A Pathologist's Guide to Evaluating the Long-Surviving Allograft; Important Features, Stains and Approach to Better Understanding of Chronic AMR

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Plan of Talk

1. **Review of histological features recommended for the diagnosis of chronic AMR** (Banff Working Group, Am J Transplant 2016)

2. **Discussion of current studies of long-term biopsies from patients with possible chronic AMR**

American Journal of Transplantation 2016; 16: 2816–2835
Wiley Periodicals Inc.

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doi: 10.1111/ajt.13909

Meeting Report

2016 Comprehensive Update of the Banff Working Group on Liver Allograft Pathology: Introduction of Antibody-Mediated Rejection

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Diagnostic Criteria for Chronic AMR

(Demetris et at al, Am J Transpl 2016)

Table 7: Criteria for chronic active liver allograft AMR

Probable chronic active AMR (all four criteria are required):

(1) Histopathological pattern of injury consistent with chronic AMR: both required:

(a) Otherwise unexplained and at least mild mononuclear portal and/or perivenular inflammation with interface and/or perivenular necro-inflammatory activity (Figures 4 and 5).[1]

(b) At least moderate portal/periportal, sinusoidal and/or perivenular fibrosis.[2]

(2) Recent (for example, measured within 3 months of biopsy) circulating HLA DSA in serum samples;

(3) At least focal C4d-positive (>10% portal tract microvascular endothelia) (Figure 5).

(4) Reasonable exclusion of other insults that might cause a similar pattern of injury (see text).

Possible chronic active AMR:

(1) As above, but C4d staining is minimal or absent

ADCC, antibody-dependent cellular cytotoxicity; AMR, antibody-mediated rejection; DSA, donor-specific antibodies; HLA, human leukocyte antigen; TCMR, T cell–mediated rejection.

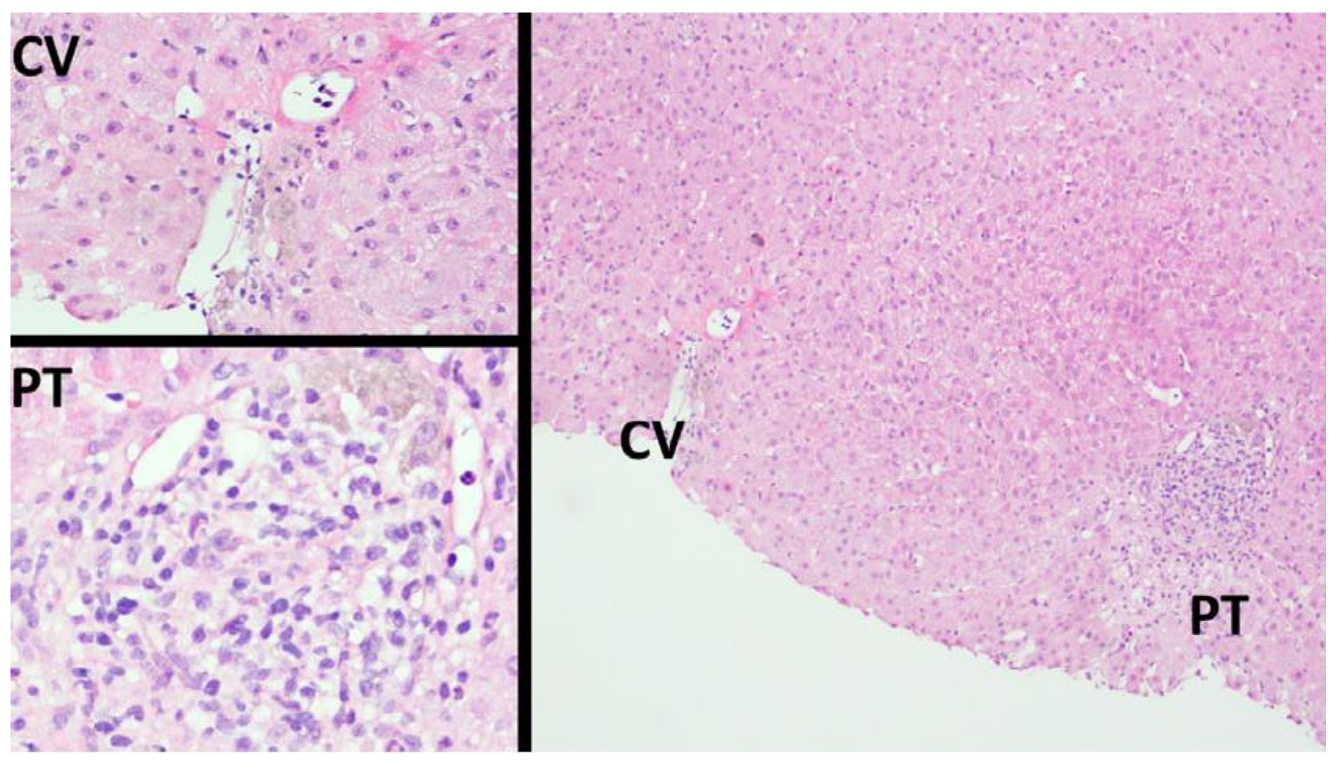
[1]It is difficult, at this time, to determine whether the mononuclear infiltrates are related to AMR (e.g. ADCC with capillaritis) or TCMR (mostly T effectors cells) or mixed AMR and TCMR. More research is needed on this topic.

[2]CD34 and SMA stains might be considered to study sinusoidal capillarization and stellate cell activation.

Diagnostic Criteria for Chronic AMR (Demetris et at al, Am J Transpl 2016)

(1) (a) Otherwise Unexplained Inflammation

- At least mild portal and/or perivenular inflammation
- With interface and/or perivenular necro-inflammatory activity



→ **Same features used to diagnose idiopathic chronic hepatitis / idiopathic post-transplant hepatitis** (Banff Working Group, Hepatology 2006)

"Idiopathic" Post-transplant Chronic Hepatitis

(Shaikh & Demetris 2007, Banff Working Group 2012)

Prevalence **Common(est) diagnosis in late (protocol) biopsies (>12 months post-LT)**

- 20-70% of late biopsies from children have unexplained inflammation that could be classified as IPTH
- Prevalence increases with time
  - 22% of children at 1 year, 43% at 5 years, 64% at 10 years (Evans 2006)
- Frequently present in patients with normal LFTs
- Similar changes occur in adults, but may be difficult to distinguish from recurrent disease (e.g. HCV, AIH, PBC)

Natural History **Associated with progressive graft fibrosis**

- 50-70% of children with IPTH have bridging fibrosis or cirrhosis by 10 years (Evans 2006, Herzog 2008)
- Idiopathic chronic hepatitis also associated with fibrosis/cirrhosis in adults (Seyam 2007, Syn 2007)

Otherwise Unexplained Late Graft Inflammation (Idiopathic Post-transplant Hepatitis) Is This a Form of Late Rejection?

- Many cases probably represent a form of late rejection (particularly in the paediatric population, in whom recurrent disease can be excluded as a cause of graft inflammation)
  - Some cases have overlapping features with acute cellular rejection (TCMR)
  - Other cases have overlapping features with de novo AIH (plasma-cell rich rejection)

- Antibody-mediated mechanisms have been implicated in the pathogenesis of IPTH and de novo AIH
  - Antibodies include donor-specific antibodies (Aguilera 2004 & 2005, Rodriguez-Mahou 2007, Salcedo 2009, Wozniak 2015, Kivela 2016)
  - Microvascular C4d deposits identified in some studies of IPTH /de novo AIH (Aguilera 2011, Trivedi 2014)

Otherwise Unexplained Inflammation in Late Post-Transplant Biopsies

1. **Are there any features which favour chronic AMR rather than other causes of late graft inflammation?**
   - late cellular rejection (TCMR) , de novo AIH (plasma-cell rich rejection)
   - recurrent disease (e.g. HCV, AIH, PBC)
   - de novo disease (e.g. hepatitis E)

2. **Is the distinction between chronic AMR and other causes of late graft inflammation (including late TCMR) clinically important?**
   - Possible treatment strategies (Demetris, Curr Opin Organ Transplant 2015)
     1. Optimize immunosuppression to facilitate/ensure compliance
     2. Utilize tacrolimus-based immunosuppression regimen, whenever possible
     3. Increase intensity of immunosuppression , unless non-compliance is strongly suspected
     4. Treat/cure any concomitant disease that may be contributing to chronic allograft inflammation

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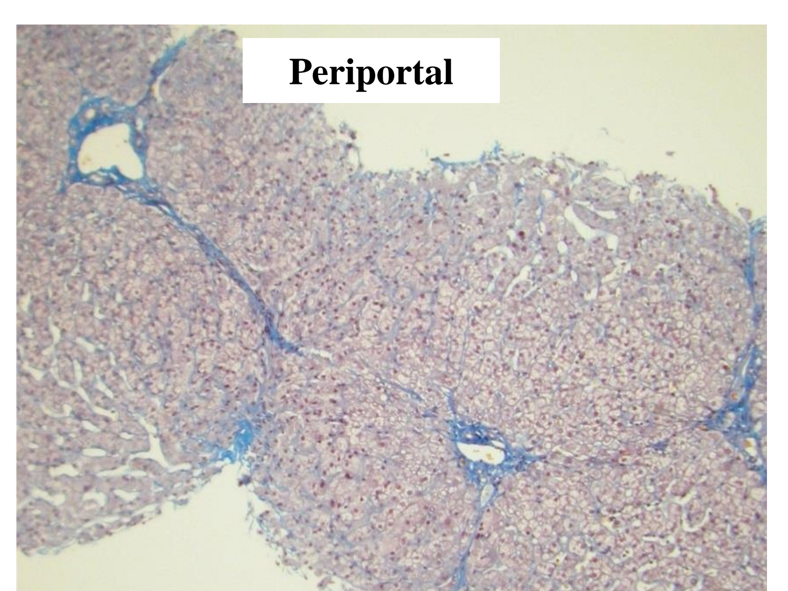
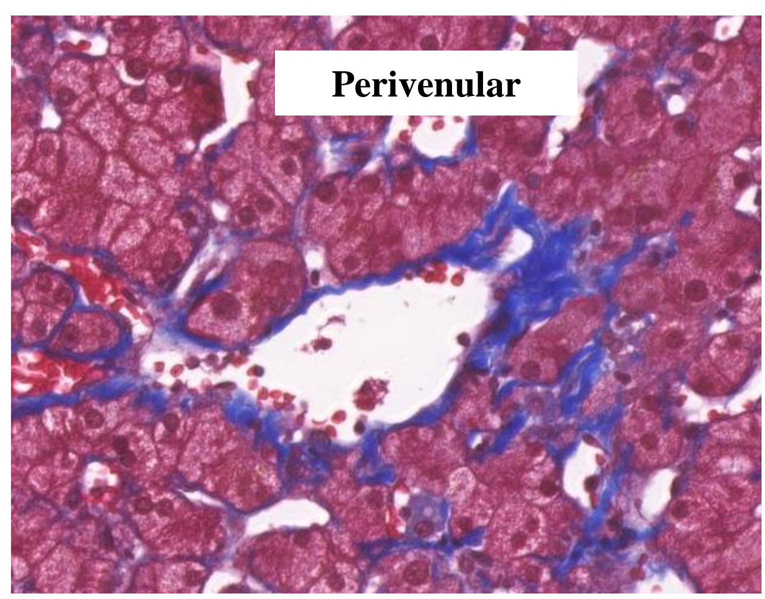
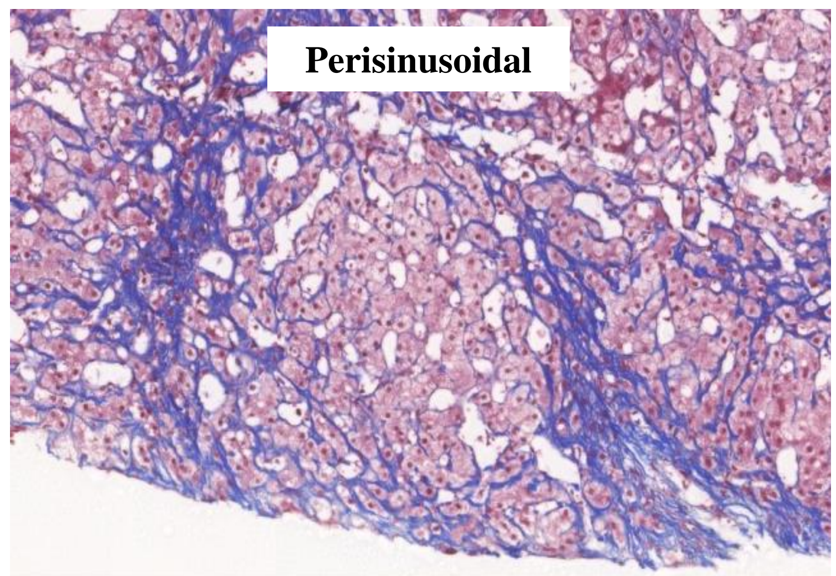
Fibrosis in Late Post-Transplant Biopsies - Histological Patterns Mainly Recognised in Children







Fibrosis in Late Post-Transplant Biopsies from Children – Scoring Histological Severity

Venturi C, Am J Transpl 2012

Table 1: Histologic features and staging definitions of the liver allograft fibrosis semiquantitative scoring system (LAFSc)

Structure	0	I	II	III
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Portal Tract	No Fibrosis	Non-expanding fibrosis in less than 50% of portal tracts.	Fibrosis in more than 50% of portal tracts and/or expansion into short fibrous septa into periportal parenchyma.	Marked expansion of most or all portal tracts with bridging fibrosis expanding to other portal tracts or central areas with or without occasional nodules.
Sinusoids (zones 1, 2)	No Fibrosis	Little fibrosis with thin focal collagen deposits involving less than 50% of sinusoids.	Little fibrosis with thin diffuse collagen deposits involving more than 50% of sinusoids, or thicker but focal fibrosis in less than 50% of sinusoids.	Thick, marked, diffuse sinusoidal fibrosis.
Centrolobular Vein (zone 3)	No Fibrosis	Circular perivenular fibrosis involving less than 50% of central veins without invasion into the perivenular parenchyma.	Circular perivenular fibrosis in more than 50% of central areas and/or expansion into short fibrous septa into perivenular parenchyma.	Marked centrolobular fibrosis with bridging to other central areas and/or portal tracts.

Unexplained Fibrosis in Late Post-Transplant Biopsies Relationship to Graft Inflammation

- All three patterns of fibrosis (periportal, perisinusoidal and perivenular) have been associated with the presence of graft inflammation
- Studies of idiopathic chronic hepatitis focused mainly on the relationship between portal inflammation/interface hepatitis and **periportal fibrosis**
- Centrilobular necro-inflammation (central perivenulitis) implicated in the pathogenesis of **centrilobular fibrosis**
- **Sinusoidal fibrosis** most strongly linked to antibody-mediated mechanisms (Egawa 2012, Miyagawa-Hayashino 2012, Yamada 2012, Tomita 2013, O'Leary 2016)
  - intially described in the absence of inflammation - "Non –inflammatory centrilobular sinusoidal fibrosis" (Egawa 2012)
  - Subsequent studies found that mild inflammatory changes (portal and/or centrilobular) also present in up to 60-87% biopsies showing centrilobular /perisinusoidal fibrosis (Miyagawa-Hayashino 2012, Yamada 2012, O'Leary 2016)

Published studies investigating relationship between graft inflammation and fibrosis

- Almost all cross-sectional
- Predictive value of different patterns of graft inflammation uncertain

Factors Predicting Graft Inflammation and Fibrosis – Studies of Serial Biopsies

(Varma, EBioMedicine 2016)

- 281 liver biopsies from 89 children with stable graft function
- Protocol biopsies 1 -10 years post-transplant
- Assessment of fibrosis - portal, sinusoidal and central – LAFSc (Venturi 2012)
- Assessment of inflammation – portal and lobular – mild, mod, severe

Histological Feature	Predictive Features in Preceding Biopsies	Other Risk Factors
Portal fibrosis	Portal inflammation (moderate -OR =10, severe-OR = 148)	Class II DSAs
Sinusoidal fibrosis	None	None
Central fibrosis	Central fibrosis	Deceased donor
Portal inflammation	Portal inflammation	Class II DSAs Non-HLA antibodies
Lobular inflammation	Lobular inflammation	Non-HLA antibodies

Assessment of Fibrosis in Late Post-Transplant Biopsies

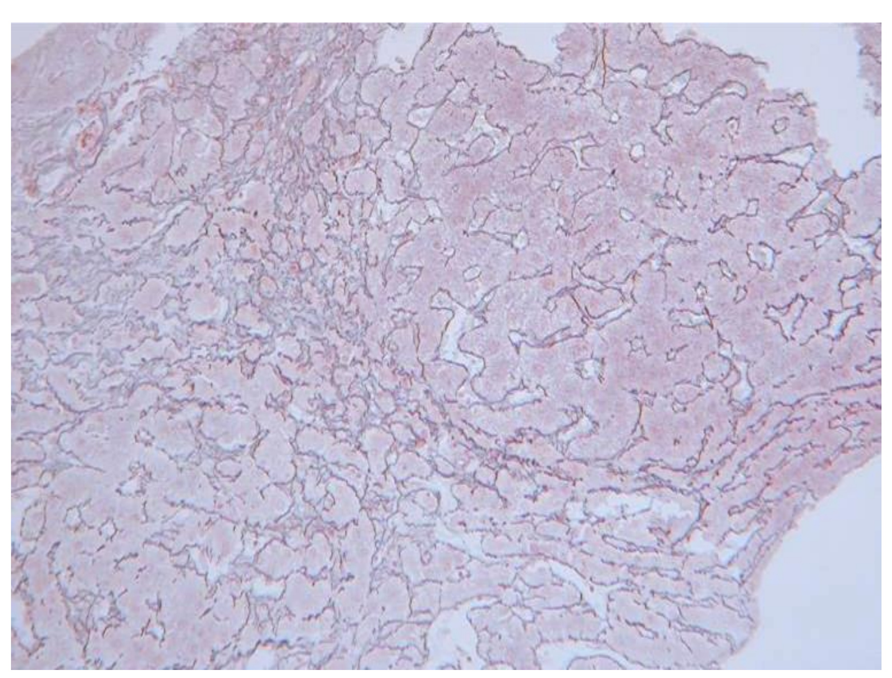
Connective Tissue Stains Essential

- Subtle patterns of fibrosis (especially perisinusoidal) may be missed in routinely-stained sections

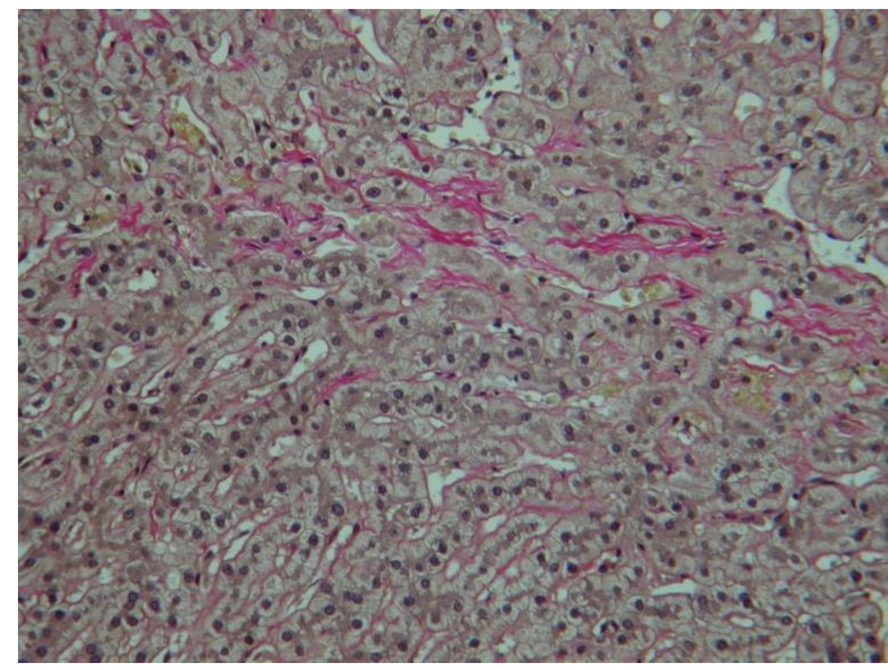
- Trichrome/HVG preferable to Sirius Red
  - Sirius Red may “overestimate” peri-sinusoidal collagen

- Reticulin staining helpful to identify nodular changes, frequently present in late post-transplant biopsies (esp in recipients of reduced-size grafts)
  - May be associated with the development of perisinusoidal fibrosis independently of immune-mediated mechanisms

Nodular Regenerative Hyperplasia in Liver Allografts



Protocol biopsy – 12 months post-transplant
(Reticulin)



Perisinusoidal collagen fibres at periphery of hyperplastic nodules
(HVG)

Unexplained Inflammation/Fibrosis in Late Post-Transplant Biopsies
Are There Histological Features Suggestive of Chronic AMR?

American Journal of Transplantation 2016; 16: 603–614
Wiley Periodicals Inc.

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doi: 10.1111/ajt.13476

Proposed Diagnostic Criteria for Chronic Antibody-Mediated Rejection in Liver Allografts

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Donor-specific alloantibodies (DSA) can cause acute

Introduction

Pre- and periodic posttransplant monitoring of donor-specific alloantibodies (DSA) currently dictates donor organ triage and immunosuppression management of kidney and heart allograft recipients. DSA in these populations represent a risk factor for premature allograft failure (1,2). Conversely, DSA determinations rarely impact organ triage or immunosuppression management of liver allograft recipients. This is because liver allografts are relatively resistant to acute antibody-mediated rejection (AMR), which likely results from the liver's large size, and regenerative capacity; secretion of soluble class I HLA antigens that bind DSA that are likely cleared by Kupffer

Proposed Diagnostic Criteria for Chronic AMR

(O'Leary, Am J Transpl 2016)

Study Population

- 45 protocol biopsies (40 at 1 year, 5 at 2 years) from patients with DSA $\geq$ 10,000 MFI
- 45 biopsies from matched DSA-negative patients (MFI < 1,000)

Histological Assessments

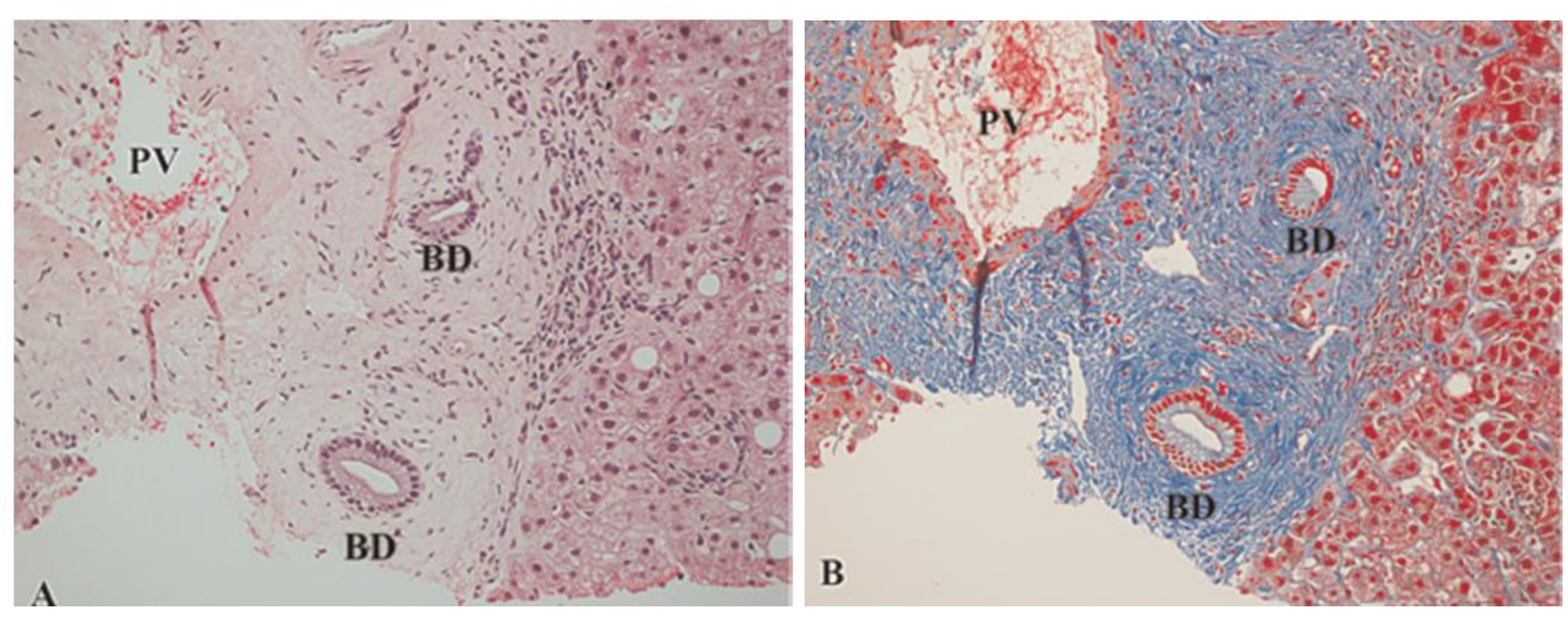
- 34 features associated with necroinflammatory damage and fibrosis scored semi-quantitatively on scale of 0-4 (none, minimal, mild , moderate, severe)
- Portal venopathy (vein loss) and portal tract collagenisation scored according to proportion of portal tracts involved
- Immunostaining for C4d and MHC scored in various structures on a scale of 0 = none, 1 = minimal (<10%), 2 = focal (10-50%), 3 = diffuse (>50%)

Statistical Analysis

- Univariate logistic regression used to correlate histologic data with (a) DSA in serum and (b) death-censored allograft loss
- Stepwise multivariable modelling used to create chronic AMR score to predict allograft failure

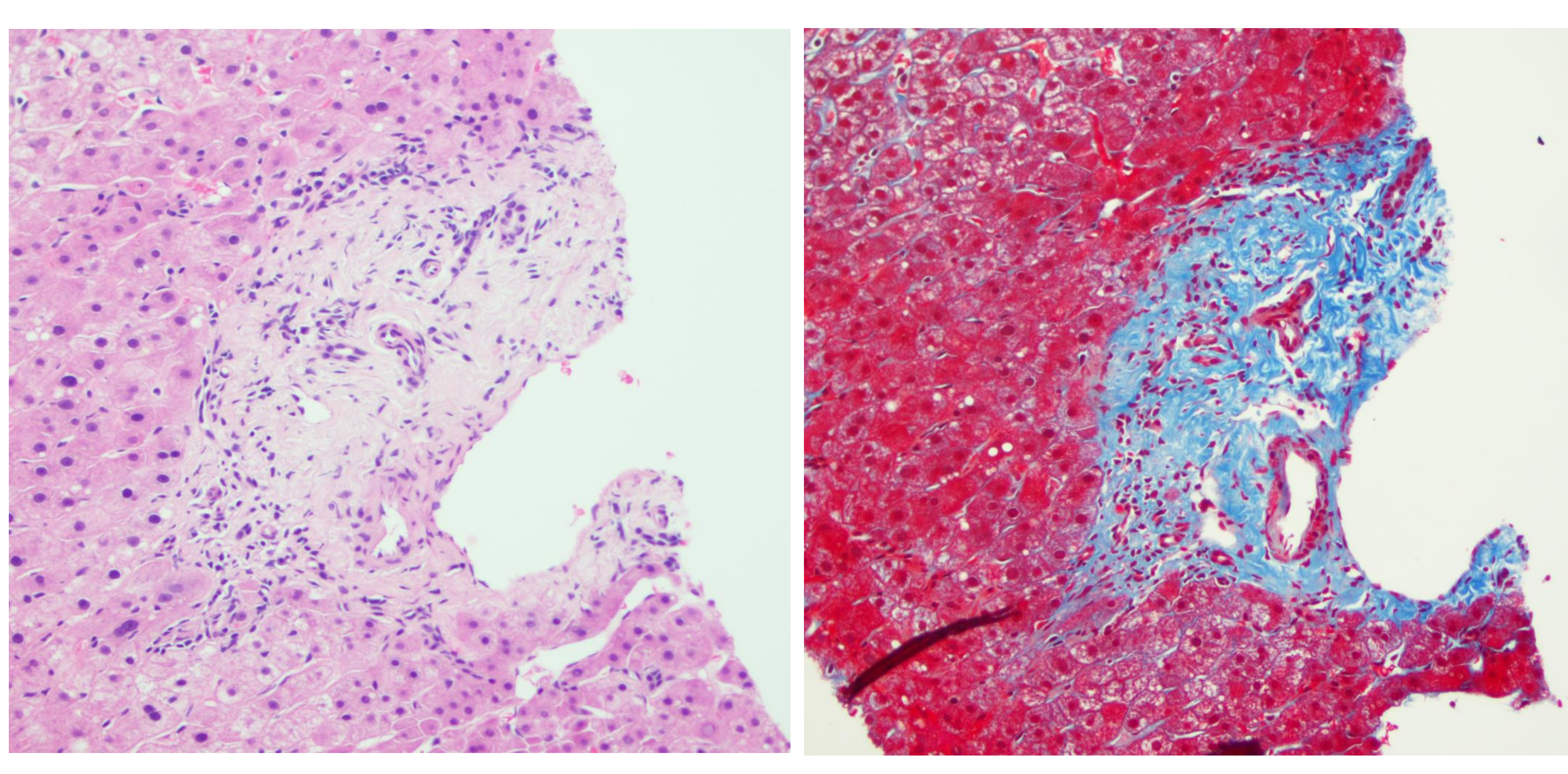
Portal Tract Collagenisation

(From O'Leary, Am J Transpl 2016)



Portal Tract Collagenisation (DSA-positive patients)

(Images courtesy of Jake Demetris)



Chronic AMR Score Predicts Graft Survival

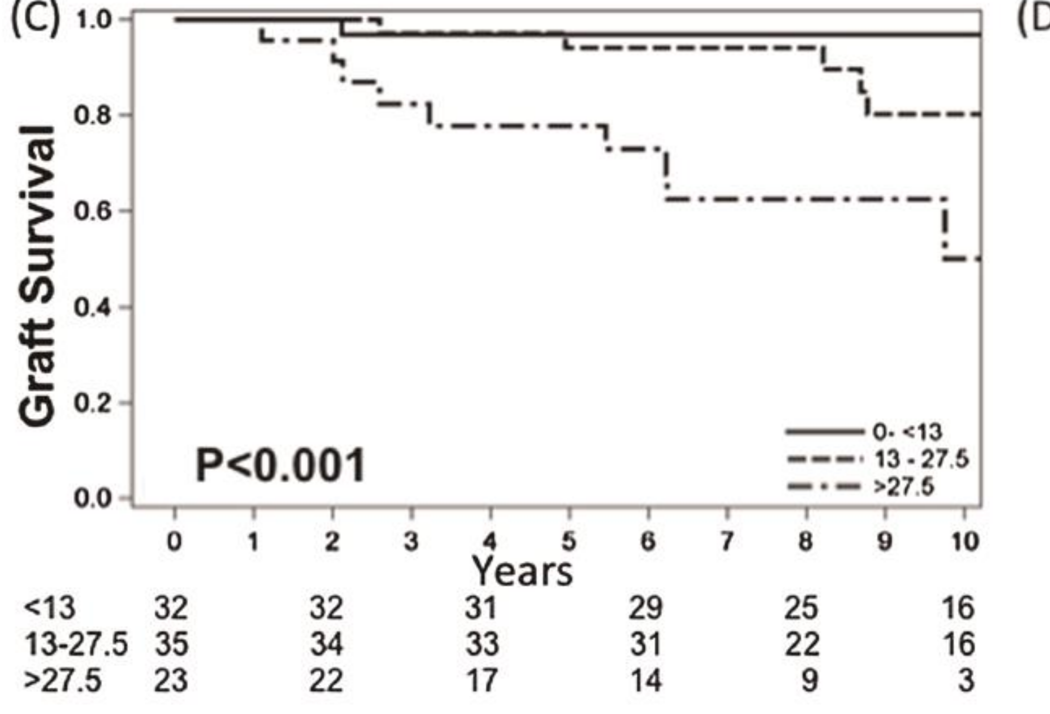
(O'Leary, Am J Transpl 2016)

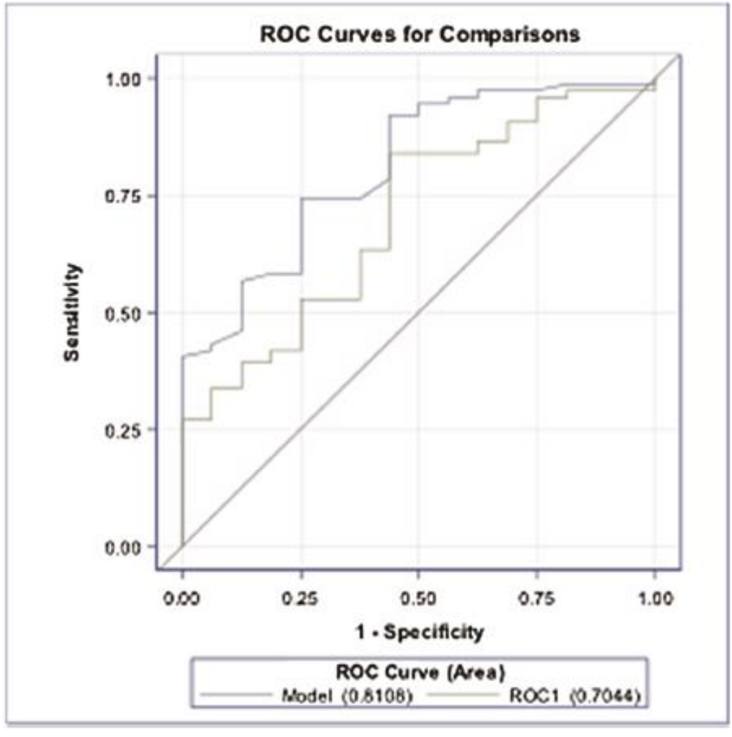
(A)

$$\left(\begin{array}{l} 0.226(\text{HCV}) + \\ 0.7748(\text{Lobular inflammation}) + \\ 0.3901(\text{Interface hepatitis}) + \\ 0.3561(\text{Portal collagenization}) + \\ 0.2886(\text{Portal venopathy}) + \\ 0.1116(\text{Subsinusoidal fibrosis}) \end{array}\right) \times 10$$

(B)

	cAMR variables			Outcome		
	OR	95% CI	P-value	No Allograft Loss	Allograft Loss	P-value
Lobular Inflammation	2.17	0.82-5.72	0.12	1(0-2)	2(1-2)	0.002
Interface Activity	1.48	0.61-3.61	0.39	1 (0-2)	2 (1-3)	<0.001
Portal Tract Collagenization	1.43	0.58-3.50	0.44	0 (0-1)	1(0-2)	0.02
Portal Venopathy	1.33	0.54-3.27	0.53	0 (0-1)	1(0.5-2)	0.005
Sinusoidal Fibrosis	1.12	0.54-2.32	0.76	1(0-2)	2(1-2.5)	0.03
HCV	1.25	0.23-6.95	0.80	0 (0-1)	1(0.5-1)	0.057





Proposed Diagnostic Criteria for Chronic AMR

(O'Leary, Am J Transpl 2016)

C4d Immunostaining

- Not used in diagnostic algorithm for chronic AMR score

Association with DSA-positivity

- Positive staining (C4d score of 2 or 3) in portal capillaries and stroma was strongly associated with DSA-positivity
- Other staining patterns (portal veins, sinusoids, central veins, central stroma) not associated with DSA-positivity

Portal capillary C4d staining (score 2 or 3) also associated with:

- Inflammation severity (portal, interface, lobular)
- Portal tract collagenisation

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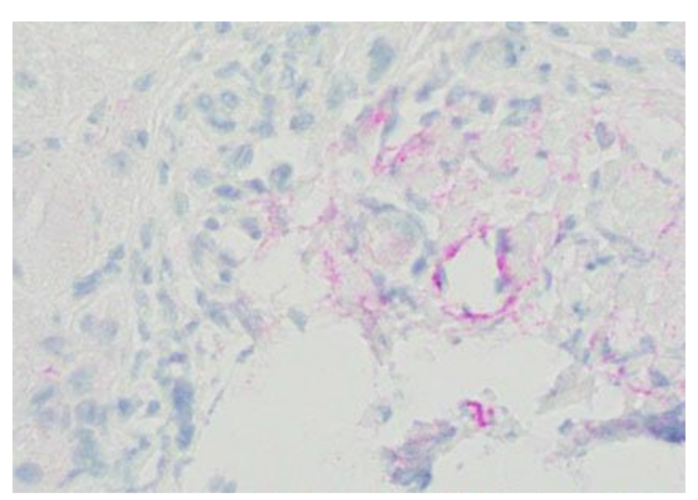
C4d Staining in Chronic AMR



Portal capillary positivity in long-surviving OLT recipient with a positive DSA
(from Banff Working Group paper, Am J Transpl 2016)

Current Recommendations for Assessing C4d Staining in AMR

(Banff Working Group paper, Am J Transpl 2016)

- C4d (immune) score based on **FFPE sections**
- Deposits assessed mainly in **portal microvasculature**

- Diagnosis of **definite acute AMR** requires C4d score of 3 - deposits in >50% of microvessels in > 50% of portal tracts
- Diagnosis of **probable chronic active AMR** requires at least focal C4d deposits >10% portal tract microvascular endothelia (C4d $\geq$ 2)

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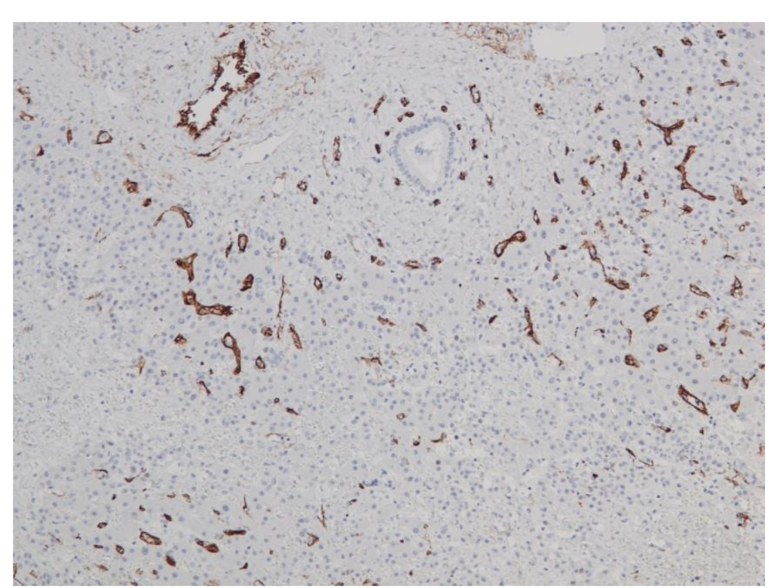
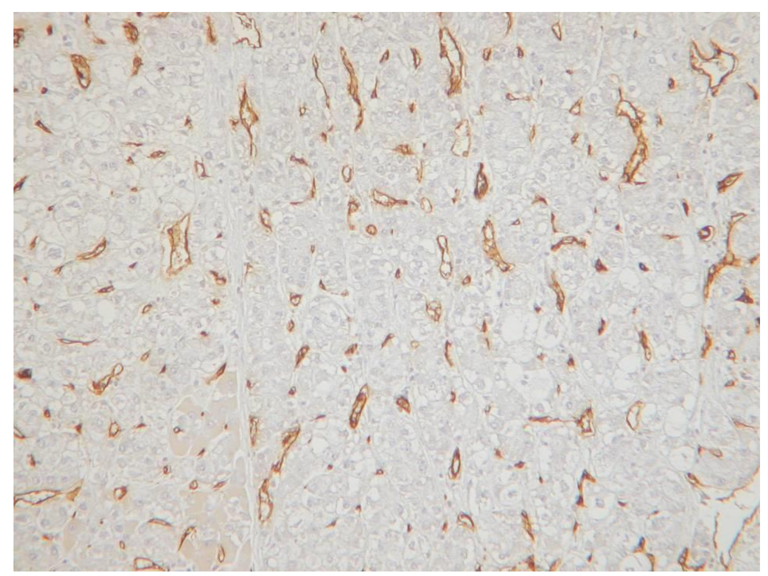
- Sinusoidal capillarisation and hepatic stellate cell activation may precede fibrosis
- Normal LSEC phenotype required to maintain HSC in quiescent state (DeLeve 2015)

Sinusoidal Endothelial Cell (SEC) CD34 Expression



Budd-Chiari Syndrome (acute-on-chronic)
Sinusoidal CD34 expression in surviving areas of periportal parenchyma



Well-differentiated HCC
Diffuse sinusoidal CD34 expression

Causes of "capillarisation" of sinusoids:

- SEC injury (e.g. chemotherapy-induced)
- Altered sinusoidal blood flow (e.g. obliterative portal venopathy, venous outflow obstruction, cirrhosis)
- Hepatocellular neoplasia (HCC, HCA)

Sinusoidal Endothelial Cell (SEC) CD34 Expression in Chronic AMR

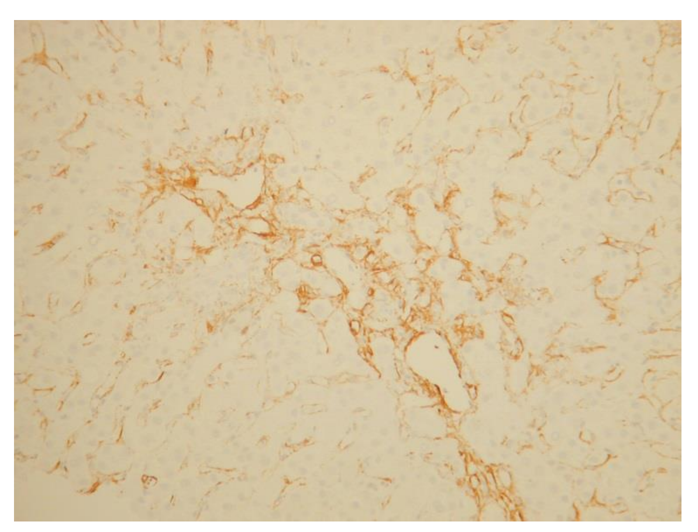
Antibody-mediated mechanisms could predispose to altering SEC phenotype

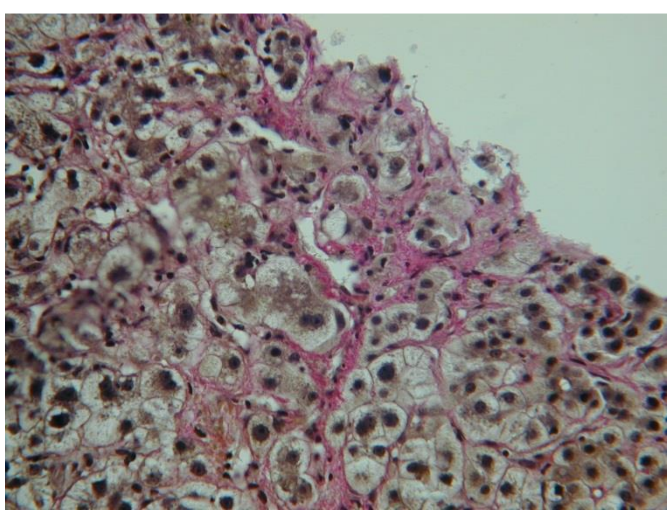
- Directly by antibody deposition on SECs
- Indirectly by altering sinusoidal blood flow (e.g. following antibody-mediated damage to portal vessels)

BUT

- No published studies documenting changes in SEC CD34 expression as a feature of liver allograft injury
  - recent study documented **stable** sinusoidal CD34 expression (and stable SMA expression in stellate cells) in biopsies obtained up to 5 years post-weaning from operationally tolerant paediatric liver allograft recipients (Feng, Hepatology 2017)

Hepatic Stellate Cell Smooth Muscle Actin Expression in Liver Allografts





- Stellate cell activation (SMA, GFAP, vimentin) in early post-transplant biopsies predicts subsequent fibrosis progression in recurrent HCV infection (Guido 1997, Carpino 2005, Gawrieh 2005, Russo 2005, Carpino 2008, Meriden 2010)
- SMA+ stellate cells co-localise with foci of perisinusoidal fibrosis in early recurrent HCV

- Recent studies have suggested that quantification of SMA-positivity in serial biopsies predicts subsequent fibrosis progression in paediatric liver allograft recipients (Metavir and LAFSc) (Venturi 2016, Varma 2017)

Other Patterns of Graft Injury Associated with Donor-Specific Antibodies Possibly Related to Chronic AMR

(O'Leary 2014, Demetris 2015, Del Bello 2016, Banff Working Group 2016)

Pattern of Injury	Comment
Chronic rejection	Many cases of "acute VBDS" probably antibody-mediated. Antibodies also implicated in pathogenesis of obliterative arteriopathy
De novo AIH (plasma cell-rich rejection)	Donor-specific antibodies include anti-GSTT-1 antibodies and anti-HLA class II antibodies
Biliary fibrosis/strictures	May reflect damage to peribiliary microvessels Consider other causes - e.g. peri-transplant bile duct ischaemia – see studies from Groningen (Peeters 2000, Scheenstra 2009, Gouw 2010)
Nodular regenerative hyperplasia	May reflect AMR-mediated portal venopathy Consider other causes – e.g. hepatic structural abnormalities occurring with restoration of hepatic volume in reduced-size allografts

What is the Relationship Between Acute & Chronic AMR?

Pathogenesis

- Different ends of spectrum of antibody-mediated injury?
- Distinct processes with different immunopathogenetic mechanisms?

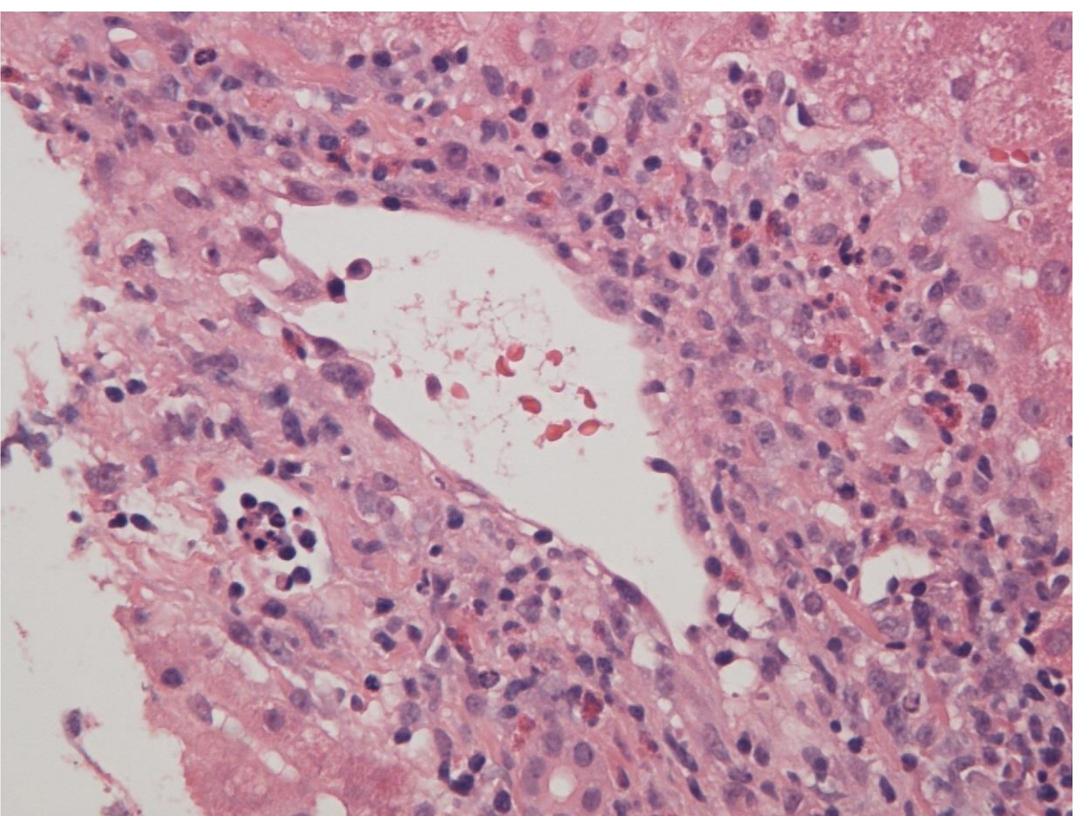
Histological Features

- Typical features of acute and chronic AMR are different
- Can they occur concurrently?

Typical Histological Features of Acute AMR

(O'Leary, Am J Transpl 2014)

- **Microvasculitis**
- **Portal eosinophilia**
- **Portal vein endothelial hypertrophy**
- **Eosinophilic venulitis (portal and/or central)**



Female, age 30

- 7 days post-retransplant (initial transplant for PSC)
- Worsening liver biochemistry (rising bilirubin and transaminases)
- Over next few days had rising antibodies to HLA-DQ (MFI up to 17,000)

Relationship Between Acute & Chronic AMR

(Del Bello, J Gastroenterol Hepatol, 2017)

9 patients with diagnosis of acute AMR and follow-up biopsy

Initial diagnosis of acute AMR

- 2 early (10-11 days) – both had preformed DSAs
- 7 late (3-24 months) – all had de novo DSAs
- Mean chronic AMR scores – 36.5 (early) and 32.6 (late)

Follow-up biopsies (5 - 48.5 months)

- Persisting features of acute AMR
  - portal eosinophilia (7), portal vein endothelial hypertrophy (5), eosinophilic venulitis (1)
  - Slight decrease in acute AMR score from 2.0 to 1.6 (p = NS)
- Chronic AMR score improved from 37 – 25 ($p = 0.003$)
- Worsening fibrosis, mainly periportal
  - Metavir score increased from 1.2 to 2.1

Significance of AMR scores

- Acute AMR score > 1.75 – 88% specificity for acute AMR (O'Leary 2014)
- Chronic AMR score > 27.5 – 50% risk of graft failure within 10 years (O'Leary 2016)

Plan of Talk

1. **Review of histological features recommended for the diagnosis of chronic AMR** (Banff Working Group, Am J Transplant 2016)

2. **Discussion of current studies of long-term biopsies from patients with possible chronic AMR**
   - Hannover Medical School - adults
   - **G**raft **I**njury **G**roup **O**bserving **L**ong-term **O**utcomes (GIGOLO) - children

Histological Assessment of Chronic AMR – Summary and Conclusions

- Antibody-mediated mechanisms are involved in the pathogenesis of late graft inflammation and fibrosis
- Paediatric liver allograft recipients appear to be more susceptible to chronic antibody-mediated graft injury, possibly reflecting differences in immature immune system
- Antibody mediated graft fibrosis has distinctive patterns (perisinusoidal fibrosis, portal tract collagenisation) not typically seen in other causes of late graft injury
- Antibody mediated mechanisms may also be involved in other patterns of late graft injury (chronic rejection, de novo AIH/plasma cell-rich rejection, biliary strictures, NRH) – for some of these other causes should be considered in the differential diagnosis

Histological Assessment of Chronic AMR – Summary and Conclusions

Areas which require further consideration

- Distinguishing chronic AMR from other causes of late graft inflammation and fibrosis (particularly in adults)
- Interactions between chronic AMR and other causes of late graft injury (e.g. late TCMR, recurrent disease, chronic hepatitis E, ischaemic cholangiopathy)
- Implications of making a diagnosis of chronic AMR for clinical management
- Relationship between acute and chronic AMR