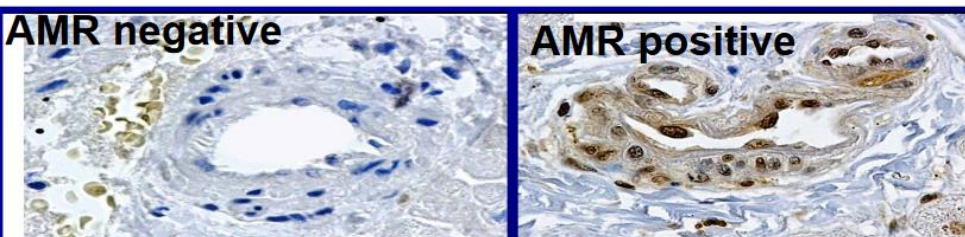
No AMR

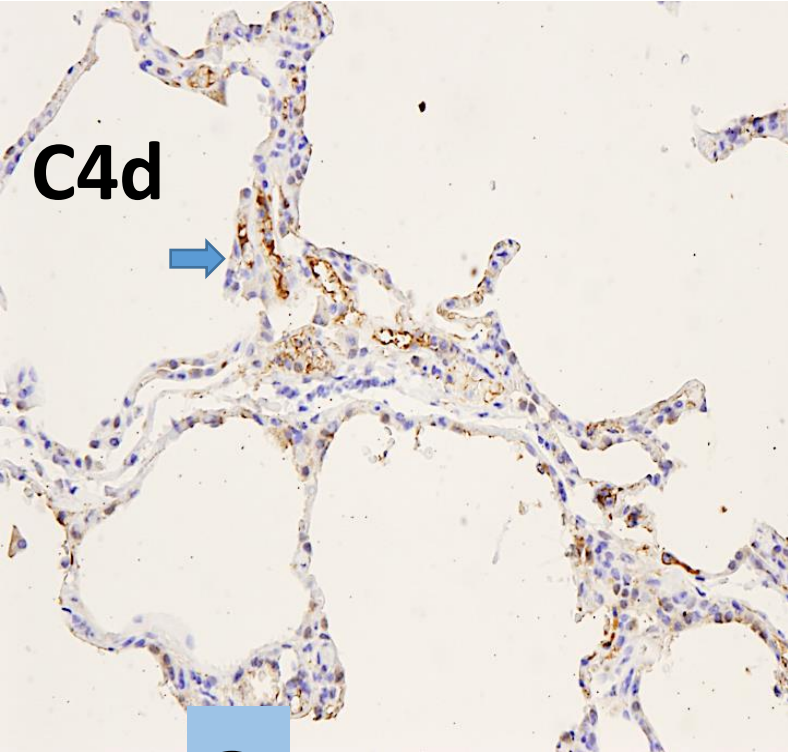
Definite AMR



AMR negative

AMR positive



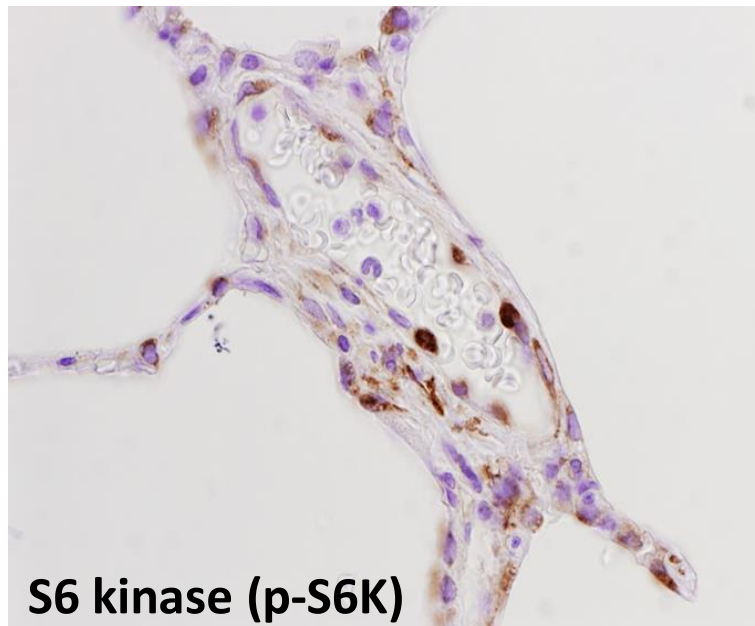
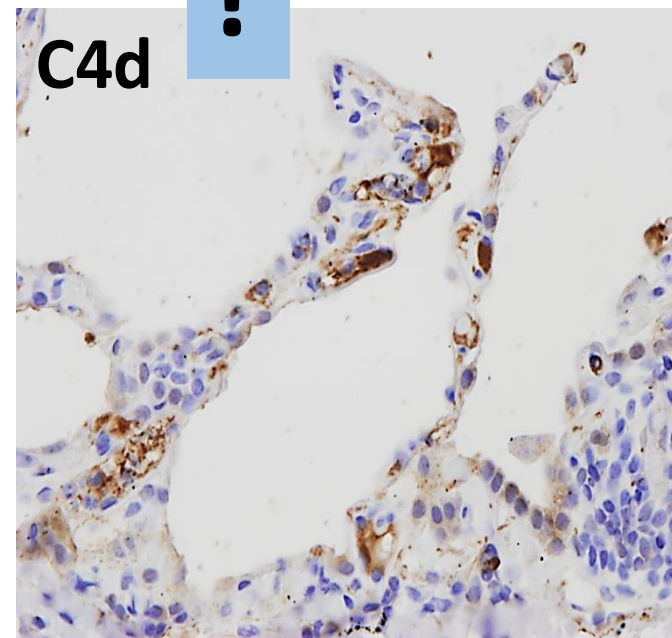


**Case Z.G., 34 yrs old (BLTX for Cystic Fibrosis; June 2015)**

After LT: acute respiratory failure , severe pulmonary hypertension with right ventricle dysfunction.  
Unresponsive to any medical treatment

Death: 7 days after LTx

**Autopsy:** ALI (AMR) + foci of ACR (A2B0)

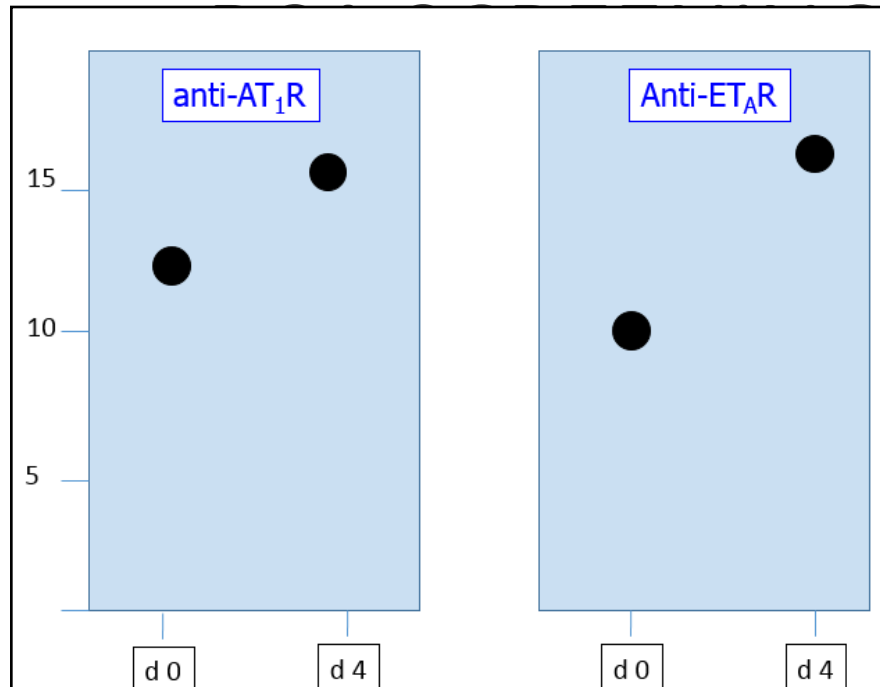


## Case Report

# Immediate and Catastrophic Antibody-Mediated Rejection in a Lung Transplant Recipient With Anti-Angiotensin II Receptor Type 1 and Anti-Endothelin-1 Receptor Type A Antibodies

E. Cozzi<sup>1,2,\*</sup>, F. Calabrese<sup>1</sup>, M. Schiavon<sup>1</sup>,  
P. Feltracco<sup>3</sup>, M. Seveso<sup>2</sup>, C. Carollo<sup>3</sup>, M. Loy<sup>1</sup>,  
M. Cardillo<sup>4</sup> and F. Rea<sup>1</sup>

Abbreviations: AMR, antibody-mediated rejection; AT<sub>1</sub>R, angiotensin II receptor type 1; CF, cystic fibrosis; CO, cardiac output; CT, computed tomography; DSA, donor-specific antibody; ECMO, extracorporeal membrane oxygenator; ET<sub>A</sub>R, endothelin-1 receptor



High levels of anti-AT<sub>1</sub>R and ETAR antibodies measured retrospectively: revealed the presence of both types of antibodies prior to transplantation which increased on p.o. day 4 from 12.8 to 15.2 Units/ml and from 15 to 18.4 Units/ml,

FEATURED PAPERS

Lung intragraft donor-specific antibodies as a risk factor for graft loss



J Heart Lung Transplant 35 (12), 1418-1426. 2016

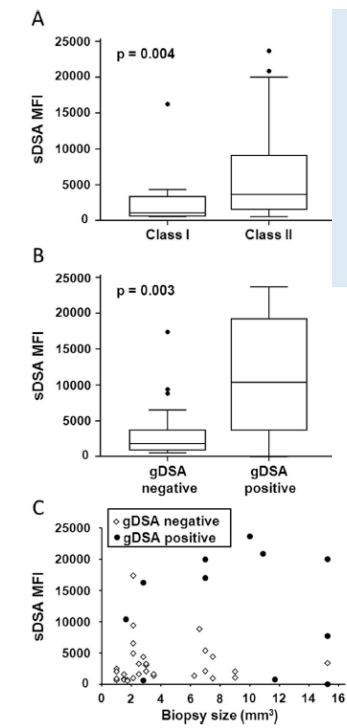
Jonathan Visentin, PharmD, PhD,<sup>a,b</sup> Albane Chartier, MD,<sup>c</sup> Layal Massara,<sup>b</sup> Gabriel Linares,<sup>a</sup> Gwendaline Guidicelli, PharmD, PhD,<sup>a</sup> Elodie Blanchard, MD,<sup>c</sup> Marie Parrens, MD, PhD,<sup>d,e</sup> Hugues Begueret, MD,<sup>d</sup> Claire Dromer, MD,<sup>c</sup> and Jean-Luc Taupin, PharmD, PhD<sup>a,b</sup>

The presence of DSA not synonymous of AMR. The lack of direct association related to:

- Inability of the DSA to bind to the graft
- Low affinity DSA and its target

On the other hand the absence of DSA not exclusion of AMR

- Entrapped within allograft (as in kidney)



**CONCLUSIONS:** In lung transplantation, gDSA appears to be a valuable biomarker to identify pathogenic DSA and LTRs with a higher risk for graft loss.



# Preliminary Data From Strasbourg LT Group

presented at 12th International Conference of Lung Transplantation  
September 15-16; 2016; Paris

Patient	Graft Dysf	DSA > 1000	Histo	C4d	Other Diag	Agreement AMR	gDSA	Last visit
1	+	+	+	+	Infection, ACR	Probable AMR	+	BOS 3
2	+	+	- /Post T	-	+	possible	+	BOS 3
3	+	+	+	+	infection	probable	+	dead
4	+	+	+	-	+	probable	+	dead
5	+	+	+	-	+	probable	+	BOS 0p
6	+	+	+	ND	+	probable	+	fit
7	+	+	+	+	CMV BAL 3 log	probable	+	fit
8	+	+	-	-	+	No AMR (BOS)	+	dead
9	+	+	-	-	+	No AMR (BOS)	+	BOS3
10	+	+	-	-	AFOP	No AMR	-	Re LT
11	no	+	-	-	systematic f/up	No AMR	-	fit

- Sensitivity = 7/7 (100%)
- Specificity = 2/4 (50%) ... gDSA+ in some CLAD?
- Positive predictive value = 7/9 (77%)
- Negative predictive value = 2/2 (100%)

## Limits:

- ✓ Tissue size
- ✓ Additional frozen sample
- ✓ Standardized processing (MFI threshold)

*Slide courtesy of Sandrine Hirshi*



# Lung AMR

## Key Questions



- AMR surrogate AMR markers: **Why** and **What** ?
- **Which** strategy to improve our knowledge ?

# Are any strategy to improve our knowledge ?

Table 3. ISHLT Recommendations for Monitoring for AMR

	Endomyocardial Biopsy	Circulating Antibody
Methodology	<p>Histological evaluation</p> <p>Immunoperoxidase: C4d</p> <p>Immunofluorescent staining: C4d and C3d</p>	<p>Solid-phase assay and/or cell-based assays to assess for presence of DSA (and quantification if antibody present)</p>
Intervals	<p>Histological evaluation of every protocol biopsy</p> <p>Immunoperoxidase/immunofluorescent staining: 2 wk and 1, 3, 6, and 12 mo after transplantation</p> <p>When AMR is suspected on the basis of histological, serological, or clinical findings</p> <p>Routine C4d(C3d) staining on subsequent biopsy specimens after a positive result until clearance</p>	<p>2 wk and 1, 3, 6, and 12 mo, and then annually after transplantation</p> <p>When AMR is clinically suspected</p>

➤ Careful scheduled

S6RP, c

➤ DSA s

clinical

in some LT centers; even not uniform)

➤ Promotion of educational activities (e.g.: WG meetings; Master courses; **Web tutorial**)



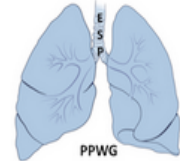
UNIVERSITY  
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Department of  
Cardiac, Thoracic and  
Vascular Sciences



Pulmonary  
Pathology  
Working  
Group



Welcome  
page

Introduction  
and Aims

The  
Tools

Clinical  
Evaluation

Serial Monitoring  
and Management

Immunological  
Screening

Lung  
Biopsy

Case  
Series

KEY

## Lung Transplant Pathology: Antibody Mediated Rejection - AMR

### Welcome

Welcome to the "lungtransplant.dctv.unipd.it", an educational forum for anyone interested in the lung transplant field. This specific web-section will focus on AMR, widely considered one of the most challenging and dynamic topics in lung transplant practice.

This tutorial will represent:

- 1) a comprehensive guide on the topic but also an opportunity to increase the interest and knowledge of various specialists, and
- 2) a good platform for exchanges and discussions in the topic.

This tutorial contains 7 sections covering all aspects of AMR and is the product of several European skilled specialists involved in this area. The menu on the top is clickable and allows the user to move directly to an area of interest. All images can be enlarged by clicking on them (click again on the picture to shrink it down).

Under → **Case Series** a subsection entitled "Required Panelist's Opinion" has been created to allow the submission of new cases by other specialists involved in this topic.

The web pages was designed by the **Informatic Education Office** of the DCTV of the University of Padova. We invite feedback from all visitors interested in improving this Tutorial. Please send comments or suggestions to [amr.dctv@unipd.it](mailto:amr.dctv@unipd.it).

**Fiorella Calabrese (Pathologist, University Hospital of Padova, I)**



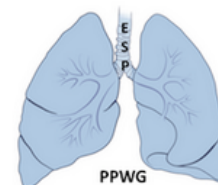
UNIVERSITY  
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Department of  
Cardiac, Thoracic and  
Vascular Sciences



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Welcome  
page

Introduction  
and Aims

The  
Tools

Clinical  
Evaluation

Serial Monitoring  
and Management

Immunological  
Screening

Lung  
Biopsy

Case  
Series

KEY

Histology

Immunofluorescence

Immunohistochemistry

Other ancillary techniques

## Lung Transplant Pathology: Antibody Mediated Rejection – AMR

### Introduction and Aims

AMR has been associated with acute and chronic allograft dysfunction, although the evidence in lung transplantation is not as robust as that in renal and cardiac transplantation (1-3).

The lung transplant scientific community is working on more precise diagnostic criteria and management protocols. The pathology council of the ISHLT recently proposed a summary statement on the pathology of lung AMR, emphasizing several histological problems due to morphological overlap with other conditions such as infections or other forms of acute lung injury (4).

The web-pages cover the main findings used to make the diagnosis of AMR and emblematic cases with clinical/morphological suspicion of AMR. The study was endorsed by the Pulmonary Pathology Working Group (PPWG) of the European Society of Pathology (ESP) and conducted by a → **Working Group** of experienced pathologists, supported by clinicians and immunologists.

The working group acknowledges the support of Biotest Italia srl through an unrestricted educational grant.

Emblematic examples with key learning points were selected and included in the specific web-section → **Case Series**. The production of this website coincides with the ISHLT working groups recommendations on the definition and diagnostic certainty of clinical pulmonary AMR led by Prof Deborah Levine.

The website represents a comprehensive guide to the problem but is not an exhaustive textbook on the subject. It aims to assist in the knowledge and experience of those working in this field. The website is designed to grow and become a forum for discussion and the exchange of ideas on this subject, and colleagues are invited to submit cases for inclusion following review by the working group.

**Martin Goddard (Pathologist, Papworth Hospital, Cambridge, UK – Chair, Pathology Council ISHLT[2015-17])**





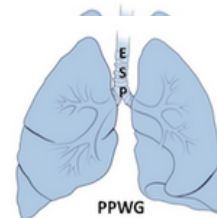
UNIVERSITY  
OF PADOVA



Department of  
Cardiac, Thoracic and  
Vascular Sciences



Pulmonary  
Pathology  
Working  
Group



Welcome  
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## Lung Transplant Pathology: Antibody Mediated Rejection - AMR

### Case Series

Case #001

Case #002

Case #003

Case #004

Case #005

Case #006

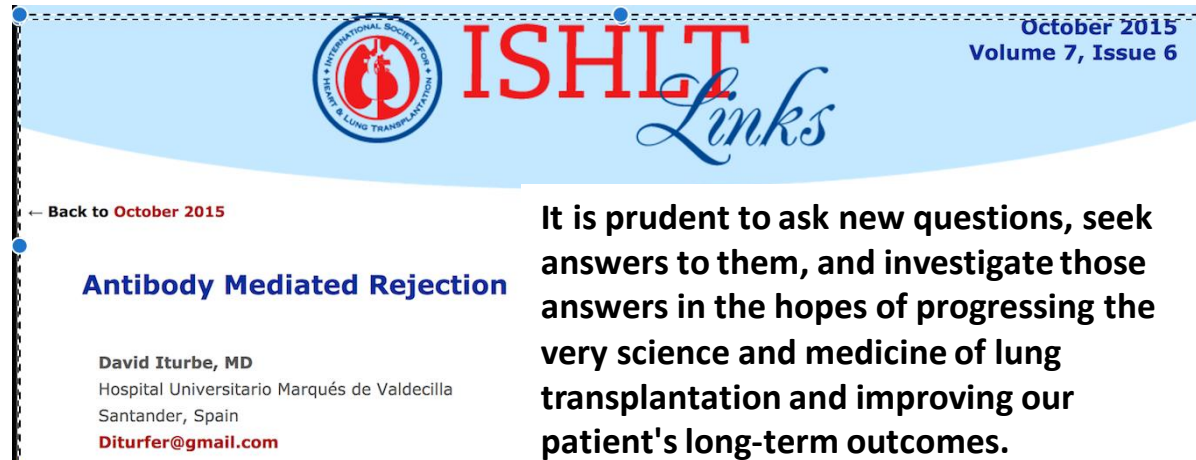
Case #007

#### *Required Panelist's Opinion*

*Specialists must send → the datasheet completed in all sections and some emblematic images (hematoxylin and eosin, C4d immunostaining...) to the Working Group ✉ [amr.dctv@unipd.it](mailto:amr.dctv@unipd.it).*



## Lung AMR Key Questions



### ➤ AMR surrogates: **Why** and **What** ?

- Current morphological features are quite weak;
- Our knowledge of AMR surrogates is gradually improving

### ➤ **Which strategy** to improve our knowledge ?

- Several LT centers now more prone to adopt new protocols, for more sensitive recipient monitoring
- Educational programs crucial for knowledge improvement and for scientific collaborations: some already planned but many others are welcome !

# THANKS

*Belgrade, September 8th 2015*

## 4 meetings :

- ✓ 2 in Birmingham,
- ✓ 1 in Belgrade
- ✓ 1 Padova

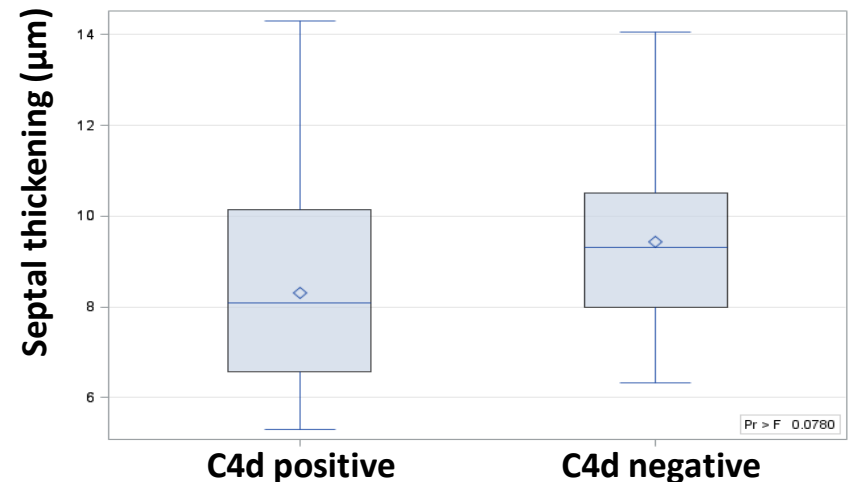
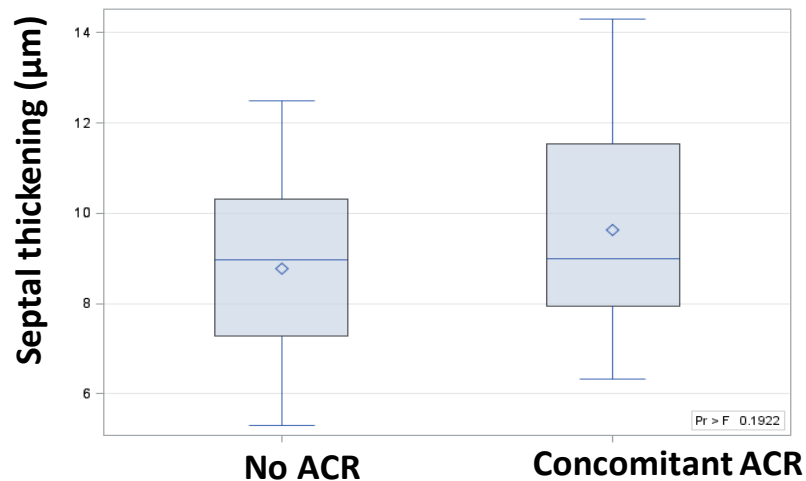
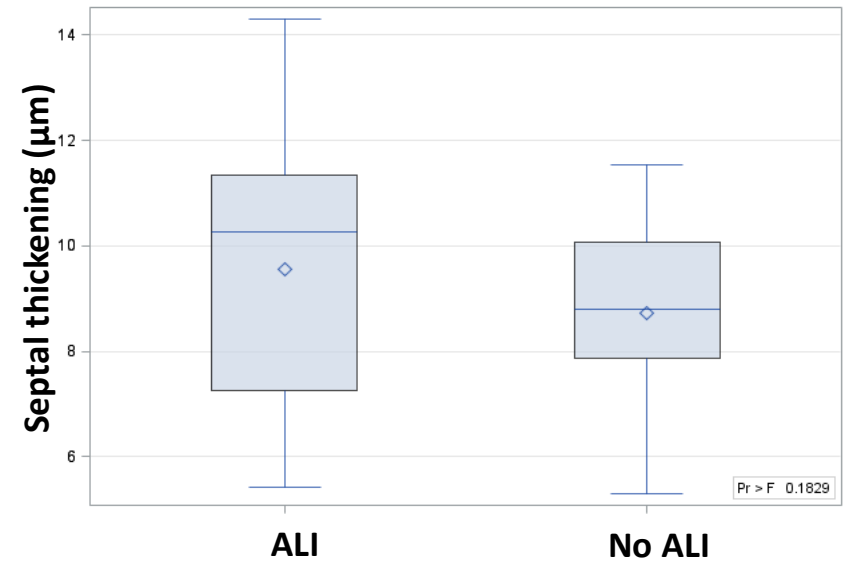
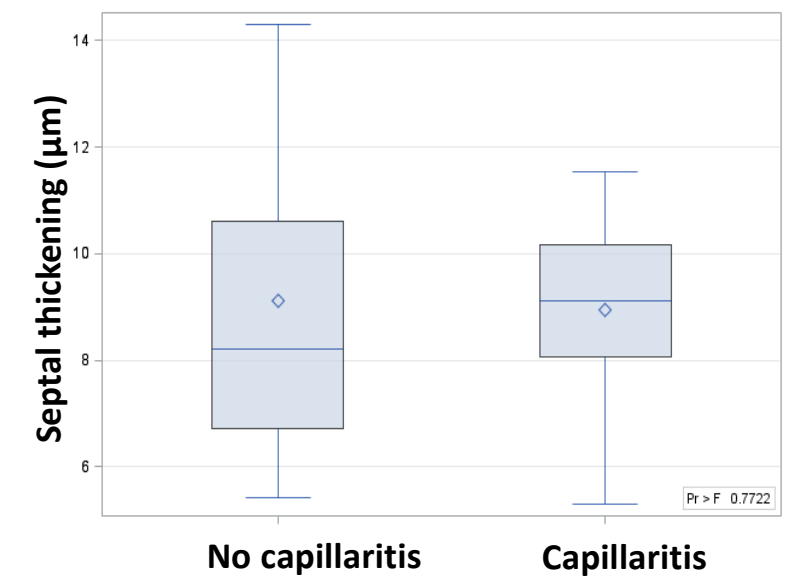
25 cases reviewed and discussed (MTD)

*Padova, February 5, 2016*

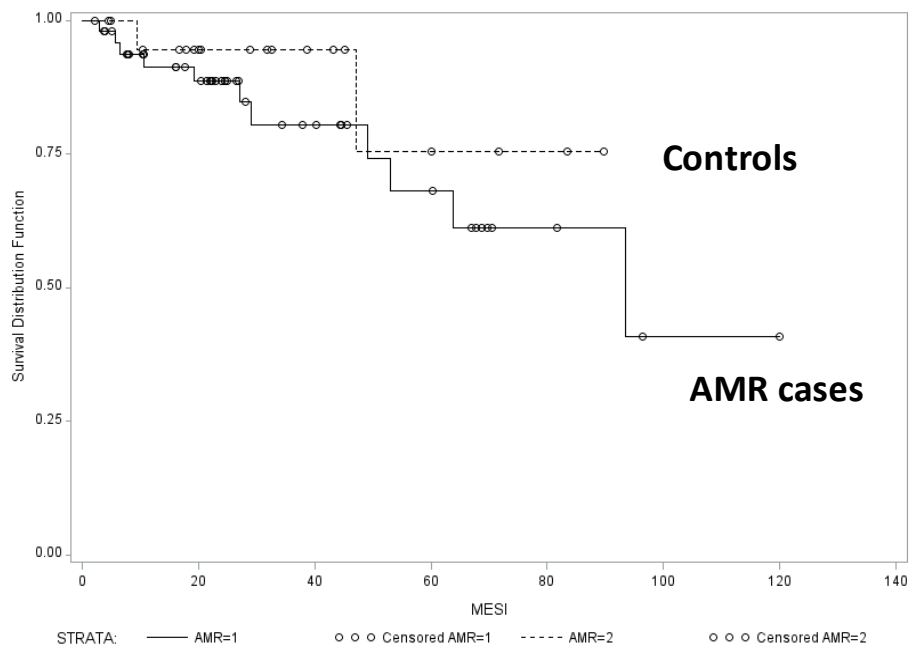


- Histological signs: capillaritis, arteritis, DAD/fibrin, **widening of interstitial spaces**
- AMR concomitant to ACR and CLAD (OB)
- C4d: low reproducibility among different labs; difficult interpretation

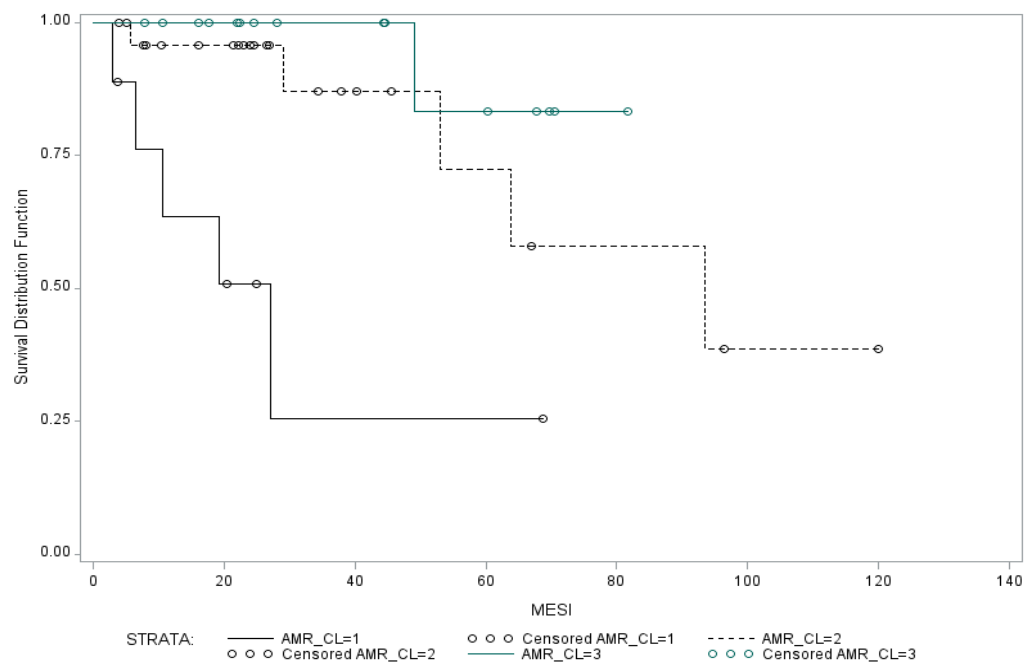
# Widening of Interstitial Spaces



*Widening was not related to age/sex/smoking status of donors and recipients.*



**Log rank test p=0.37**



**Log rank test p=0.0006**



## The SAS System

### The NPAR1WAY Procedure

Analysis of Variance for Variable THICKEN  
Classified by Variable dsa\_3000

dsa_3000	N	Mean
1	34	8.676765
0	38	6.731842

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Among	1	67.878879	67.878879	9.5063	0.0029
Within	70	499.827515	7.140393		

Average scores were used for ties.

