Antibody-Mediated Rejection in the Lung Allograft

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I have no financial relationships with commercial interests to disclose.

My presentation does not include discussion of off-label or investigational use.

Learning Objectives

- To define the current histopathologic criteria for the diagnosis of lung AMR
- To review the immunophenotypic findings in AMR
- To identify potential avenues of investigation in the pathology of AMR

TEMPORAL PARADIGM FOR LUNG TRANSPLANT PATHOLOGY

PERIOPERATIVE AND EARLY POST-TRANSPLANT PERIOD (UP TO 1 MONTH)

- Primary Graft Dysfunction/failure
- Hyperacute Rejection
- Anastomotic Complications
- Infections

INTERMEDIATE COMPLICATIONS (1 MONTH – 1 YEAR)

- Acute Cellular Rejection (ACR)
- Airway Inflammation
- Antibody-Mediated Rejection (AMR)
- Infections
- Post-transplant Lymphoproliferative Disorder (PTLD)
- Drug Toxicity
- Aspiration Changes

LATE COMPLICATIONS (AFTER 1 YEAR)

- Obliterative Bronchiolitis
- Chronic Vascular Rejection
- Restrictive Allograft Syndrome/Pleuropulmonary Fibroelastosis
- Post-transplant Lymphoproliferative Disorder and other EBV-related disorders

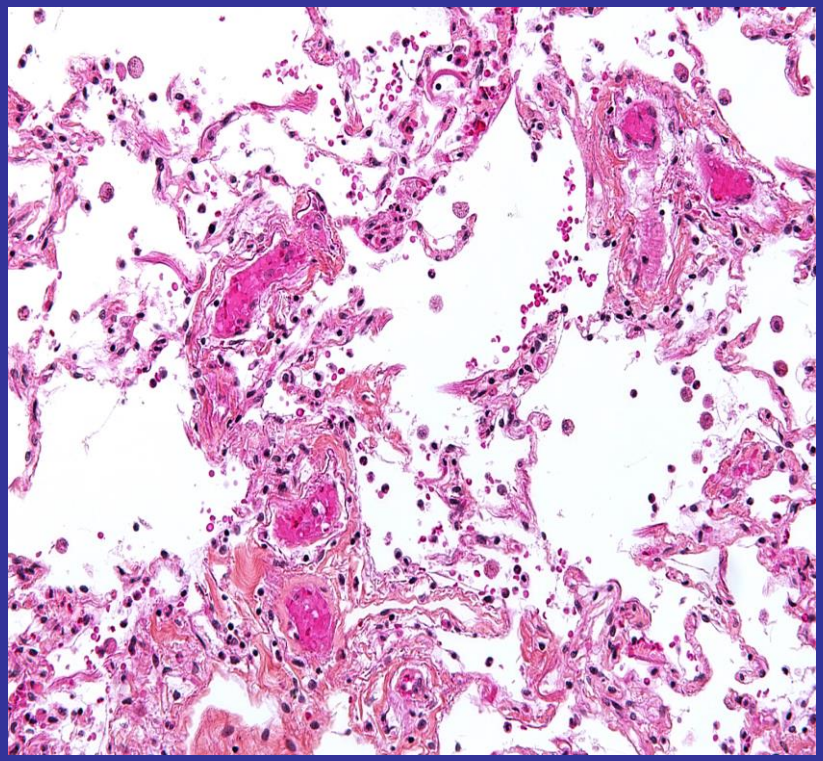
Hyperacute Rejection

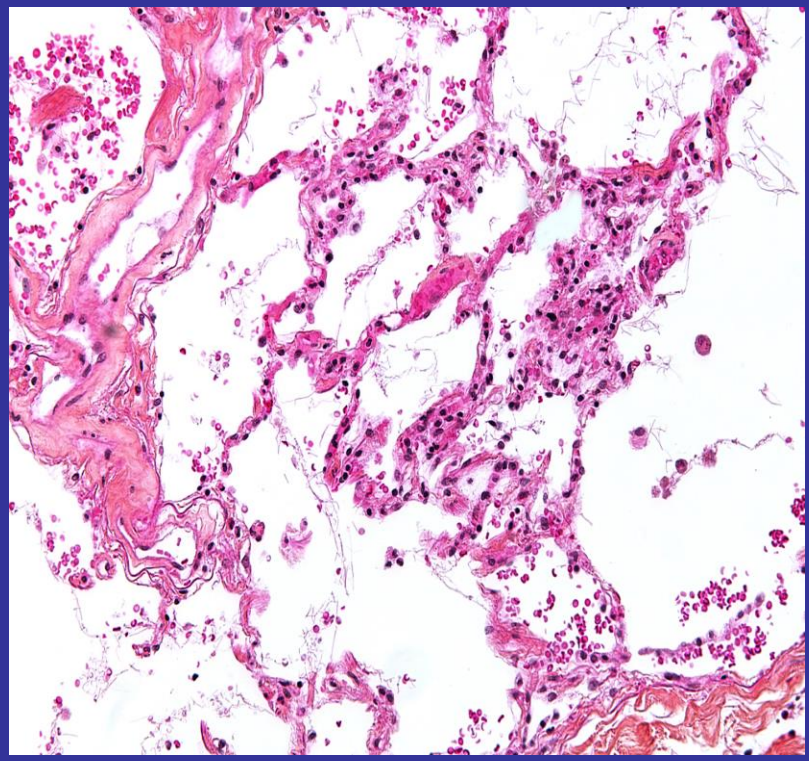
- Rare but potentially fatal complication caused by presence of pre-circulating anti-donor antibodies in recipient
- Pts. with high titer of “panel reactive antibodies (PRA) or anti-endothelial Ab are at highest risk
- Pulmonary edema, progressive respiratory failure and pleural effusions develop within minutes to hours after transplantation
- Histologic findings include DAD, interstitial neutrophilia, fibrin thrombi, vasculitis
- IF or IHC shows immunoglobulin and complement deposition in alveolar septa

Hyperacute Rejection (HAR)

- HAR reported in series of case reports (5)
- Risk factors include ABO mismatch, circulating anti-HLA Ab (anti-B8, -A2, -DR11)
- Hadjiliadis et al JHLT 2005; 24:S249 reported correlation between pretransplant high PRA and survival
- 30% of pts with high PRA died of acute lung injury within 30 days of transplantation

Hyperacute Rejection





Antibody-Mediated Rejection

- Numerous studies over last decade have reported association of *de novo* donor HLA-specific antibodies and development of persistent/recurrent ACR, LB, and CLAD
- True incidence/prevalence unknown as consensus definitions, diagnostic criteria and management protocols have been lacking until recently
- AMR has been reported in combined heart-lung, single & bilateral lung and living-donor lobar transplant adult & pediatric recipients
- Prior to 2012 variety of morphologic terms applied to AMR: “septal capillary necrosis”, “capillary injury”

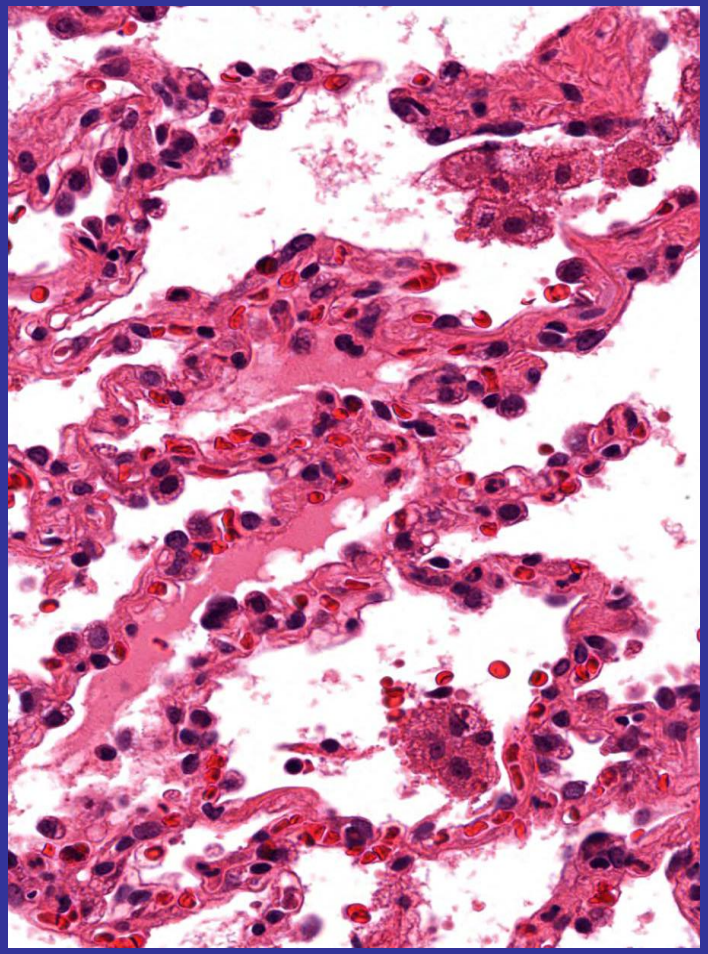
Histopathological Patterns in AMR

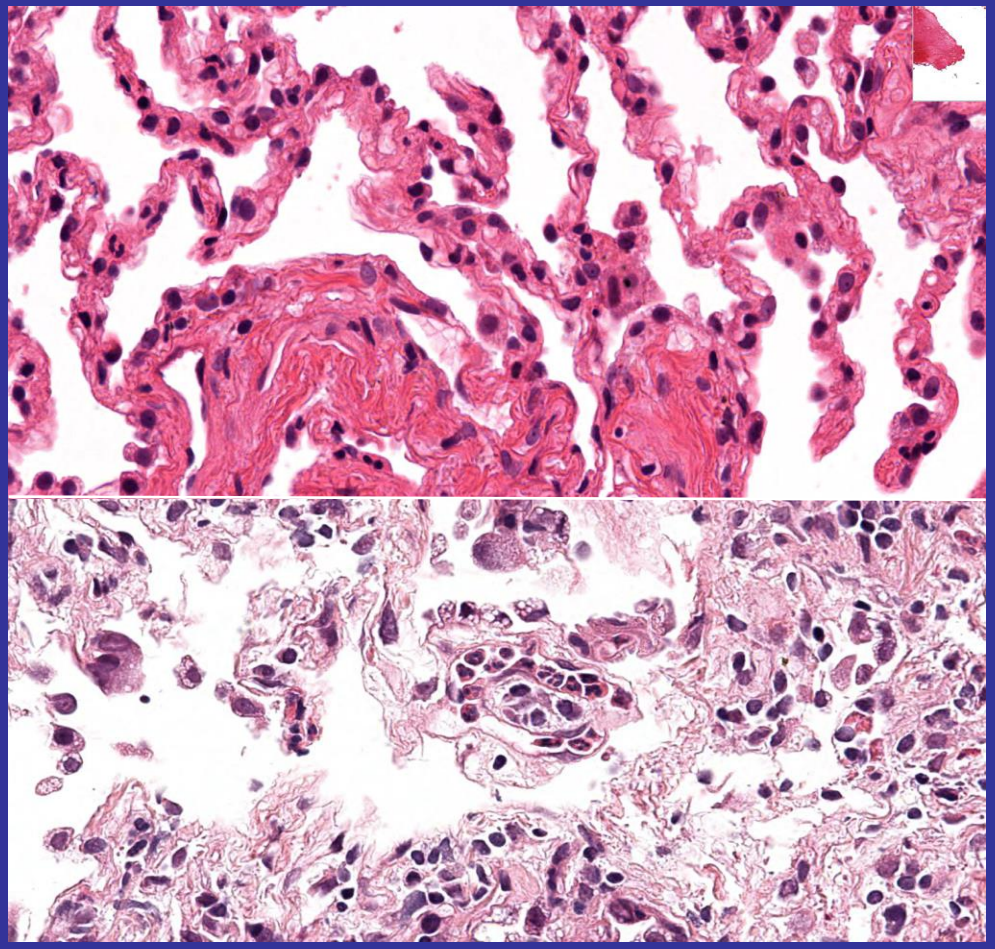
- ***"Neutrophilic Capillaritis"***: patchy or diffuse process composed of dense neutrophilic septal infiltrates associated with neutrophilic karyorrhetic debris & fibrin +/- platelet-fibrin thrombi in microvasculature, alveolar hemorrhage and flooding of PMNs into airspaces

- ***"Neutrophilic Margination"***: neutrophilic infiltrates in interstitial capillaries and septa in absence of karyorrhetic changes and fibrinous accumulations

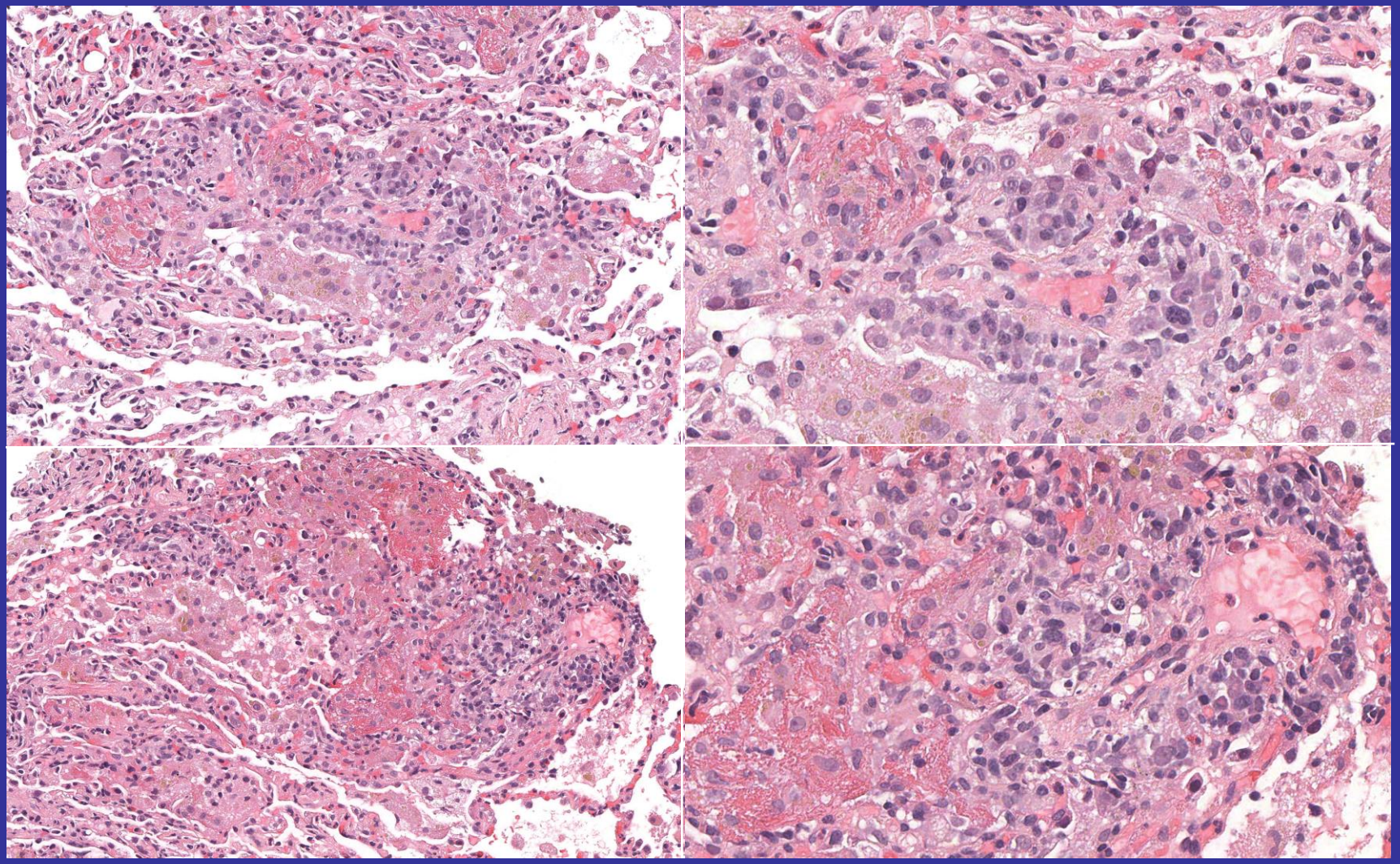
• Berry GJ et al. J Heart Lung Transplant 2013; 32:14-21.

Neutrophilic Margination

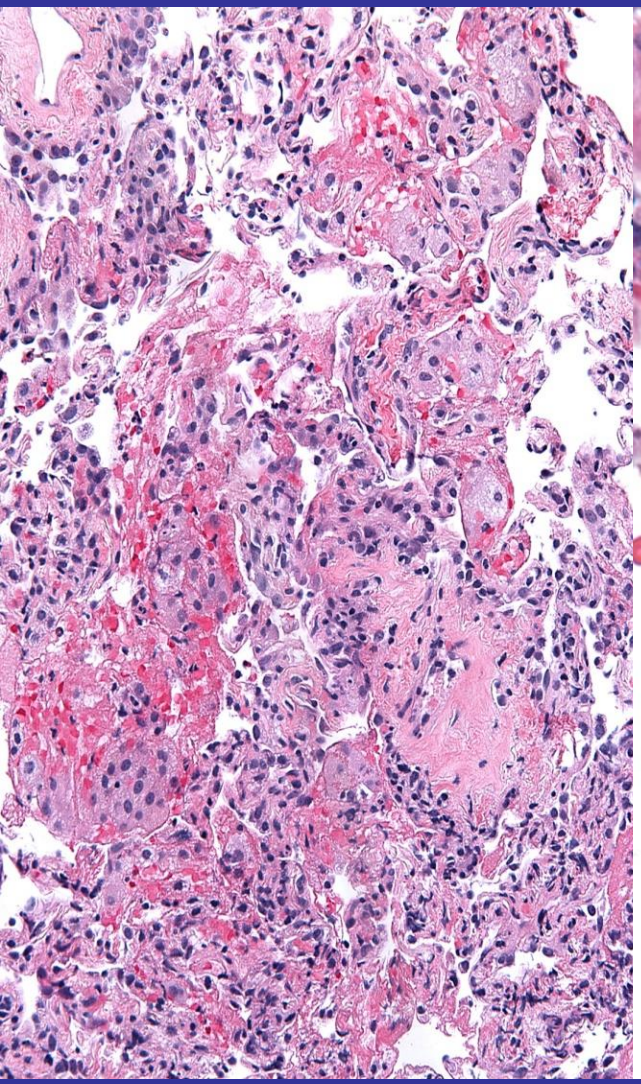


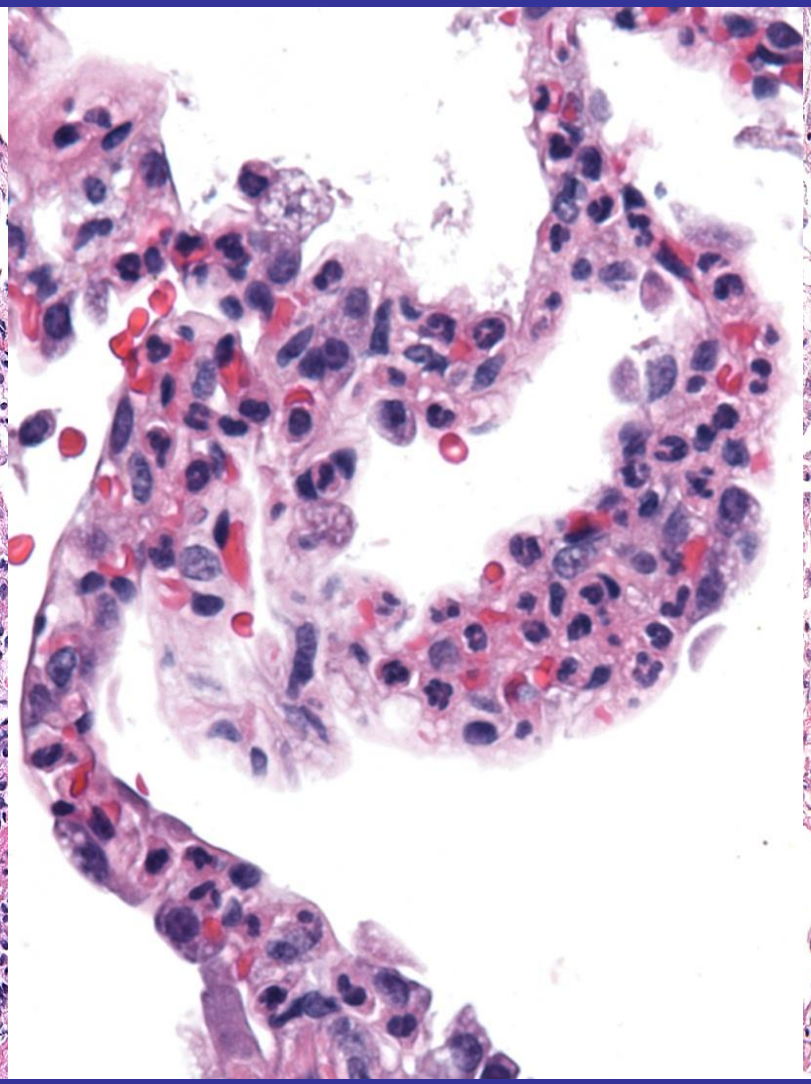


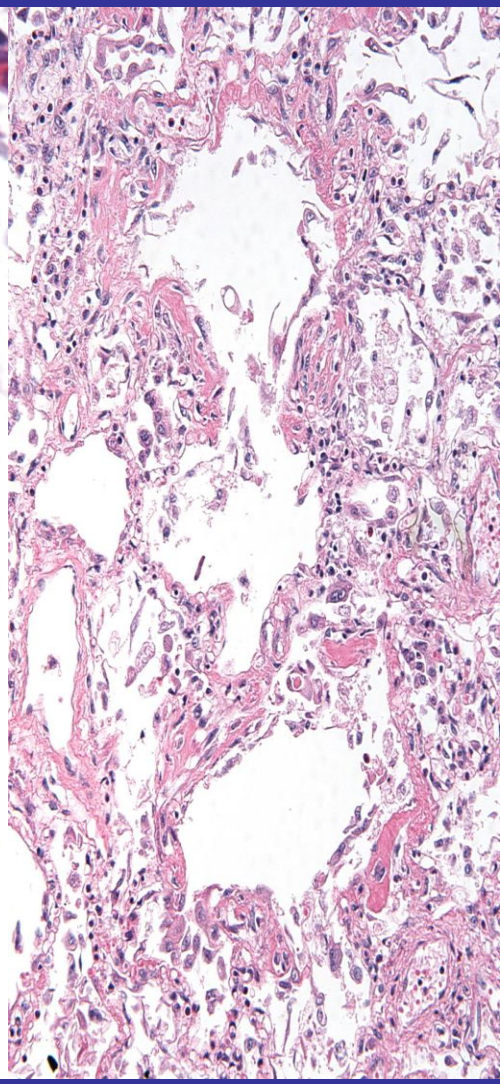
Neutrophilic Capillaritis



Lung: Spectrum of AMR Pathology







Yousem SA et al. Am J Surg Pathol 2012; 36:987-992

- **New onset DSA (anti-HLA); graft dysfunction, histopathology; biopsy at time of onset- 23 patients.**

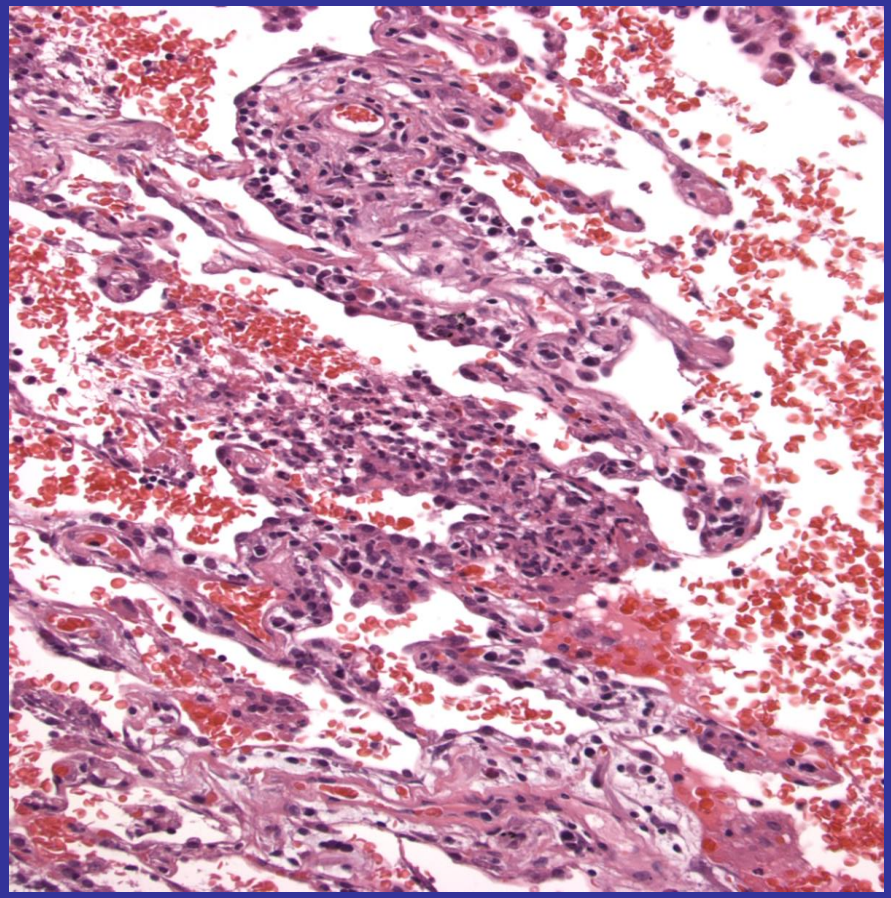
N=17 Coexistent ACR – A2 (2), A3 (14), A4 (1)

- **18% had coexistent neutrophilic capillaritis**
- **When compared to matched group of ACR patients, only capillaritis was distinct.**
- **C4D positive in 13/17 (76%); matched group 24%.**

N=5 Acute/organizing lung injury.

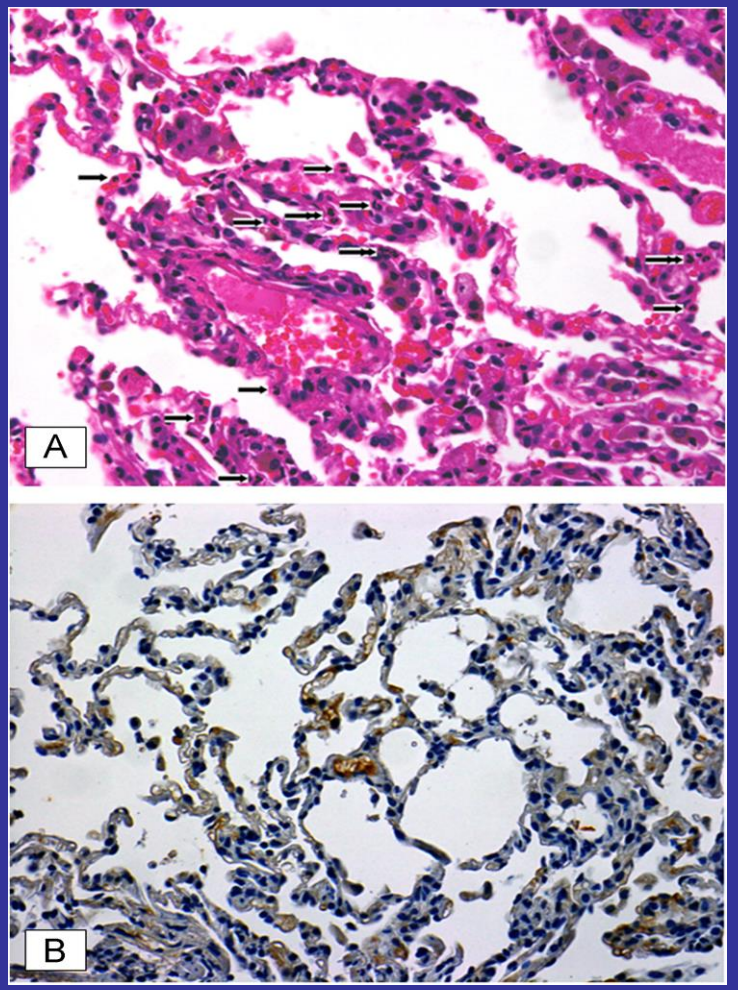
- **80% C4D positive; historical controls 50%.**

N=1 Lymphocytic bronchiolitis
C4D -ve



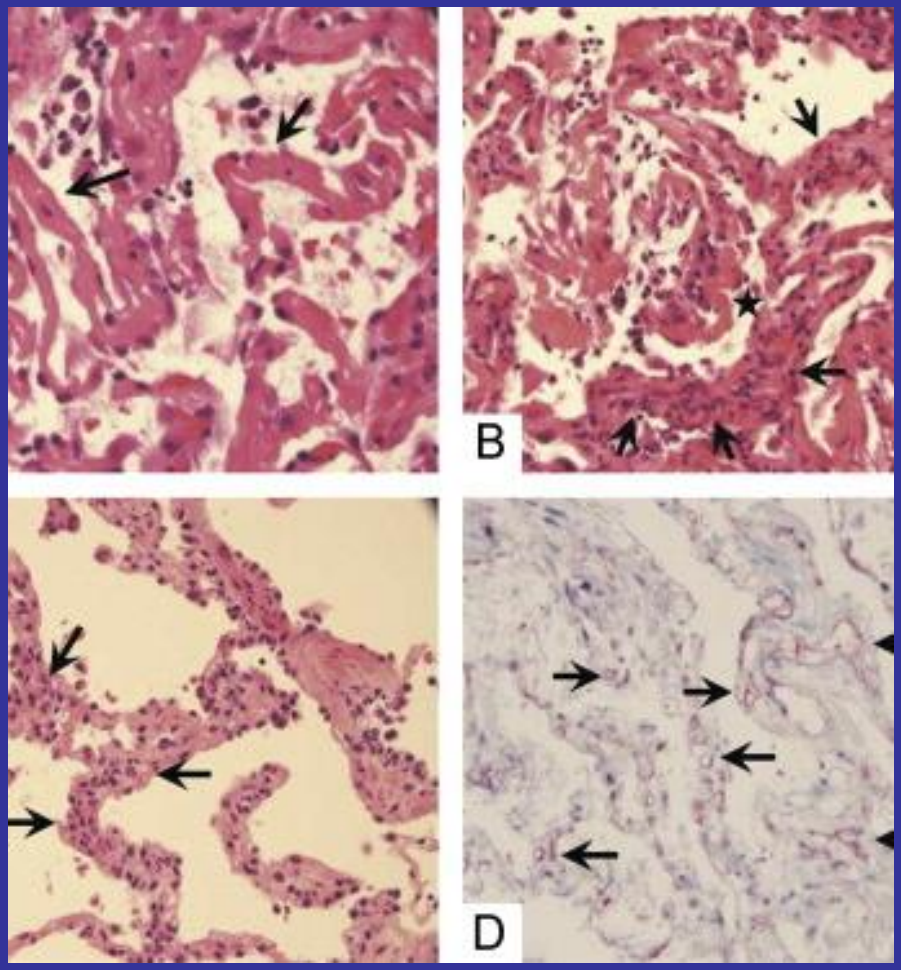
DeNicola MM, Weigt SS, Belperio JA et al. J Heart Lung Transplant 2013; 32:326-332

- Microvascular inflammation as septal neutrophilia (Gr 2-4) and/or DAD as markers marker of AMR
- 41 biopsies (16 DSA+ve; 9/25 control gr developed de novo anti-HLA Ab (not DSA))
- 17/41 had suspicious histology (11/16 vs 6/25)
- C4d and C3d not more common in DSA+ vs control group



Witt CA, Gaut JP, Yusen RD, et al. J Heart Lung Transplant 2013; 32: 1034-1040.

- 21 pts with AMR (+triple test)
- Median time to onset 258 days; 7/21 developed AMR within 45 days of Tx
- 15/21 treated and improved clinically but all developed CLAD
- 6 pts died of refractory AMR



Where are we in 2017?

- Series of individual center and Banff 2016 studies confirm lack of specific histopathologic findings in AMR
- Acute lung injury +/- DAD, neutrophilic margination or capillaritis exhibit variable sensitivity/specificity
- C4d appears to be quite specific but insensitive

Banff study of pathologic changes in lung allograft biopsy specimens with donor-specific antibodies

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METHODS: We asked 9 pathologists with experience in lung transplantation to evaluate 161 lung transplant biopsy specimens for various histologic parameters. The findings were correlated with antibody status positive for DSAs, positive for non-DSAs, and no antibodies (NABs) present. The significance of each histologic variable was reviewed.

RESULTS: We found no statistically significant association with acute cellular rejection, airway inflammation, or bronchiolitis obliterans and the presence or absence of antibodies. However, biopsy specimens with DSAs had a statistically significant difference vs NABs in the setting of acute lung injury, with or without diffuse alveolar damage ($p = 0.0008$), in the presence of capillary neutrophilic inflammation ($p = 0.0014$), and in samples with endotheliitis ($p = 0.0155$). In samples with complement 4d staining, there was a trend but no statistically significant difference between specimens associated with DSAs and specimens with NABs.

CONCLUSIONS: Capillary inflammation, acute lung injury, and endotheliitis significantly correlated with DSAs. The infrequently observed diffuse staining for complement 4d limits the usefulness of this stain.

J Heart Lung Transplant 2016;35:40–48

Table 3 Correlation with Antibody Status

Variable	Non-DSA (n = 288) No. (%)	DSA (n = 495) No. (%)	NAB (n = 657) No. (%)	*p*-value Non-DSA vs DSA vs NAB	*p*-value[a] Non-DSA vs NAB	DSA vs NAB	Non-DSA vs DSA
ACR				0.68	0.47	0.69	0.54
0 (none)	217 (76)	387 (79)	531 (81)				
1	30 (10.5)	54 (11)	75 (12)				
2	30 (10.5)	31 (6)	33 (5)				
3, 4	8 (3)	20 (4)	13 (2)				
No. missing		20					
Airway inflammation				0.90	0.84	0.68	0.82
B0 (none)	144 (84)	270 (82)	330 (85)				
B1R (low grade)	24 (14)	48 (14)	47 (12)				
B2R (high grade)	3 (2)	13 (4)	13 (3)				
No. missing		18					
Obliterative bronchiolitis				0.82	0.37	0.95	0.63
C0 (none)	162 (95)	318 (96)	380 (97)				
C1 (present)	9 (5)	12 (4)	10 (3)				
No missing		19					
ALI				0.0019	0.91	0.0008	0.0272
None	247 (87)	359 (74)	568 (88)				
ALI	36 (12)	97 (20)	75 (11)				
ALI with DAD	2 (1)	30 (6)	5 (1)				
No. missing		18					
Endotheliitis				0.0535	0.15	0.0155	0.61
No	262 (92)	448 (91)	628 (96)				
Yes	22 (8)	43 (9)	24 (4)				
No. missing		22					
Capillary inflammation grade				0.0050	0.99	0.0014	0.0230
0	235 (83)	354 (72)	544 (83)				
1	33 (11)	86 (17)	74 (11)				
2, 3	16 (6)	53 (11)	35 (6)				
No. missing		19					
C4d by IHC				0.1196	0.38	0.0322	0.67
Negative	89 (83)	131 (71)	183 (85)				
Positive < 50%	13 (12)	29 (16)	27 (13)				
Positive > 50%	5 (5)	25 (13)	5 (2)				
No. missing		127					

ACR, acute cellular rejection; ALI, acute lung injury; C4d, complement 4d; DAD, diffuse alveolar damage; DSA, donor-specific antibodies; IHC, immunohistochemistry; NAB, no antibodies of any kind.

[a]The *p*-value for significance is <0.0167.

Roux A, et al. Am J Transplant 2016; 16:1216-28.

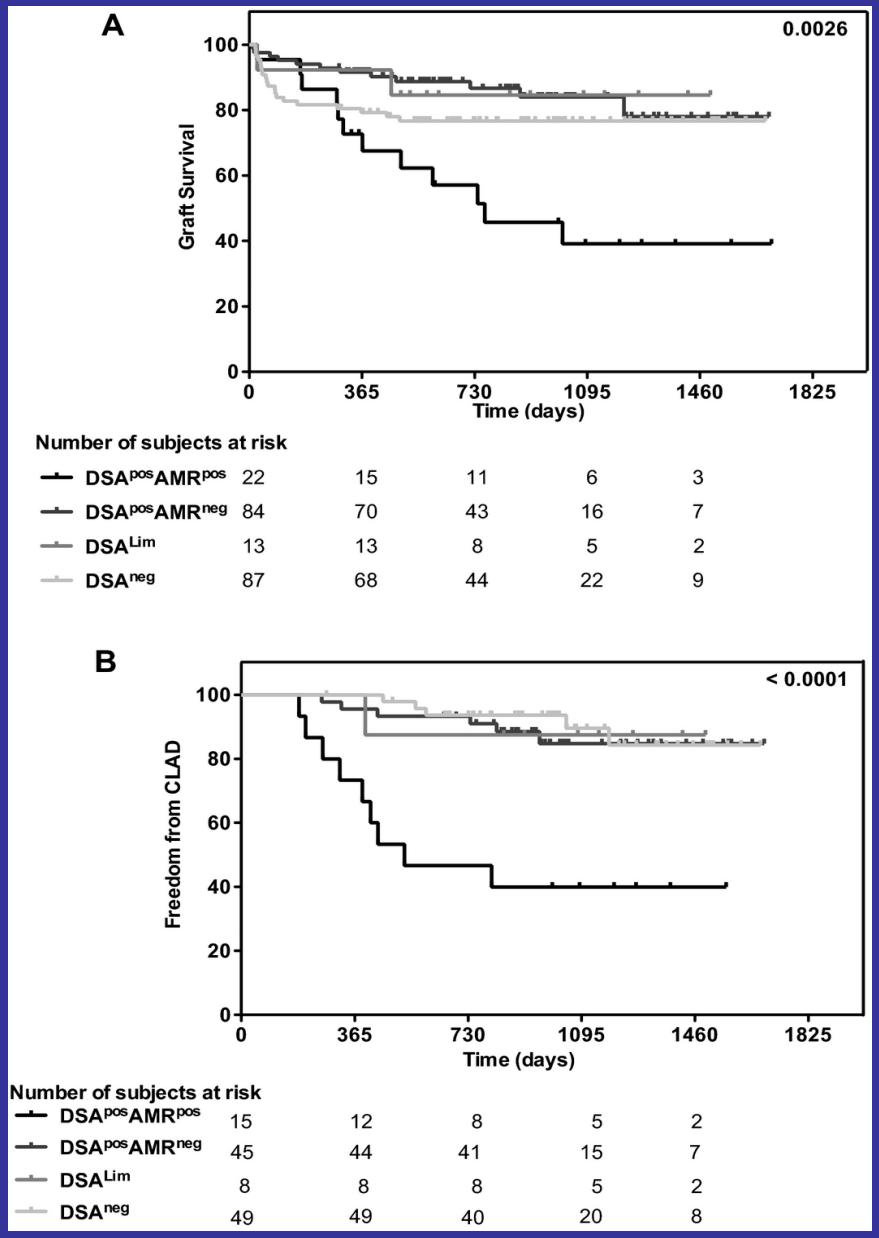
- Retrospective study of 206 pts classified into DSA+ AMR+ (11%), DSA+ AMR- (40%), DSA limited AMR- (6%), DSA- AMR- (43%) groups
- Higher incidence of ACR in DSA+ AMR+ group
- Multivariate analysis showed AMR as risk factor for CLAD and graft loss

Definitions

Table 1: Criteria for AMR-DSA status categorization

AMR patients($DSA^{pos}AMR^{pos}$)		Non-AMR patients		
		$DSA^{pos}AMR^{neg}$	DSA^{Lim}	DSA^{neg}
DSA positivity: DSA MFI >1000, or MFI of 500–1000 with more than two specificities and/or detected more than once		**DSA positivity:** DSA MFI > 1000, or MFI of 500–1000 with more than two specificities and/or detected more than once	DSA detected only once and having only one specificity with an MFI of 500–1000	All single-antigen tests with DSA MFI <500
$AMR\ C4d^{Pos}$	$AMR\ C4d^{Neg}$			
Clinical dysfunction and **DSA positivity** and **C4d positive staining with or without** histological patterns suggestive of AMR	**Clinical dysfunction** and **DSA positivity** and **negative C4d staining** with **histological patterns** suggestive of AMR: **neutrophilic capillaritis**,‡ **acute lung injury**‡	**No AMR diagnosis throughout entire follow-up**		

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



Roux A, et al. Am J Transplant 2016; 14:1216-28

Stanford Adult Pulmonary AMR Experience

- Retrospective cohort study
- Inclusion Criteria:
    - Lung transplantation at Stanford University in the period June 2008 to March 2013
    - Tested for DSA based on clinical indication, mainly evaluation of allograft dysfunction
        - Transbronchial biopsy specimens (within 30 days of serum collection for DSA) grouped according to presence or absence of complement-fixing donor-specific antibody
    - HLA antibody evaluation
        - Tested for HLA antibody by IgG and C1q methods
        - MFI > 1000 was MFI cut-off

- 116 serum samples (obtained from 60 lung transplant recipients) were tested for DSA based on clinical indication with concurrent transbronchial biopsies performed within 30 days
- Of these, 37 samples showed C1q+ DSA and 79 samples showed no DSA by C1q

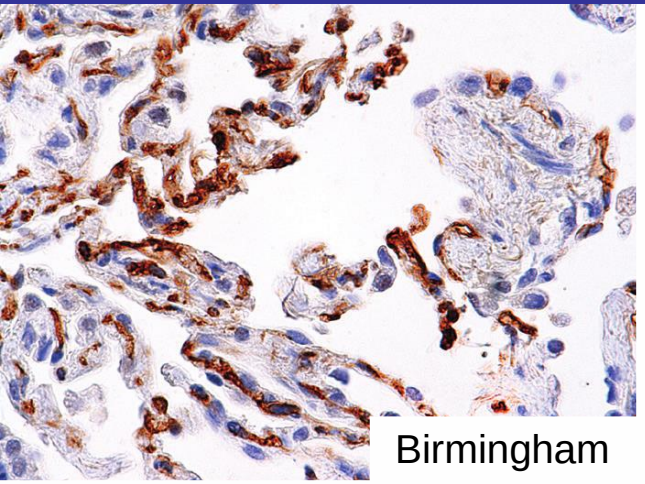
Chhatwani L et al. J Heart Lung Transplant 2016; 35:S108

Histopathologic Findings

	Serum samples with C1q DSA positive n = 37 (%)	Serum samples with no C1q DSA n = 79 (%)	p value
A grade rejection ≥ A2	13 (35.0)	11 (13.9)	0.008
B grade rejection B1R B2R	 1(2.7) 2 (5.4)	 3 (3.8) 1 (1.3)	0.4
C grade rejection C1	 3 (8.1)	 2 (2.53)	0.16
C4d Positive Negative Not done	 1 (2.7) 30 (81.0) 6 (16.2)	 4 (5.1) 67 (84.85) 8 (10.1)	0.48
Neutrophilic margination Present	 2 (5.4)	 7 (8.9)	0.14
Acute capillaritis	0	0	
Acute lung injury ALI present DAD	 0 0	 1 (1.3) 2 (2.53)	
Other Organizing pneumonia Aspiration	 0 0	 2 (2.53) 1 (1.3)	

Issues in AMR

- Is it a technical issue?
- Are there more sensitive markers than C4d
- Biomarkers of endothelial activation: mTOR effectors p70S6K, pS6R
- Are there more specific histopathologic features that might be useful; European Pulmonary Pathology digital microscopy study
- Pathology Council plans to reconvene at ISHLT 2017 to reassess histopathologic and immunophenotypic aspects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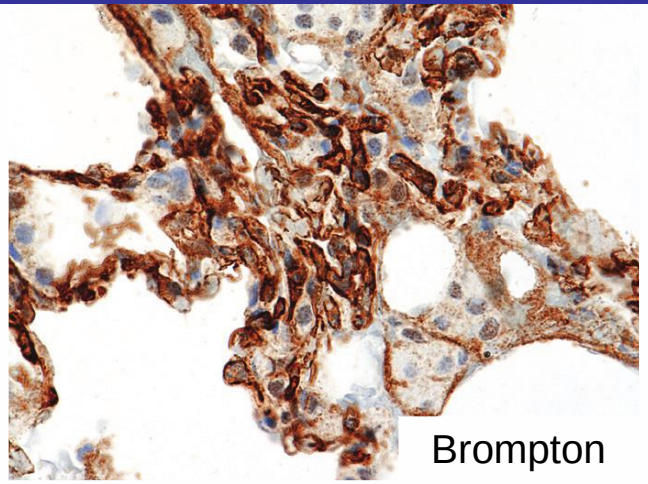
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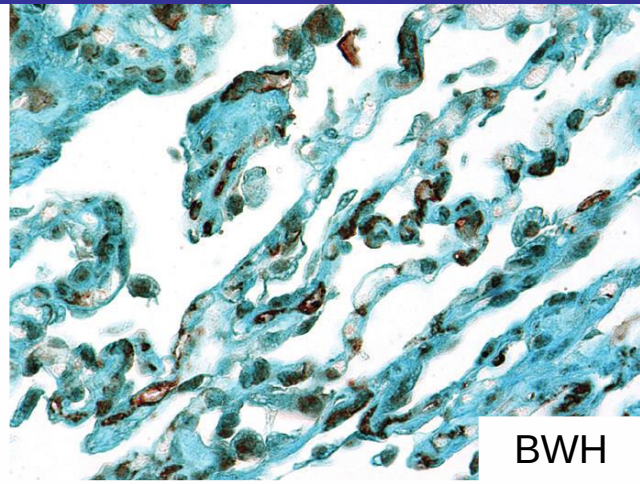
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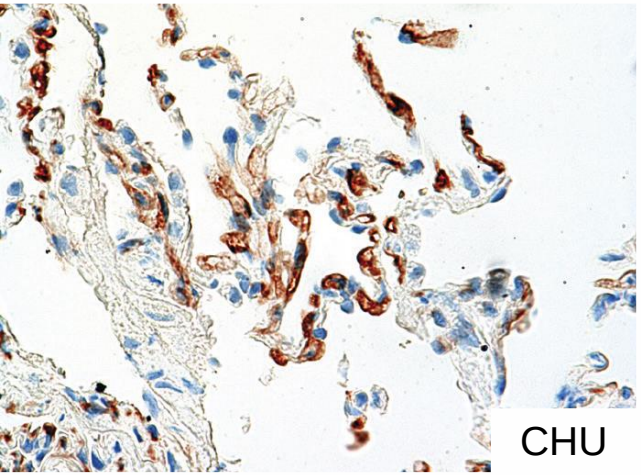
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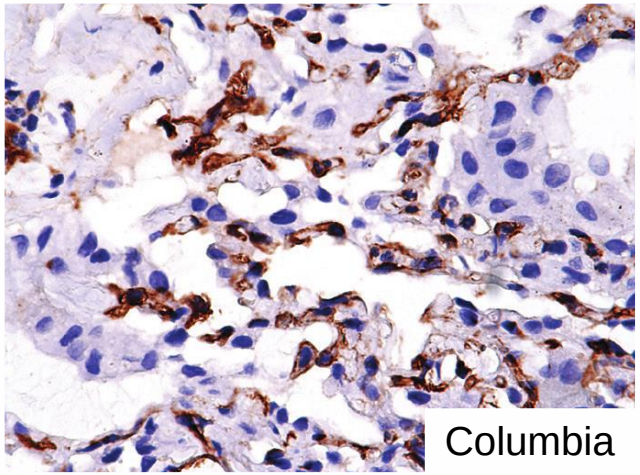
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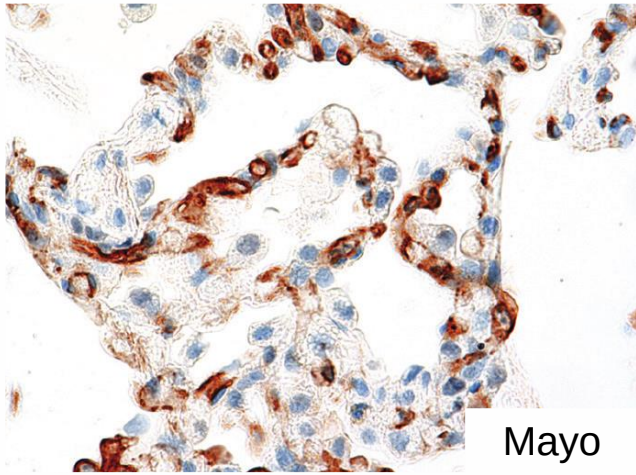
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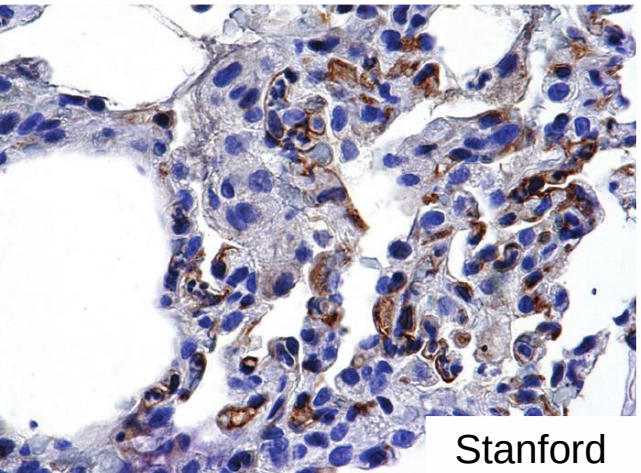
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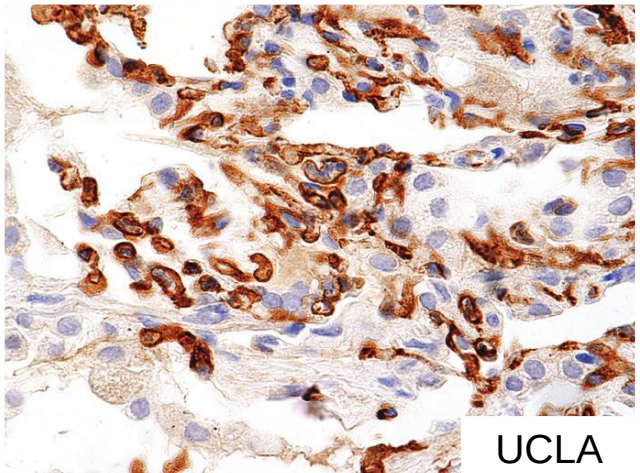
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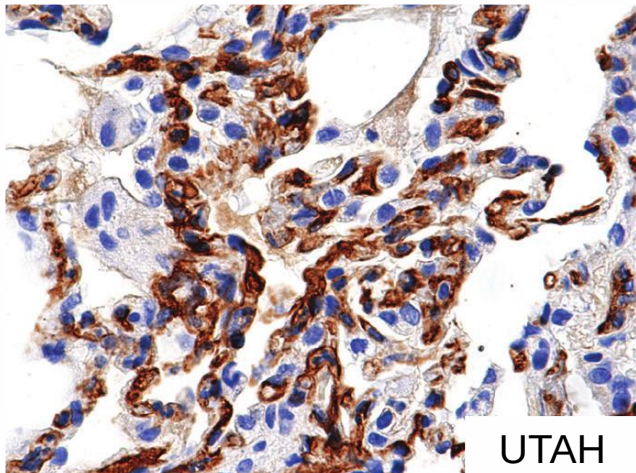
UTAH

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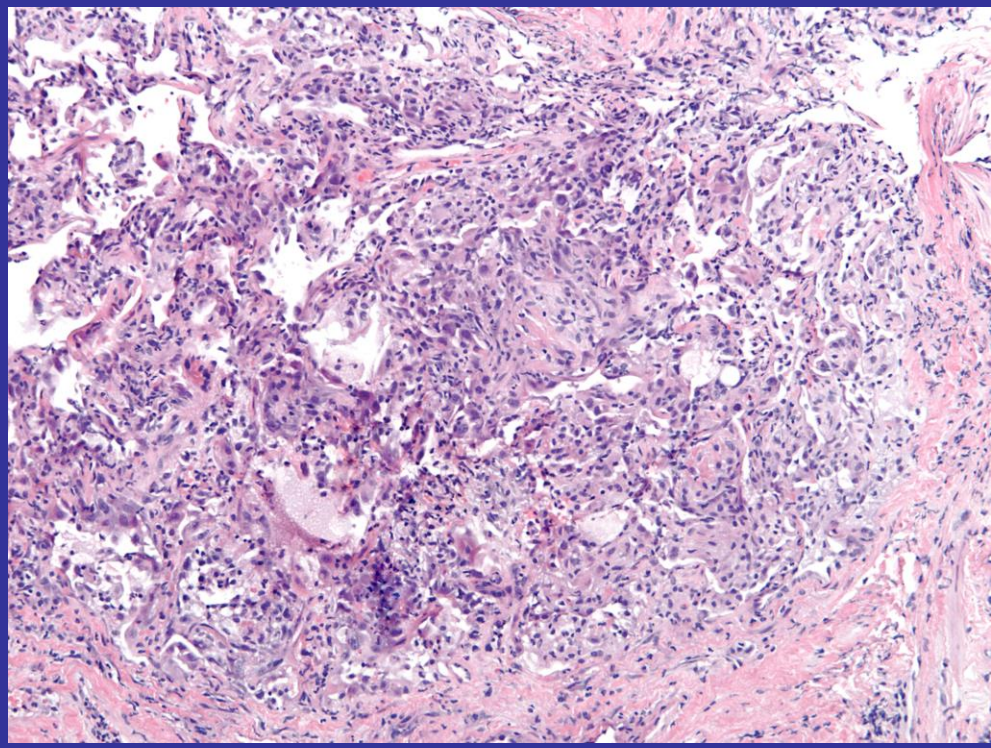
- Are there more specific histopathologic features that might be useful; European Pulmonary Pathology digital microscopy study raises new histopathologic targets

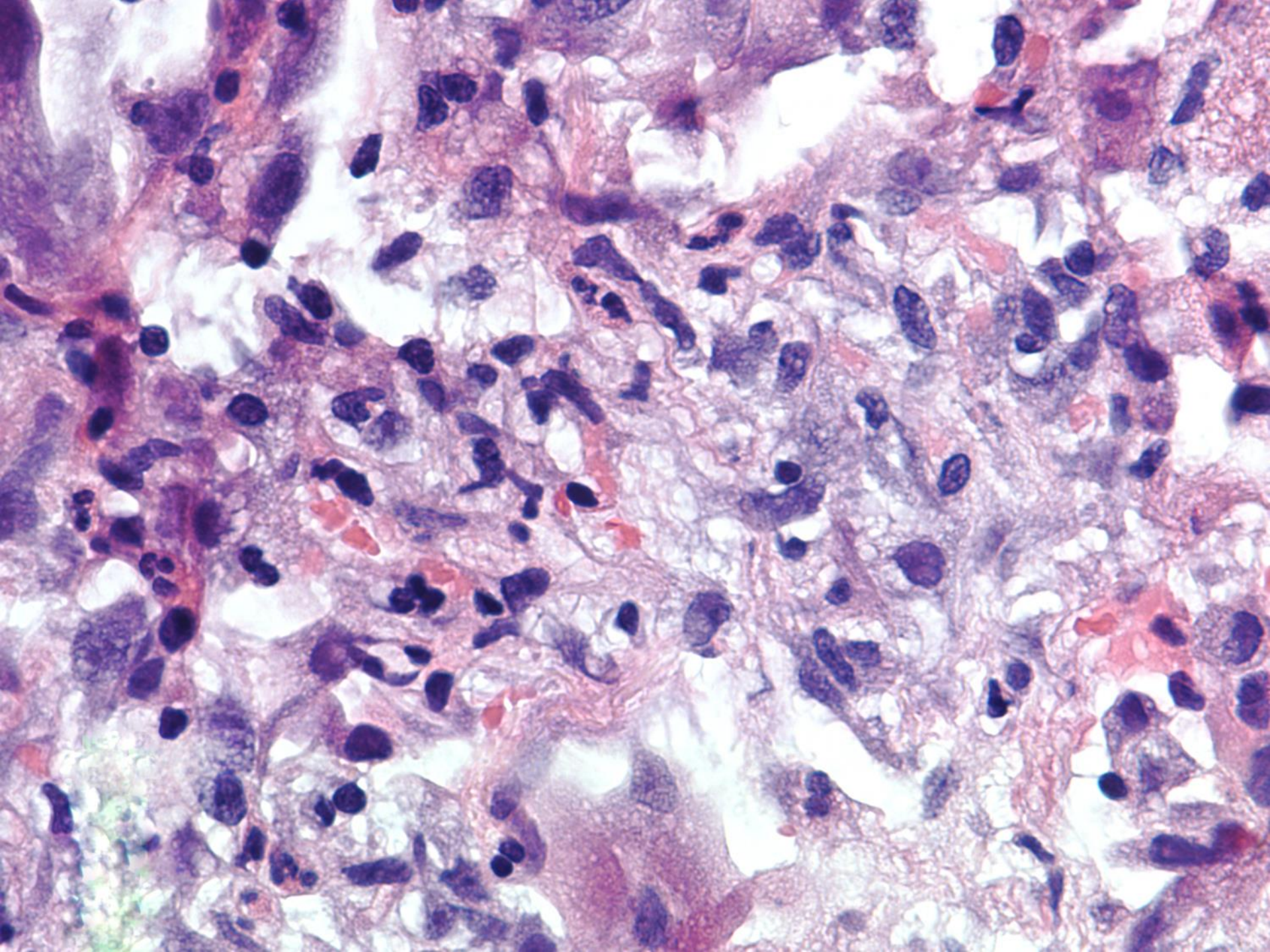
Summary & Future Directions

- Diagnosis of pulmonary AMR requires multidisciplinary approach
- Histopathological findings are nonspecific patterns of injury; patterns should trigger clinical, serologic and immunophenotypic evaluation
- Qualified terminology should be used in pathology report with final clinical diagnosis incorporating all modalities
- Centers are encouraged to develop protocols that will promote investigations addressing issues of time to onset of AMR, incidence, prevalence, spectrum of temporal, morphological and immunopathologic changes, clinical outcomes and risk for chronic allograft dysfunction
- Novel approaches to the histopathologic, immunophenotypic and molecular components of pulmonary AMR are required

Case 1

- 15-year old female s/p BSSLTx for CF
- Prior episodes of ACR and AMR
- Presents with new & rising DSA (C1q-binding Class II AB) and severe lung dysfunction (fever, dyspnea)





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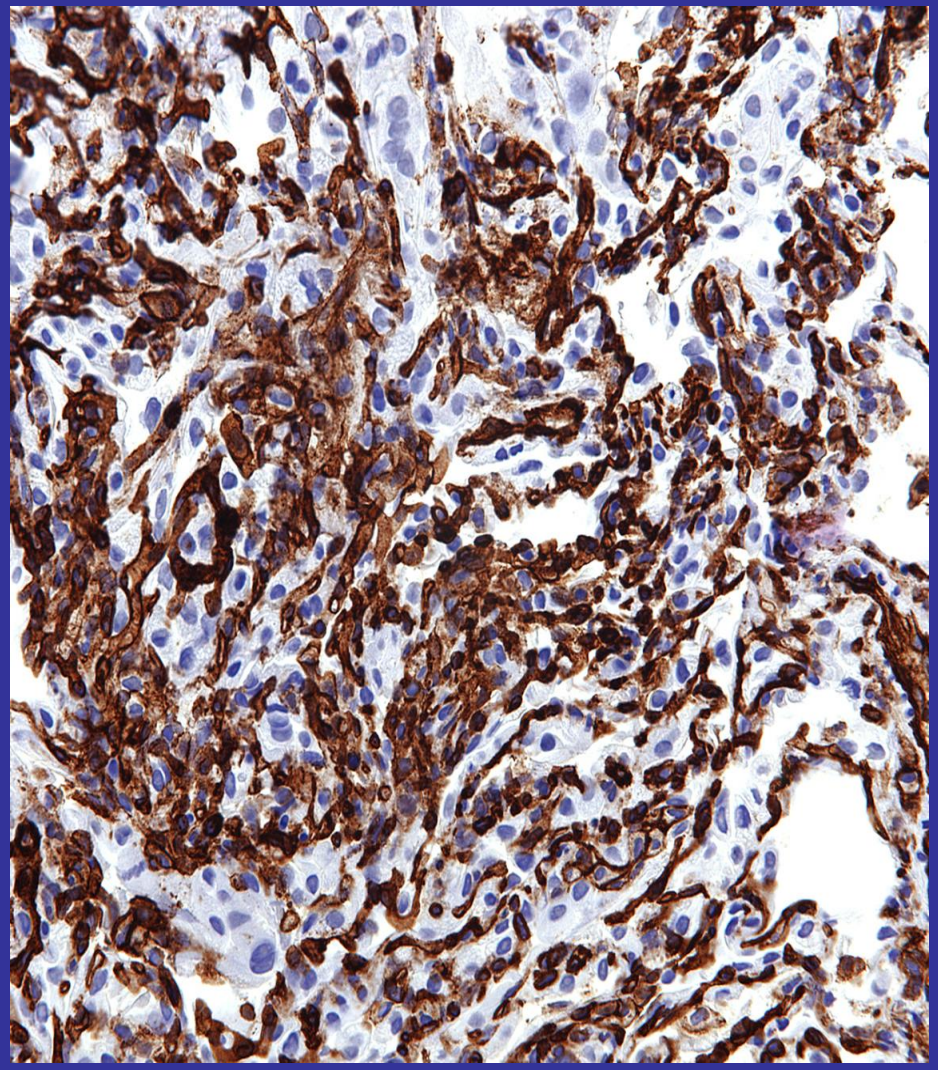
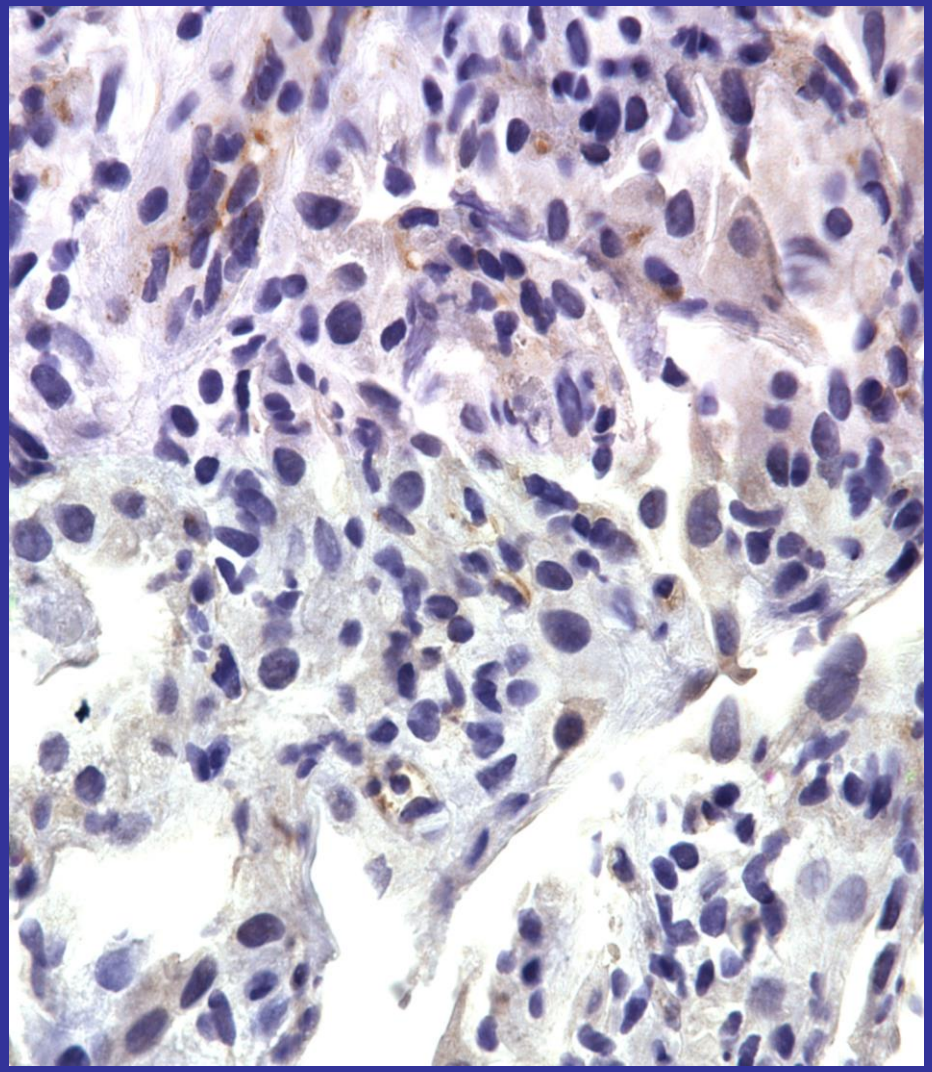
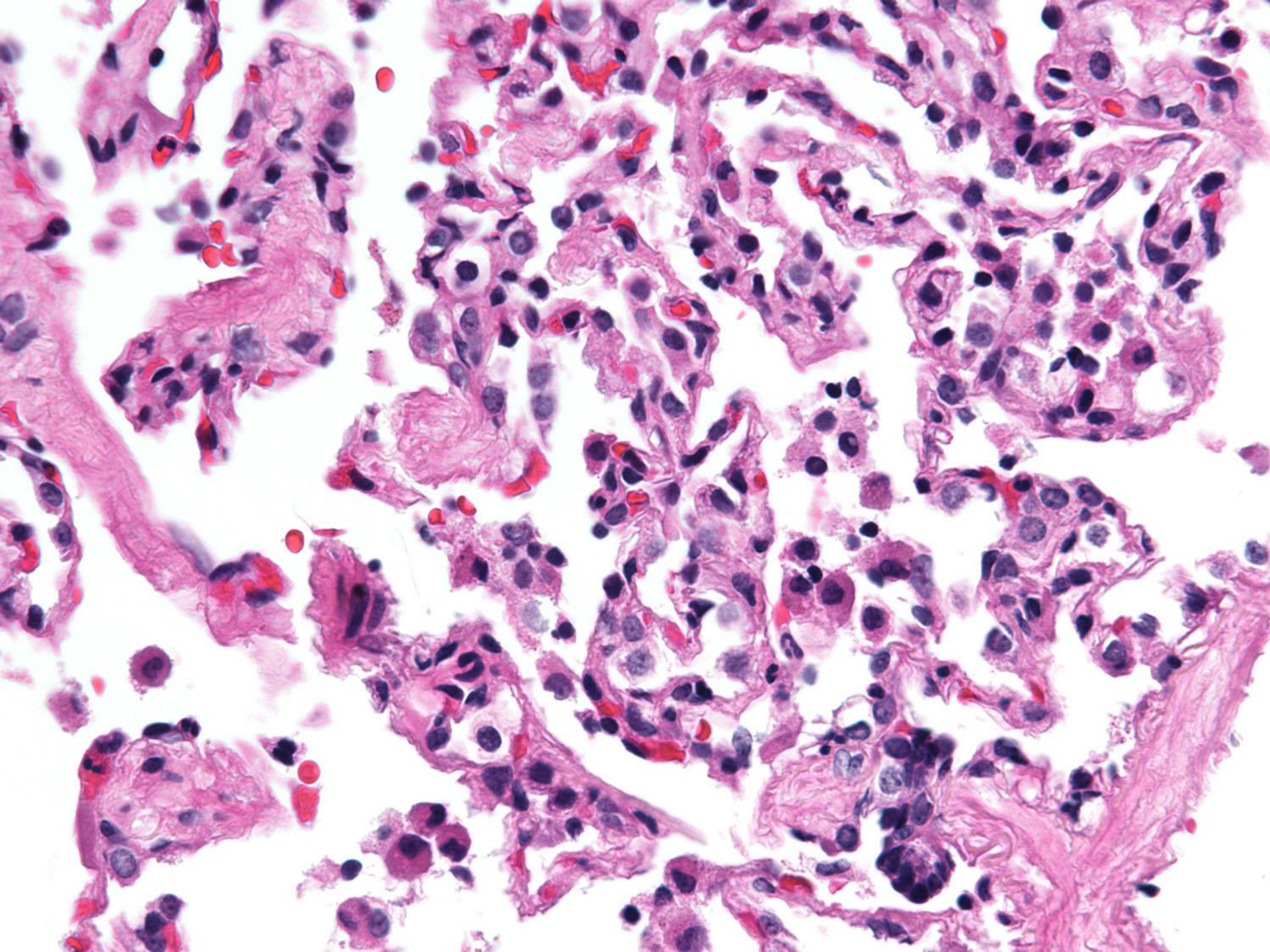


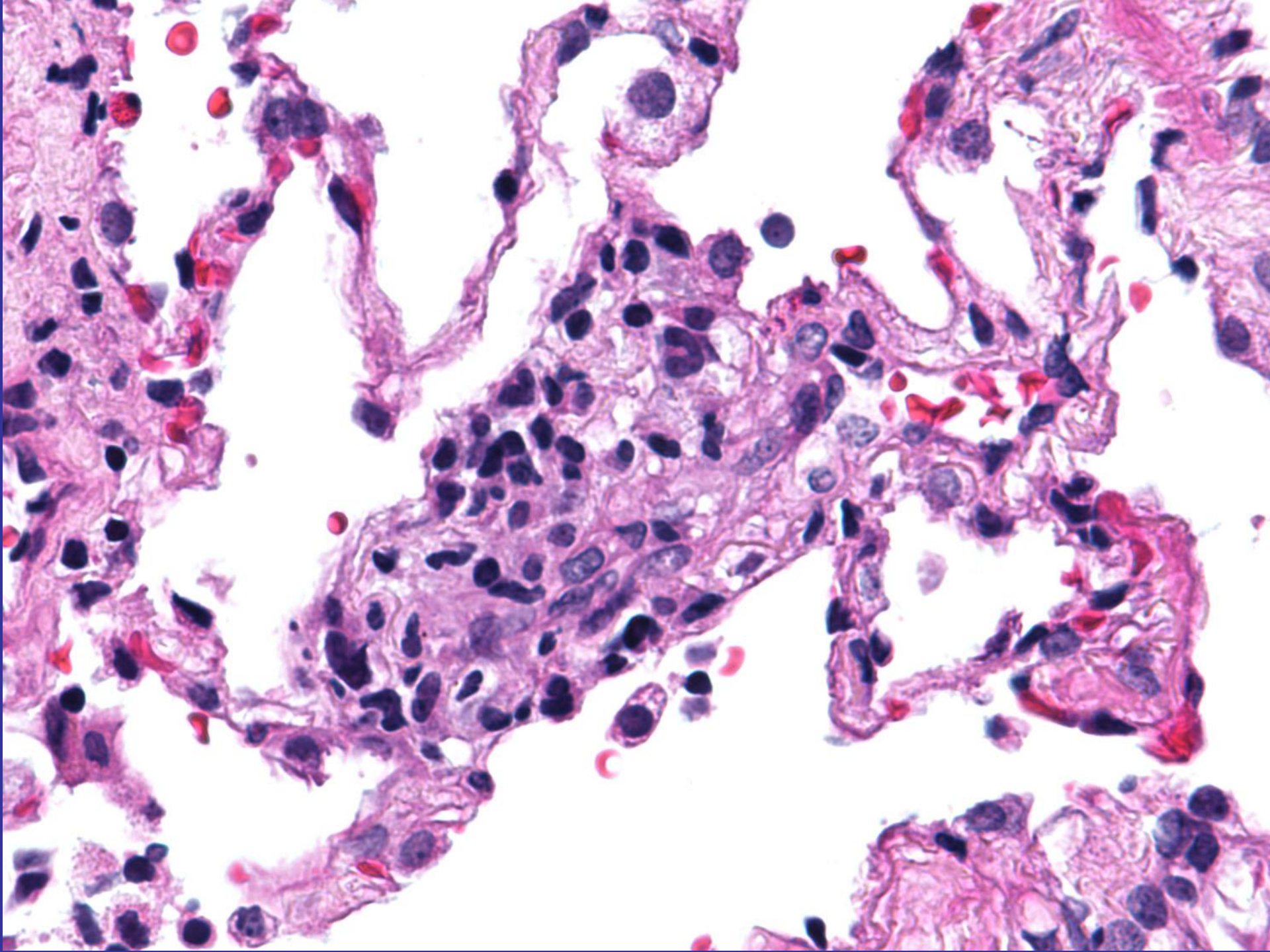
C4d

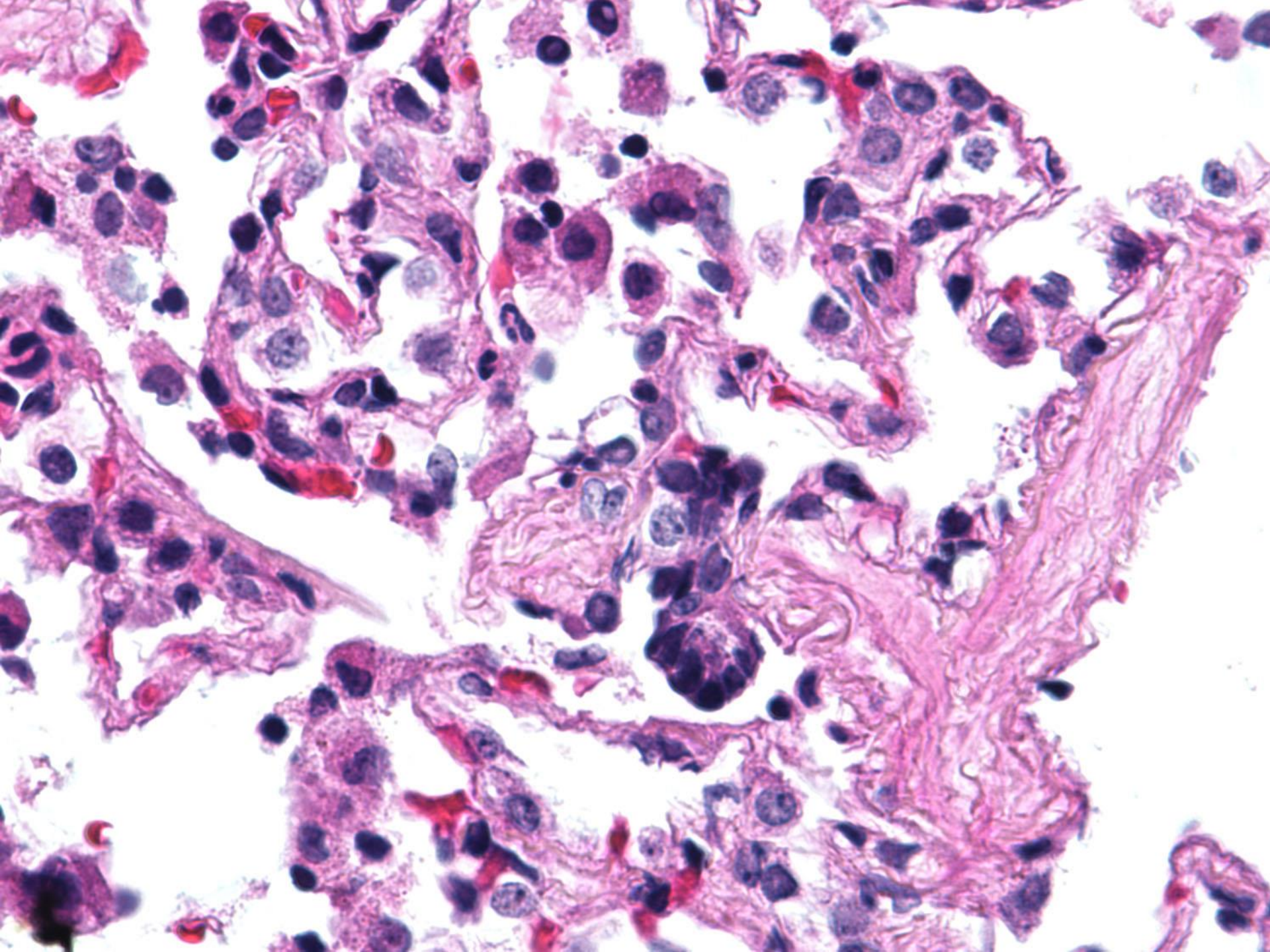


CASE #2

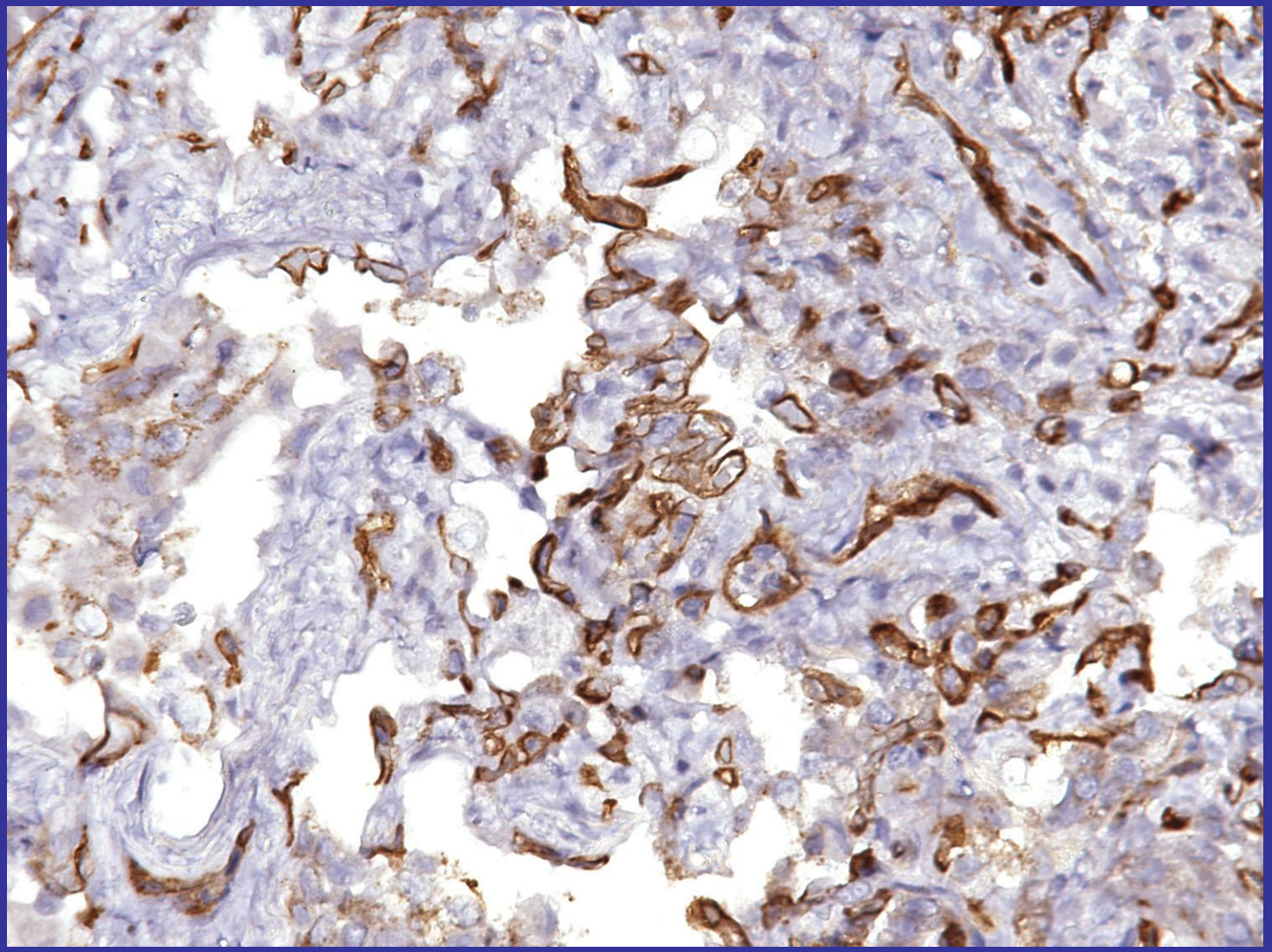
- 18-year old male s/p bilateral SSLTx in 2002 for CF
- Biopsy at 1 week ACR-A2
- Subsequent TBBx A1 or AX
- In 2008 he presented with drop in PFTs
- Underwent FOB with TBBx on 10/2/08







C4d Staining



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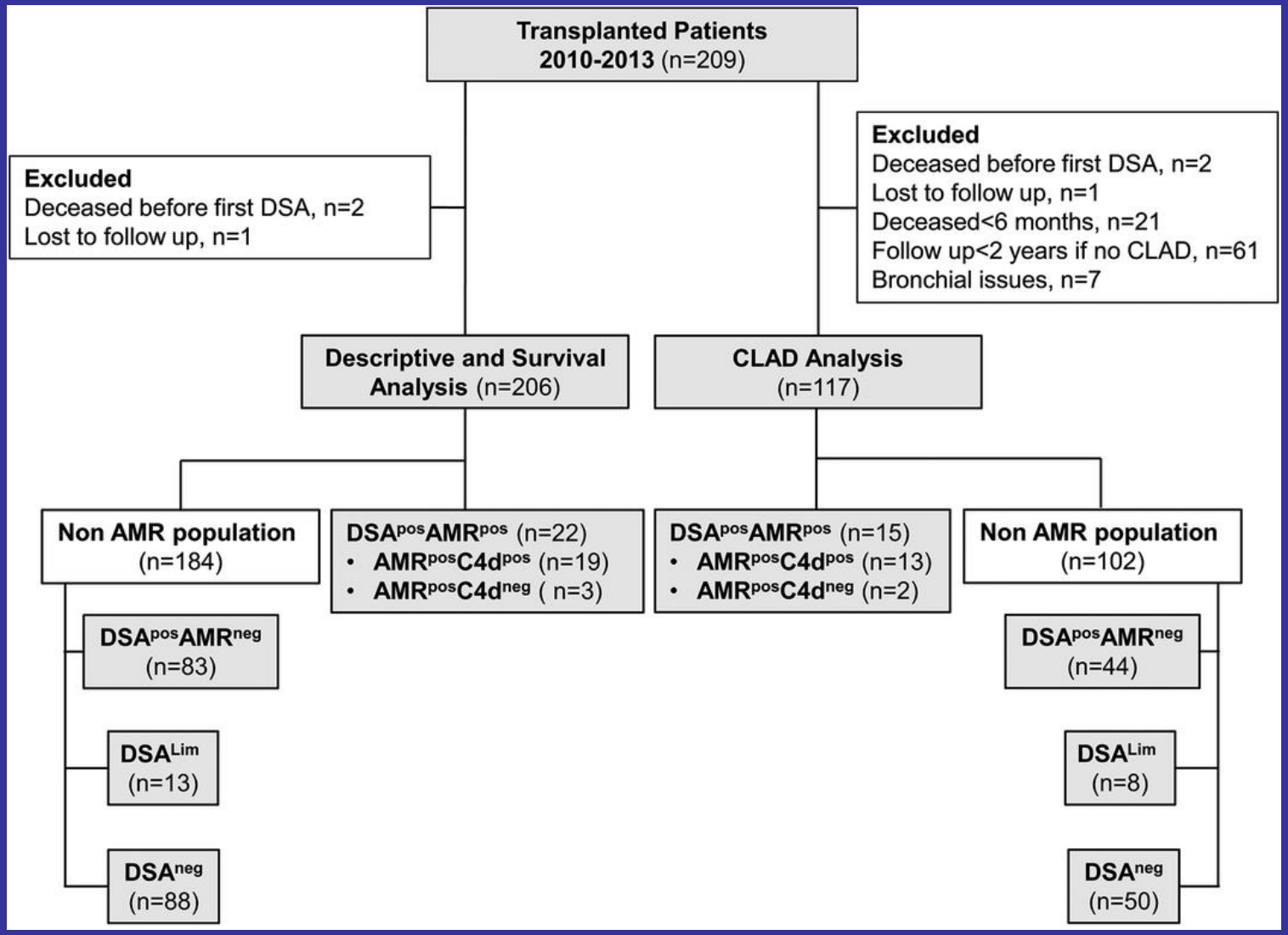

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