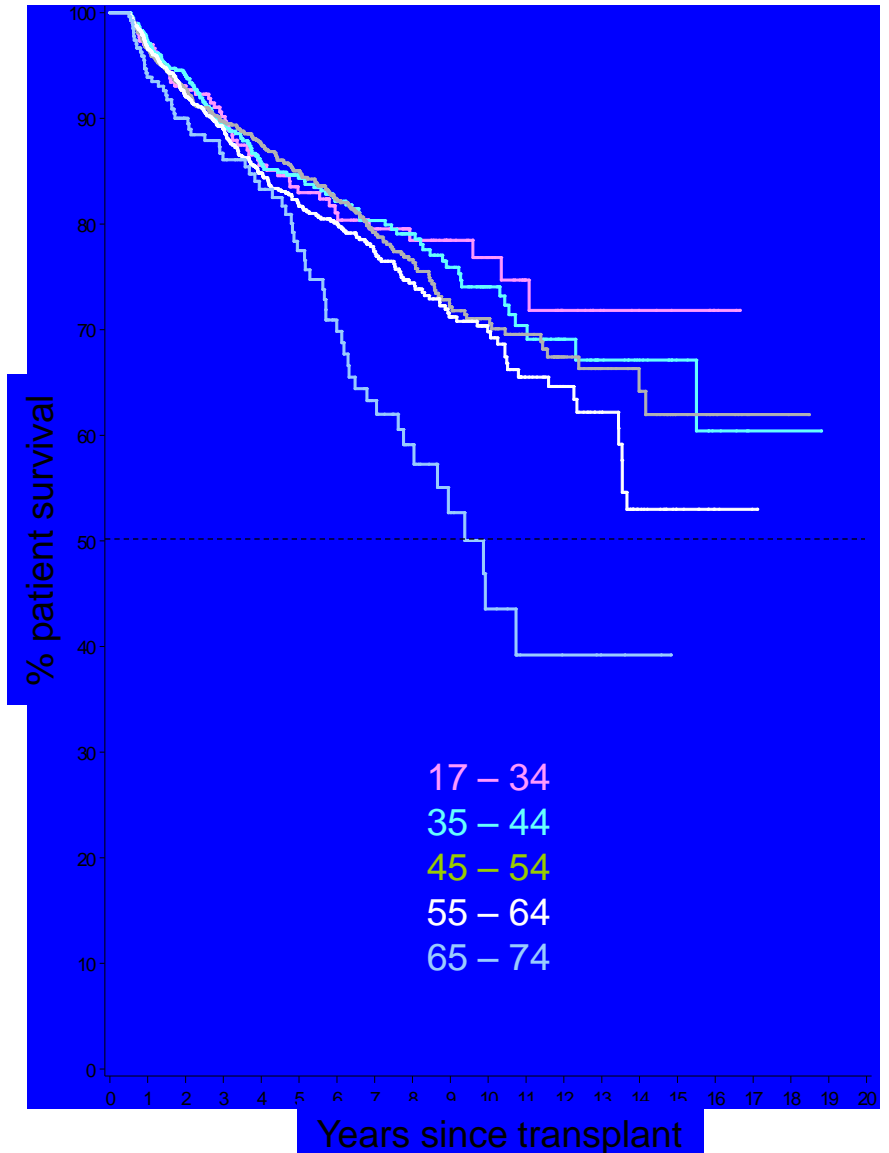# Chronic liver allograft dysfunction

An unsolved problem

Kaplan-Meier estimates by sex and age group

% patient survival

Years since transplant

Males
Females

% patient survival

Years since transplant

17 – 34
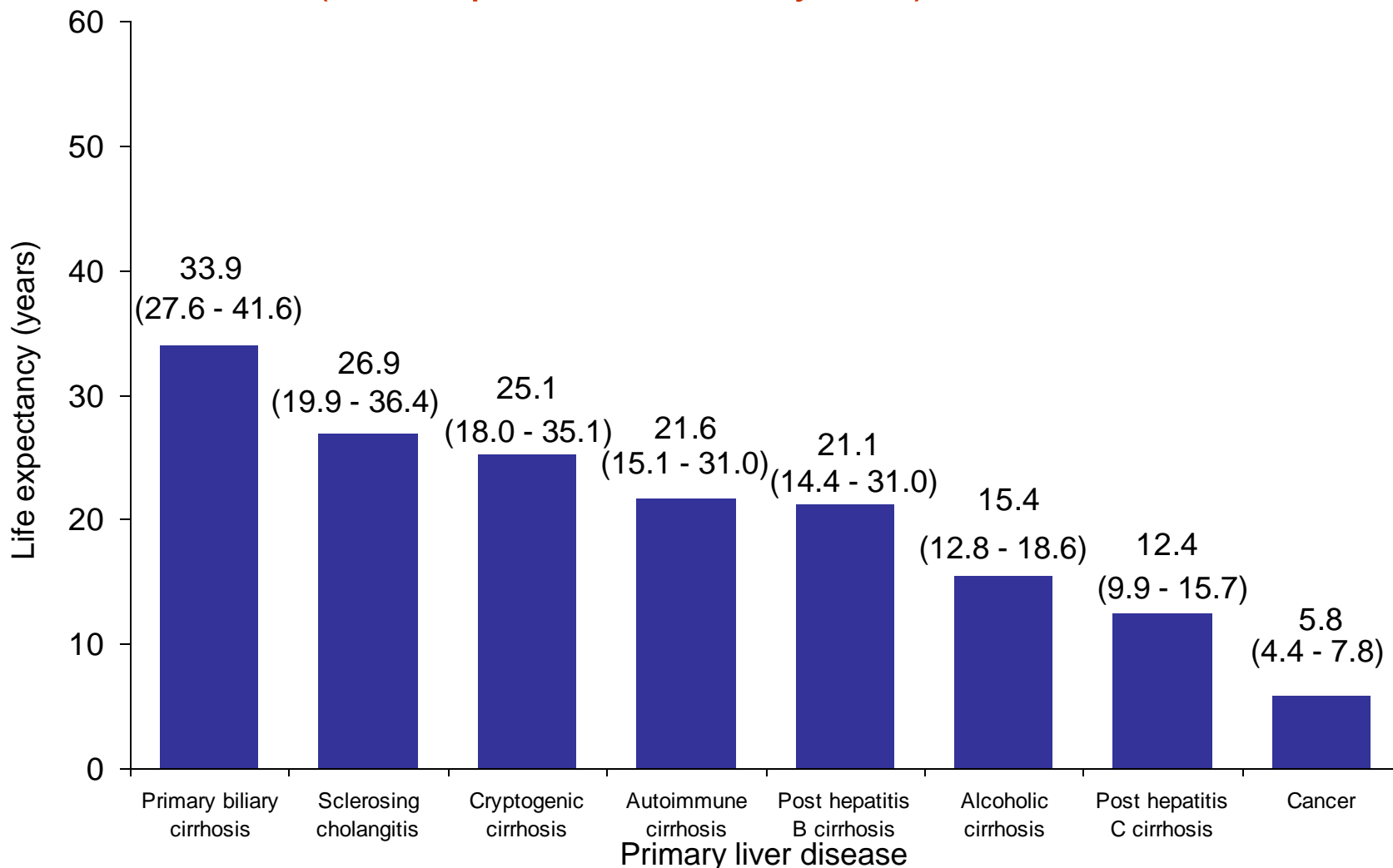35 – 44
45 – 54
55 – 64
65 – 74
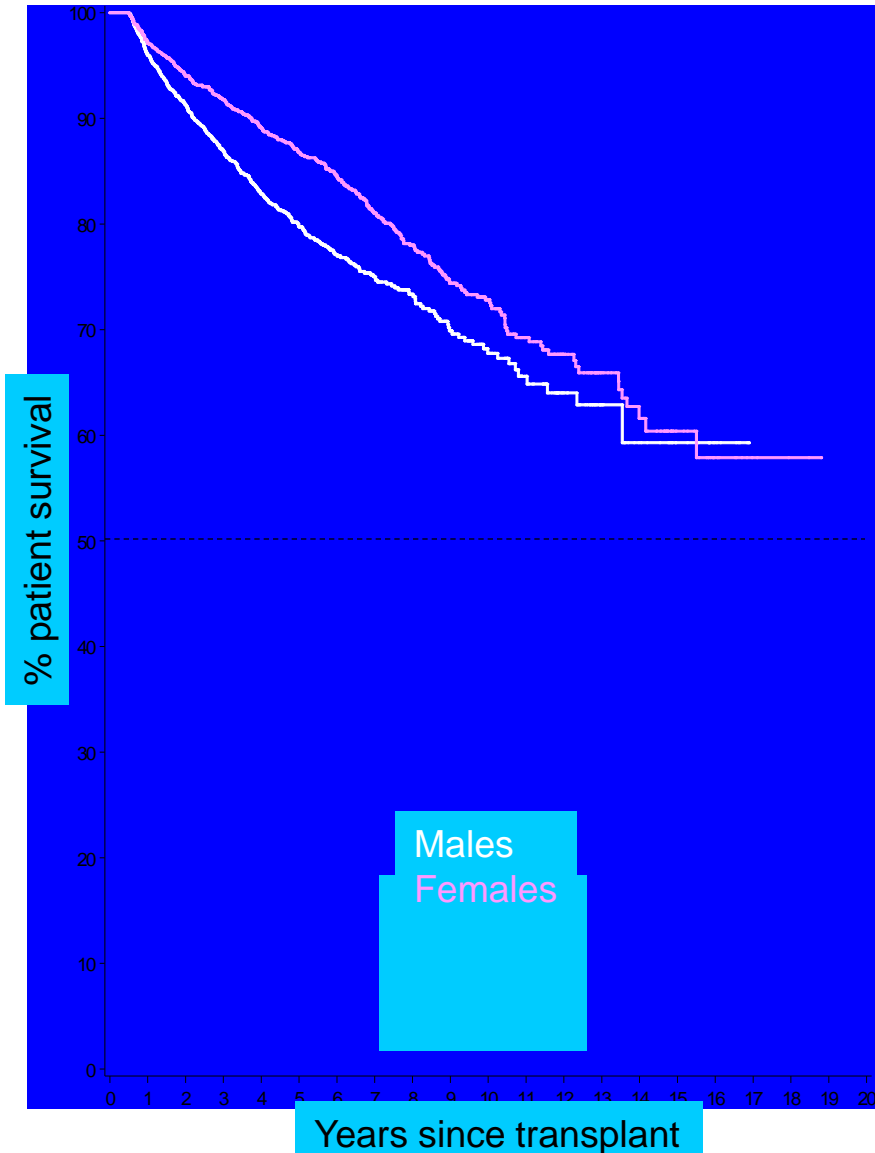
Average life expectancy by age group
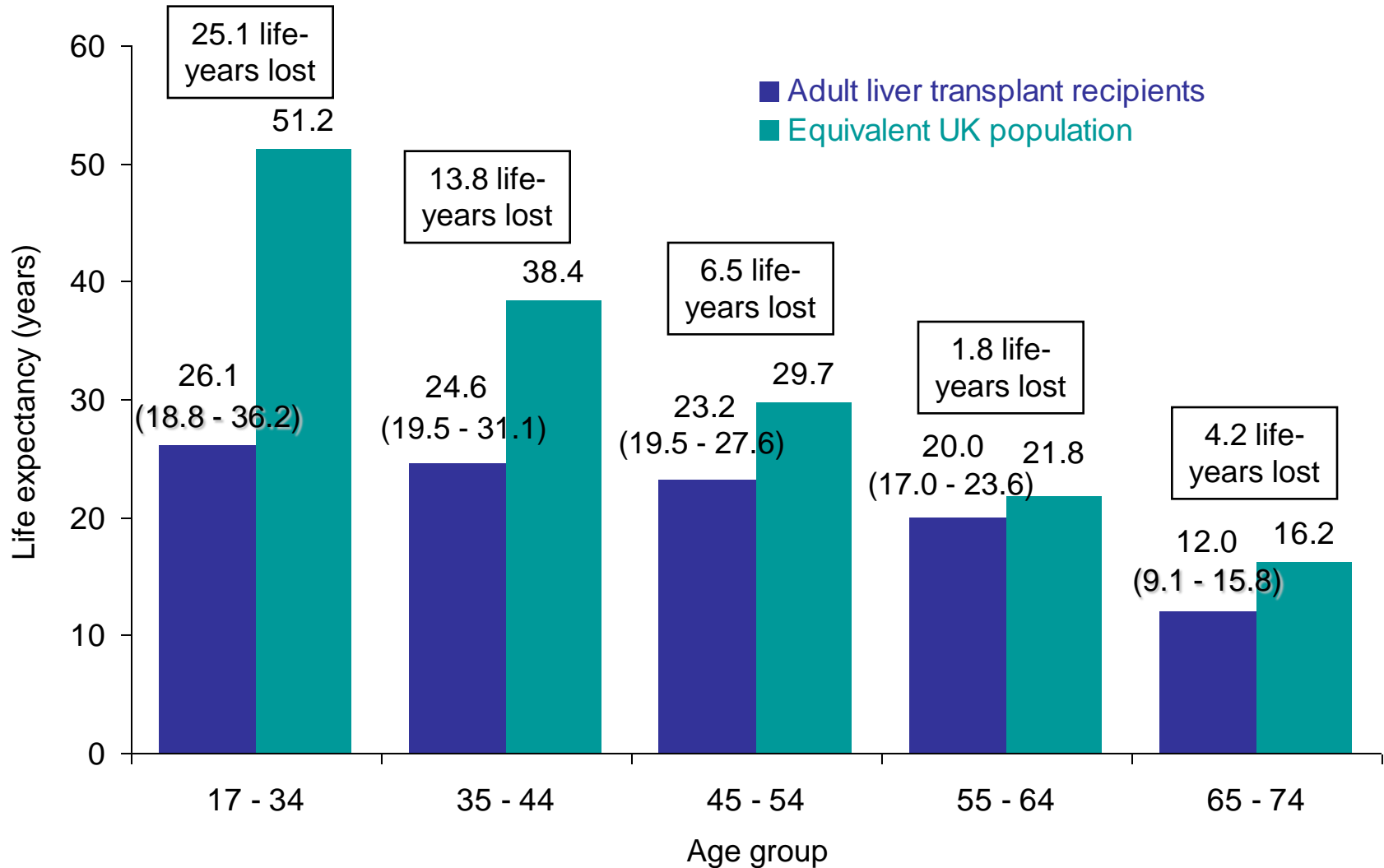
Average life expectancy by primary liver disease
(UK Population - 29.3 years)

Life-years lost by sex, age group and primary liver disease
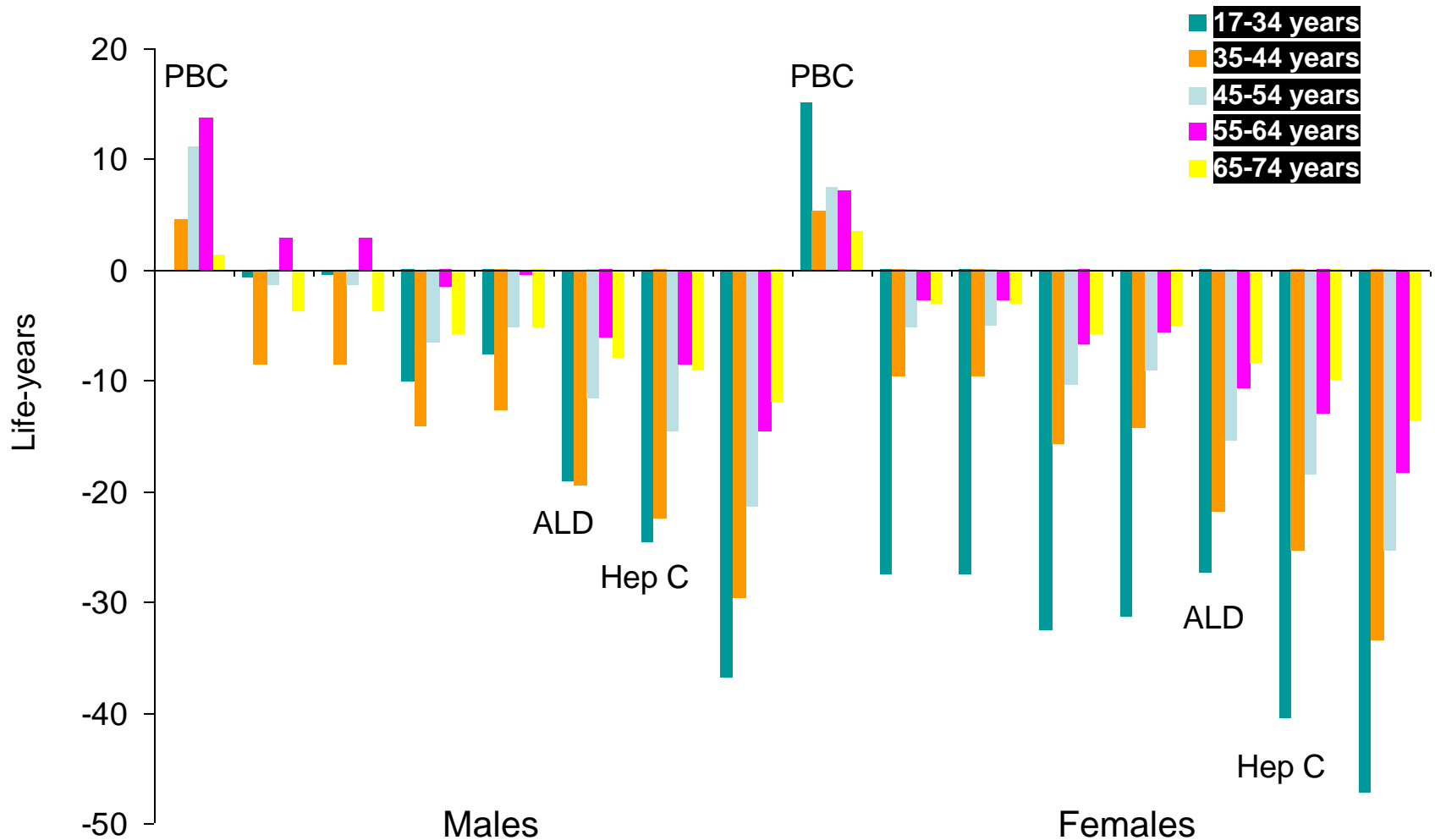
Late Liver Allograft Dysfunction

- Rejection
  - Acute/cellular
  - Chronic/ductopenic
- Technical/surgical problems
- Drug toxicity
- Late effects of ischemia/re-perfusion
- Disease Recurrence
- De novo autoimmune disease
- Idiopathic post-transplant hepatitis
- Viral
  - Recurrent
  - De novo

Histological Features of 1045 late liver biopsies (72% protocol)

Normal   15%

Biliary obstruction   1%

Chronic hepatitis

     recurrent disease   6%

     unknown cause   22%

Recurrent disease   20%

Other   28%

(fatty infiltration, fibrosis, siderosis)

(Hubscher 2012)

Abnormal liver histology in children

	N	1 year	5 years	10 years
Fouquet (2005)	67			73%
Evans (2006)	113	32%	55%	69%
Ekong (2008)	63			92%
Scheenstra (2009)	77	34%	65%	69%

Do liver tests reflect liver histology?

Review of protocol biopsies

	Normal Histology	Abnormal Histology
Normal Liver Tests	33 (4.9%)	331 (49.8%)
Abnormal Liver Tests	15 (2.3%)	276 (41.5%)

Liver tests are not a guide to normal histology

	Histology Abnormal	LFTs Normal	LFTs Abnormal
Pappo (1995)	66%	36%	75%
Slapak (1997)	72%	46%	87%
Sebagh (2003)	56% 5y 80% 10y	72%	90%
Abraham (2008)	27%	27%	
Vasani (2008)	<5%	<5%	
Mells (2009)	76%	76%	
Gelson (2010)	85%	85%	

Why the variation?

- Use of protocol biopsies
- Case mix
- Interpretation of histopathology
- Immunosuppression
- Age of recipient
- Duration of follow-up

Disease Recurrence

- Viral
  - Hepatitis A, B, C
- Immunological
  - Primary Biliary Cirrhosis
  - Autoimmune Hepatitis
  - Primary Sclerosing Cholangitis
- Malignancy
- Metabolic
  - Alcohol
  - NAFLD
  - other
- Other

Disease aetiology and follow up

Liver disease	Number of patients	Median follow up in days (interquartile range)
PBC	541	2868 (986 to 4502)
PSC	200	1957 (719 to 3913)
HCV	181	1732 (842 to 2581)
ALD	179	1758 (766 to 3426)
Non drug FHF	151	1956 (630 to 3769)
NAFLD	114	1793 (683 to 3817)
AIH	103	1834 (147 to 3563)
Paracetamol	53	1351 (26 to 3699)

Risk of graft loss from all causes

Liver disease	Hazard ratio (95% confidence interval)
PBC	N/A
PSC	1.6 (1.2 to 2.3)
HCV	2.0 (1.5 to 2.9)
ALD	1.4 (0.9 to 2.0)
Non drug FHF	1.0 (0.7 to 1.6)
NAFLD	1.3 (0.9 to 2.0)
AIH	1.6 (1.0 to 2.4)
Paracetamol	0.9 (0.4 to 1.9)

Graft loss from disease recurrence

Liver disease	Percentage of grafts lost to recurrent disease	Hazard ratio (95% confidence interval)
PBC	1.3	N/A
PSC	8.4	6.0 (2.5 to 14.2)
HCV	14.3	11.6 (5.1 to 26.6)
ALD	3.2	1.0 (0.2 to 4.9)
Non drug FHF	2.7	1.7 (0.4 to 6.6)
NAFLD	3.2	2.2 (0.6 to 8.4)
AIH	6.2	4.1 (1.3 to 12.6)
Paracetamol	0	N/A

Proportion of all grafts lost after 90 post operative days to disease recurrence. Diseases listed in descending order of proportion of grafts lost to disease recurrence (Cox regression model, 1-survival curve)
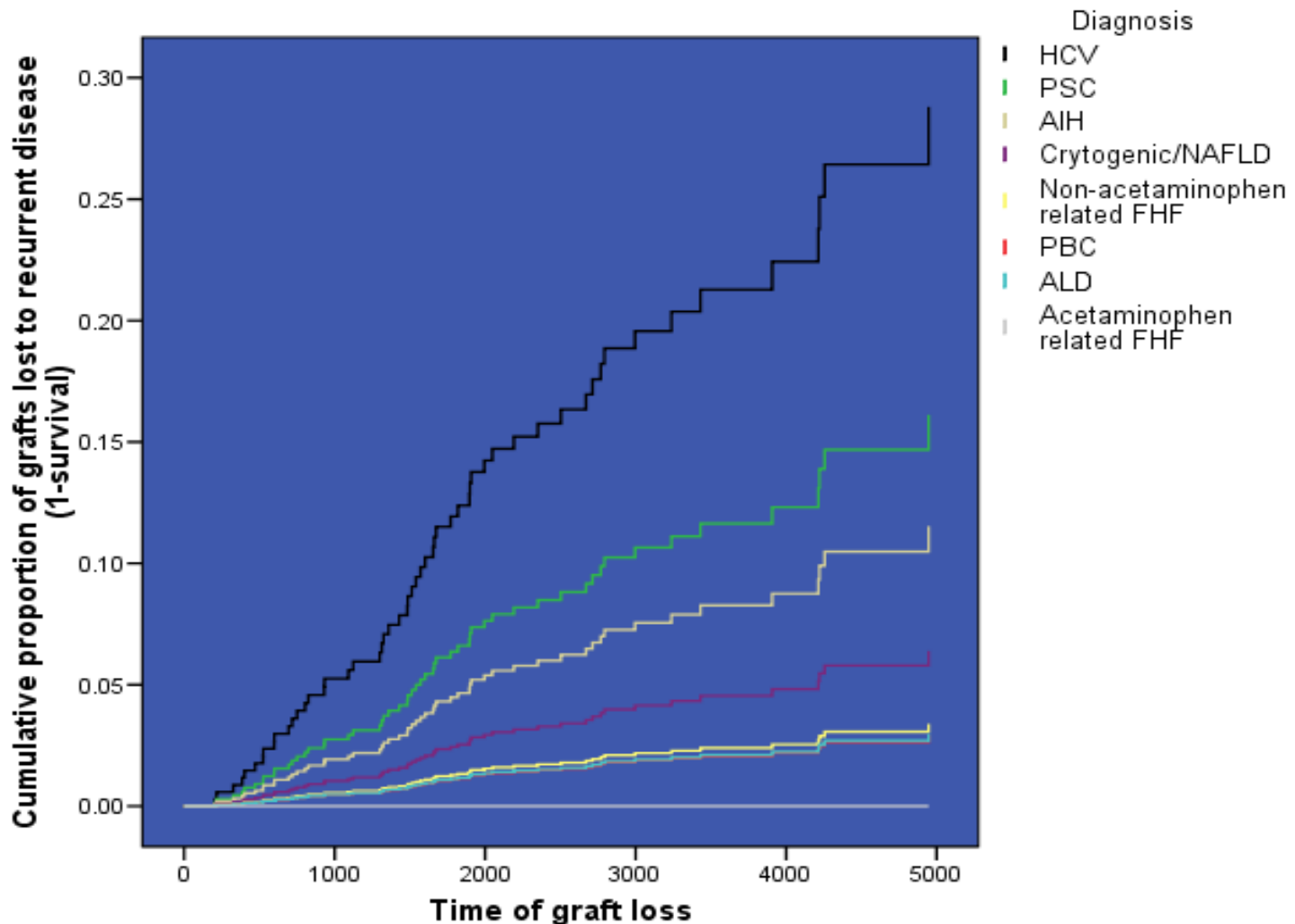
Rate of recurrence does not correlate with rate of graft loss

Liver disease (*n* surviving >90 days)	Percentage of grafts lost to recurrent disease	Published rate of recurrence	
		Overall (%)	Our centre (%)
PBC (450)	1.3	18	24
PSC (166)	8.4	11	37
HCV (161)	14.3	62-80	77
ALD (155)	3.2	5-20	24
Non drug FHF (111)	2.7		
NAFLD (93)	3.2	25-33	
AIH (81)	6.2	22	28
Paracetamol (34)	0	12	

Risk of graft loss from causes other than recurrence is not related to indication

- There is no difference in the risk of graft loss from all causes other than disease recurrence

  - PSC   HR 1.4   95% CI 0.9 to 2.0
  - HCV   HR 1.4   95% CI 0.9 to 2.2
  - AIH   HR 1.4   95% CI 0.9 to 2.2

Recurrent disease

- Alcohol
- NAFLD
  - Diagnosis based on history and clinical signs
  - Additional tests
  - Treatment largely symtomatic
    - Role of gliptans
    - Drug therapy for ALD

Detection of alcohol

- Liver tests
  - MCV, GGT – non-specific
  - Carbohydrate deficient transferrin:
    - levels vary with gender, nicotine use, BMI age
    - Good sensitivity and sensitivity; half life 14 days and normalisation takes weeks after cessation

- Specific
  - Blood, urine, breath alcohol

Ethanol metabolites

- Ethyl glucuronide
- Phosphatidylethanol
- Fatty acid ethyl esters

In blood, serum, hair, urine

Use of hair for detection of alcohol consumption

- Hair grows about 1 cm/month
  - Fatty acid ethyl esters in sebum
  - Ethyl glucuronide incorporated via blood/sweat
- Modifying factors
  - Cosmetic treatment
  - Impaired renal function

- Recent ethanol use
  - Blood or breath alcohol
  - Urine ethyl glucuronide
- Longer term ethanol consumption
  - Hair ethyl glucuronide or phosphatidyl ethanol

Disease Recurrence

Recurrent PBC 60% at 10 years

Diagnosis

Histological

Liver Tests are normal in 40% recurrent PBC

Role of immunosuppression

CyA less than Tacrolimus

Genetic role in recurrence rate

Role of UDCA uncertain

improves liver tests but ?role in outcome

Very little effect on graft function at 10 years: 4% graft loss

Disease Recurrence

Recurrent PSC (30% at 5 years)

- Transplant for PSC

- Non-anastomotic biliary strictures (MRI or PTC)

- Exclude secondary causes (ischemia (HAT), biliary infection, I/R injury, rejection, ABO incompatibility)

- Histology sometimes helpful

Treatment with UDCA – uncertain benefit on liver; reduced risk of colonic polyps/cancer

Progresses to cirrhosis in 15% at 10 years

Disease Recurrence

- Recurrent AIH (30% at 5 years)
  - Transplant for AIH
  - Raised transaminases, immunoglobulins and auto-antibodies
  - Interface hepatitis

  Role of donor/recipient HLA match controversial

  Commoner with type1 than type 2 AIH

  Interface hepatitis preceded serologic changes

  Treat with increased steroids

  Outcome variable:

Late acute rejection (Thurairajah et al 2013)

- Retrospective review of 970 consecutive adult liver recipients
- Incidence 11%
- Onset median 565 days (90-2922)
- Risk factors
  - Younger age
  - PBC
  - Previous graft
- Median trough Tac
  - 1 week prior   5.5ng/mL
  - 4 week prior   7.7ng/mL
- Outcome: poor response in about 30% proceeding to CR

Hepatitis E viral infection

- 4 Genotypes
  - G1: SE Asia, Middle East, Africa
  - G2: Mexico, Africa
  - G3:Europe, N America, Japan
  - G4: SE Asia
- G1 and 2: sporadic and outbreaks associated with faecal contamination; clinical attack rates greatest 15-35 year old with fatality rates 0.2-0.4% (10-25% in pregnancy)
- G3 and 4: sporadic, linked to food (especially pork, deer, wild boar): commoner in older males, no increased risk in pregnancy, usually self limiting
- Vaccines being evaluated

HEV and Blood donation

- HEV rates in blood donors
  - Seroprevalence rates vary with age and time with increase in younger donors and decrease in older donors but around 21% (Netherlands)
  - Viremia rates around 1:2800 (Hogema, 2014)
- Transmission rates
  - vary

Outcome of 18 recipients infected with HEV by transfusion
(Public Health England)

Inferred IMS	N	Anti-HEV	Clearance	Clinical Hepatitis
None	8	8 (100%)	8 (100%)	1
Mod	6	5/6 (83%)	3/4 (75%)	0
Severe	4	2/3 (66%)	2/3 (66%)	0

HEV and organ transplantation

- Evidence base primarily on relatively small series and case reports
- Both *de novo* and recurrent infections may occur
  - Of 283 organ recipients, 38% had evidence of HEV infection at time of transplant with 3 *de novo* and 3 recurrent infections (Abravanel 2014)
  - Le Grand (2011) estimated in France 3.2 cases/100 person years
- Outcomes: follow-up of 217 SOTR, 14 developed acute HEV and abnormal liver tests: 8 developed chronic hepatitis and resolution occurred in 43% within 1-3 months (Kamar 2008)

HEV and Transplantation

- More likely to develop chronic disease
- Both IgM and IgG may remain negative so diagnosis made on HEV PCR
- Usually have biochemical and histological hepatitis but less severe than non-immuosuppressed
- High risk to develop chronic disease with progressive fibrosis

  (Kamar 2008, Kamar 2011, Zhou, Wang)

- In vitro effect of IMS
  - mTORi and CNI support HEV replication
  - MMF inhibits replication
  - Steroids: no effect
- Treatment
  - Reduce immunosuppression
  - Interferon
  - Ribavirin

HEV and Transplantation

- Both de novo and recurrent HEV occurs and may progress to graft cirrhosis
- Diagnosis requires
- Treatment
- Strategies
  - Screen donors?
  - Screen recipients pre transplant
  - Screen all recipients at one year
  - How best to screen?

Drug Toxicity

- Azathioprine associated with nodular generative hyperplasia and with hepatitis
- Ciclosporin associated with cholestasis
- Other drugs may cause DILI

Graft cirrhosis

(Seyyam et al)

- Retrospective study of 1647 adults liver allograft recipients, of whom 1287 had survived > 1 year

- Cirrhosis developed in 48 patients
  - 29 recurrent disease
  - 19 non-recurrent disease

Cirrhosis from disease recurrence

HCV	9%	11/129
PBC	0.5%	2/398
PSC	2%	3/145
AIH	10%	4/41

Others: alcohol 1, NASH 4, HBV 4

Graft Cirrhosis
Acquired disease

De novo AIH   4

Biliary cirrhosis   4

HBV   1

Unknown   10*

*6 were transplanted for sero-negative FHF

Cirrhosis after OLT (Tabatabai 1999)

- 33 cases of cirrhosis in 493 grafts from 435 patients
  - Recurrent disease   24
    - Viral   20
    - PBC   1
    - AIH   1
    - Alcohol   1
   - Tumour   1
  - Acquired HCV   3
  - Budd Chiari   1
  - De Novo AIH   1
  - Biliary obstruction   1
  - Ischaemia   1

FHF and graft fibrosis (Mohamed, 1997)

	Sero-negative	Other
N	41	16
Fibrosis	Mild 13 Moderate 3 Severe 3	Mild 3
Cirrhosis	1	0

Cryptogenic cirrhosis and allograft fibrosis

- Of 39 patients grafted for cryptogenic cirrhosis who survived for >1 year, steatosis and steatohepatitis were present in 38% (control 17%); 19% had moderate steatosis at 1 year and half progressed to cirrhosis at 4 years (Sutedja 2004).

- Autoantibodies may indicated greater risk of progression to cirrhosis

Chronic Hepatitis
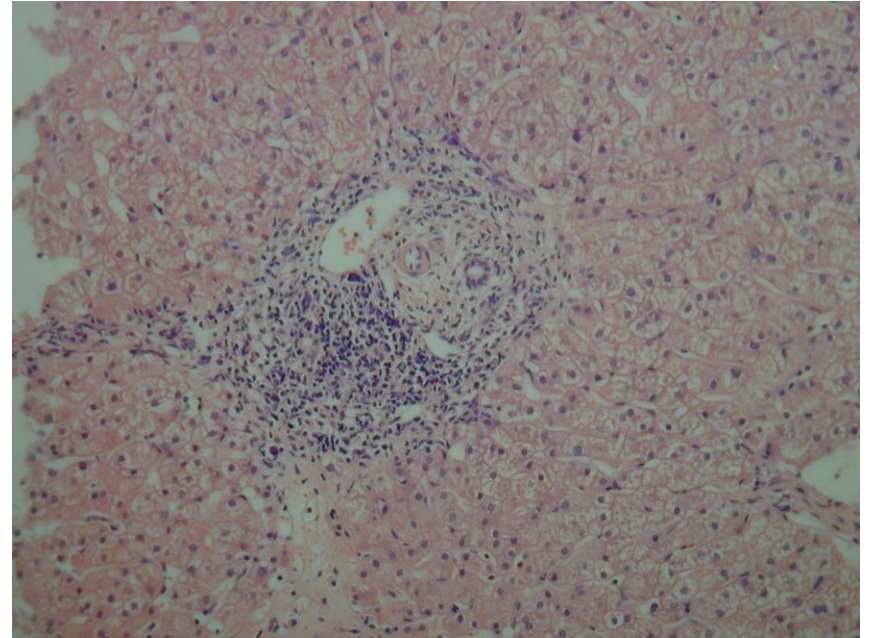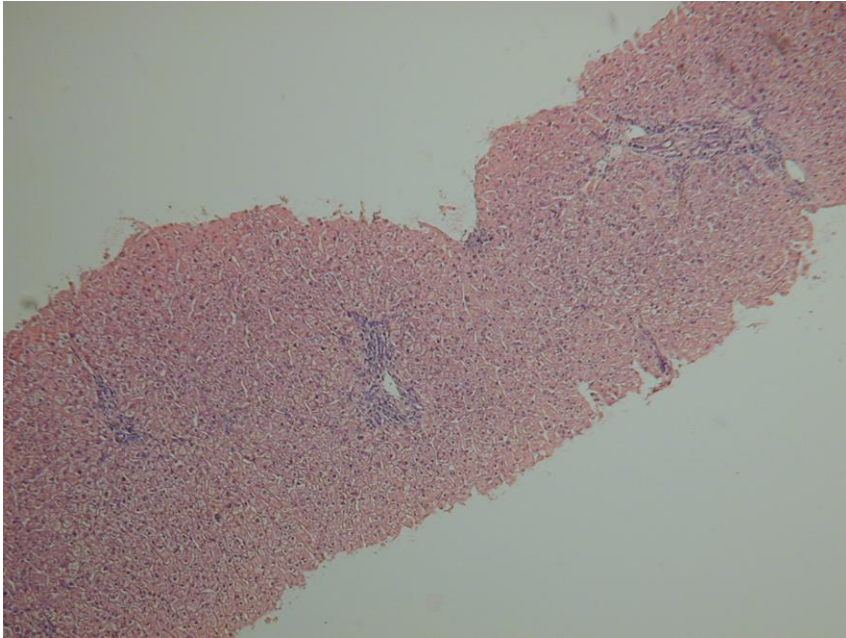
Graft Hepatitis

- Portal and lobular mononuclear portal infiltrate

- Variable interface hepatitis

- Absence of rejection and other identifiable causes of graft dysfunction

Is chronic allograft hepatitis progressive?

- 30 patients
  - alcoholic liver disease (abstinent)
  - Liver failure following acetaminophen/drugs
- Paired liver biopsies
- Time of first demonstration of CH: 22 months
- Second biopsy: 48 months

Findings on initial biopsy

- Inflammation
  - Mild   19
  - Moderate   2
- Fibrosis
  - Mild   8

Liver tests: normal serum AST and bilirubin

elevated Alk Phos in 16

Progression

- Fibrosis
  - Increase   12
  - Decrease   3
  - No change   6
- Necro-inflammatory score:
  - Increase   5
  - Decrease   10
  - No change   6

Graft hepatitis of unknown cause (Nakhleh et al, 2005)

Of 704 patients received a liver transplant

  282 at low risk of disease recurrence

  31 cases of chronic hepatitis

cryptogenic cirrhosis	13
steatohepatitis	12
α_1 anti-trypsin deficiency	3
tumour	2
acetaminophen toxicity	1

- Diagnosis   8 months (0.5-72)
- Histological activity
  - Mild 19,moderate 21
- Progression
  - 10 resolution
  - 15 persistence

Autoantibodies

- Autoantibodies occur more commonly after LT

- Higher titres in association with abnormal liver tests suggest graft hepatitis (Foschi 2015)

Liver tests and liver histology

- Auto-antibodies are seen in up to 70% liver allograft recipients, and do not correlate with graft function

- Histologic abnormalities were present in 80% of 143 patients at 10 years; in 52% liver tests were normal (Sebagh 2003)

Antibody-mediated rejection in liver transplants – a real problem?

- Donor specific HLA-alloantibodies are well recognised as a risk factor for premature graft loss in kidney and heart transplantation
- DSA cause damage by
  - Complement activation via classic pathway
  - Direct interaction with cell surface Ags
  - Activation of pro-inflammatory cells
- DSA detected by
  - Complement dependent cytotoxicity (CDC)
  - Flow cytometry (FC)
  - Solid phase immunoassay (such as Luminex)

- Criteria for AMR in kidney (Banff 2003)
  - Circulating DSA
  - Graft dysfunction
  - Tissue injury
  - Antibody action (C4d or Ig deposition)

Histology of AMR
(Bellamy 2007, Sakashita 2007, Ali 2012, Demetris 1988)

- Centrilobular hepatocyte swelling

- Hepatocanalicular cholestasis

- Acute cholangiolitis (early)

- Ischemic cholangiopathy (late)

- C4d staining

  – Arterial, portal venous and sinusoidal endothelial staining more common in DSA+ than DSA- grafts

  – Non specific

  – Technically challenging

- Association between DSA and ACR is conflicting (see O'Leary 2013, Taner 2014)

- Association between DSA and chronic rejection is also conflicting (O'Leary 2011, Musat 2011, Goh 2010)

- Recent large scale retrospective studies from Europe, US and Asia have suggested that pre-sensitisation has no impact on patient or graft survival (Goh 2010, Ruiz 2012, Shin 2013)
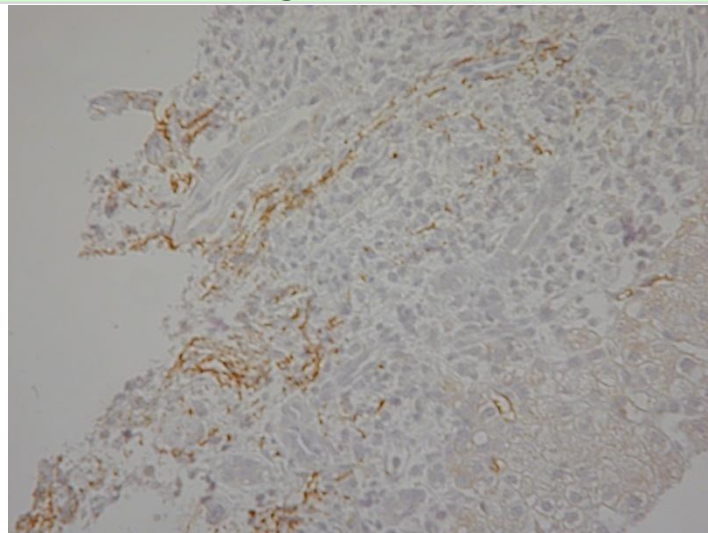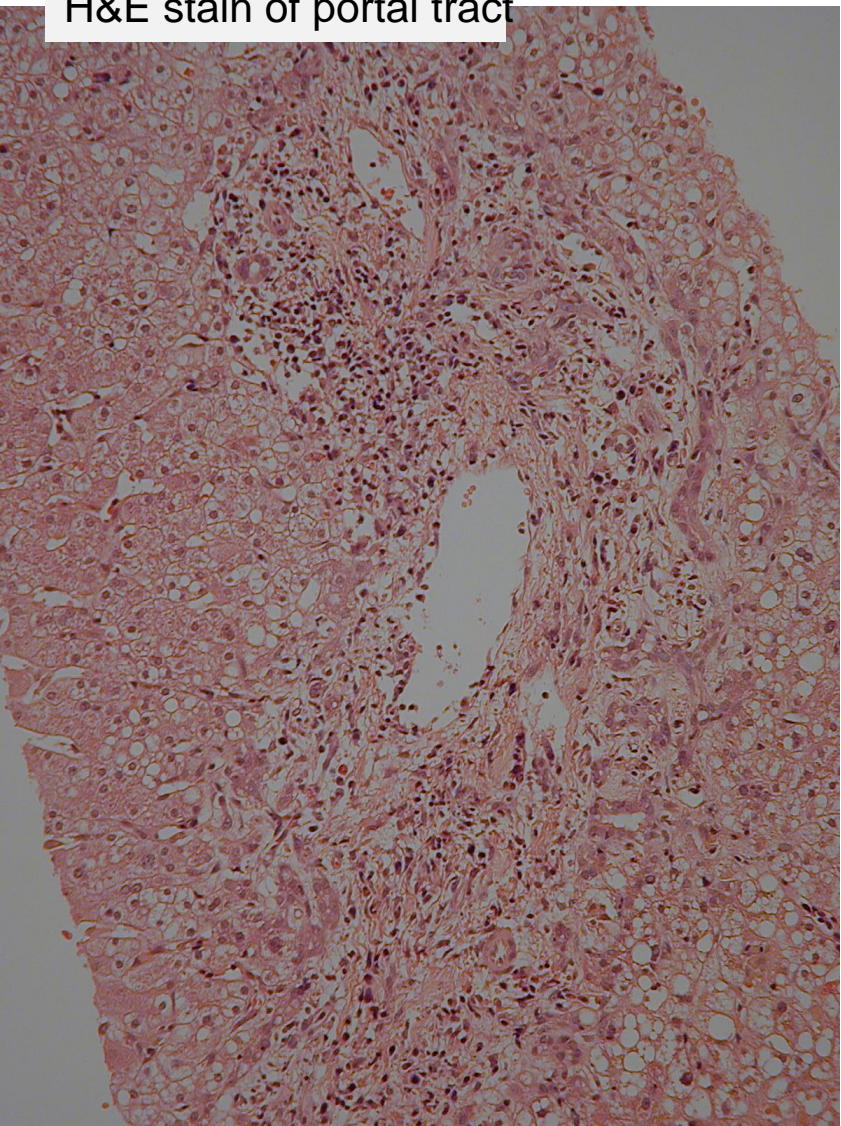
Donor Specific Antigen Titres
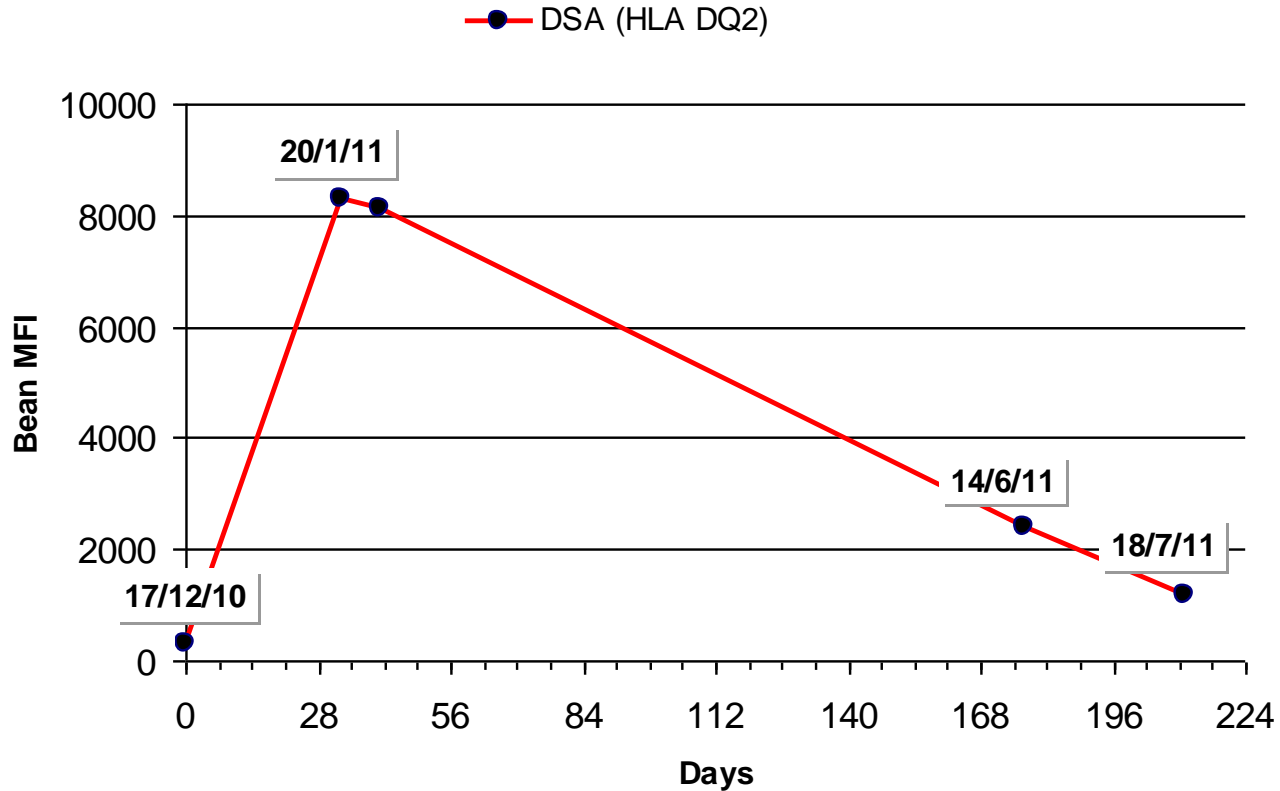
Biopsy: 25 weeks post OLTx

Portal oedema and Ductular reaction
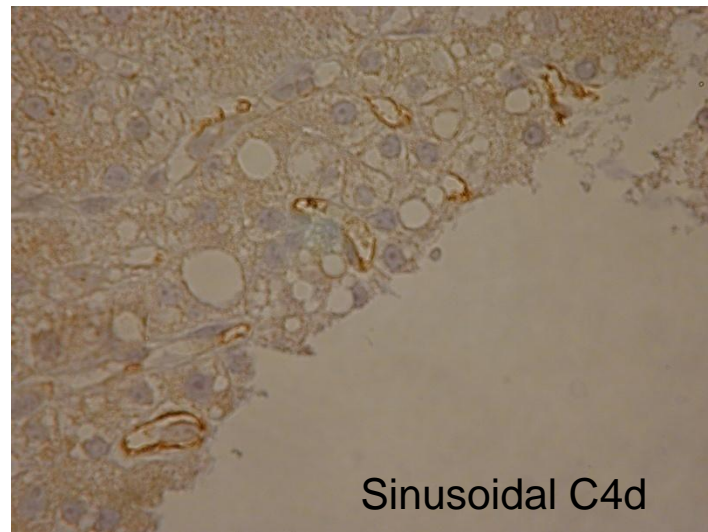

Portal C4d
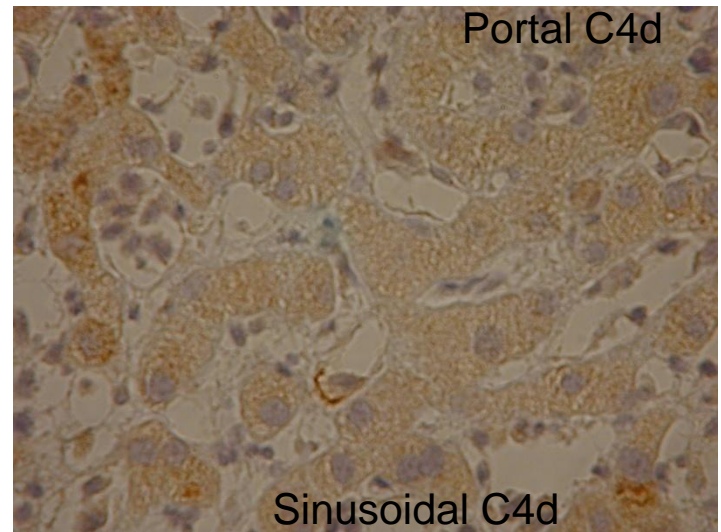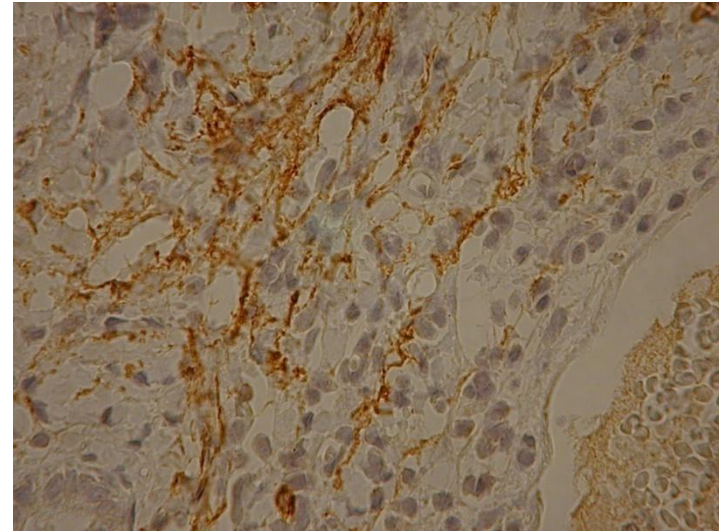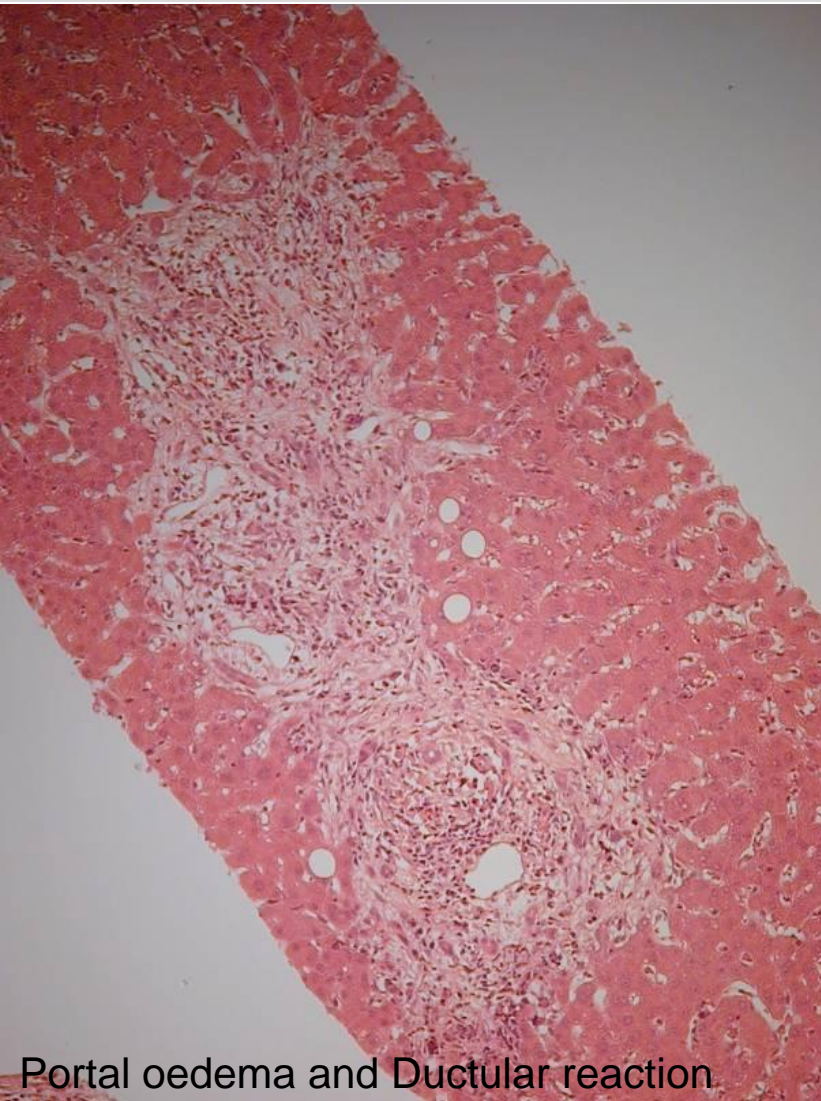
Sinusoidal C4d

Biopsy: 1ˢᵗ Episode of Acute Rejection
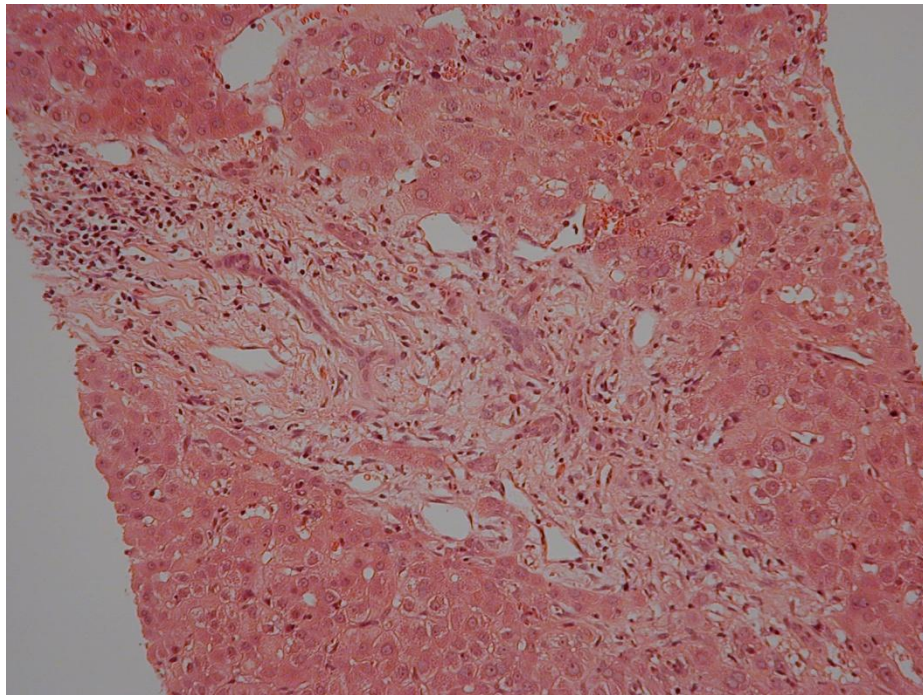

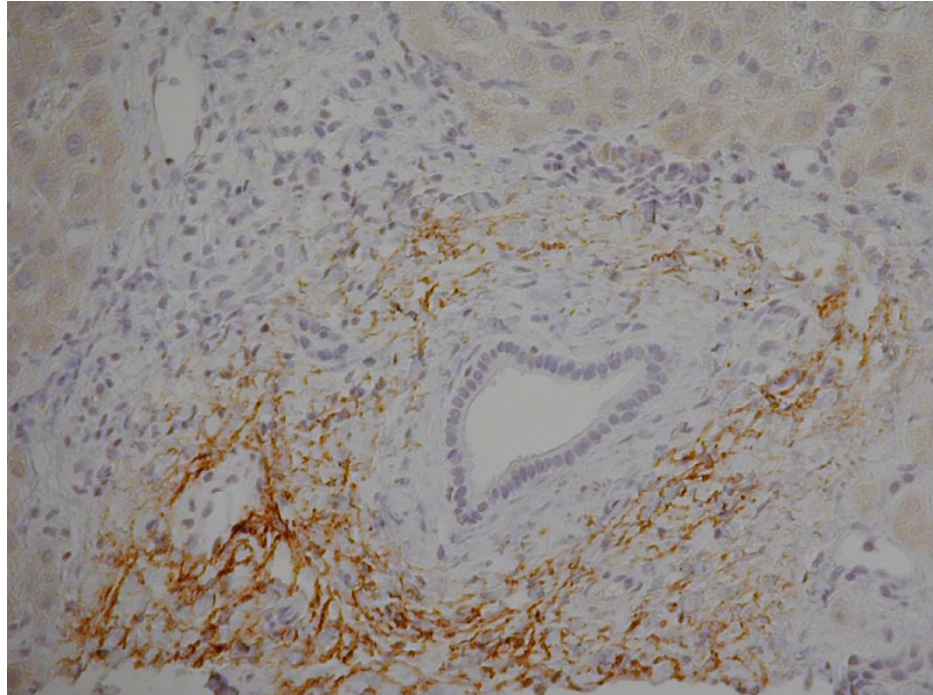H&E stain of portal tract

Portal and periportal C4d

Sinusoidal C4d

Biopsy: 9 months post OLTx

Portal tract less inflammed

Stromal C4d staining / peribilary

DSA, AMR and Liver Transplants (O'Leary et al, 2015)

- Role of DSA remains uncertain
- Variations in technology such as testing, titers,
- Diagnostic criteria not clear
- DSA probably do cause graft damage in liver recipients
- DSA may be associated with ductopenia, biliary strictures, plasma cell hepatitis, accelerated fibrosis
- However, the diagnosis and treatment of AMR remains unclear

However

- Iacob (2015) undertook a cross-sectional study of 174 prospectively studied liver allograft recipients

- Association between endothelial C3d deposition and Class II DSA (p<0.001)

- Patients with C3d deposition
  - 4.3 greater risk of graft failure
  - Shorter time to graft failure (95 vs 176m)

De novo AIH

- Initially described in children but seen in adults (<1%) developing 4 months – 10 years post OLT
- Features of AIH associated with LKM, ANA or SMA and high IgG with interface hepatitis.
- Variable response to corticosteroids; may progress to graft loss
- Autoimmune or alloimmune – effect of isotypes of Glutathione-S-transferase
- Graft dysfunction mimicking autoimmune hepatitis (Heneghan et al, 2001)
- Plasma cell hepatitis (Demetris 2008)

Plasma cell hepatitis

- Common features of AIH and plasma cell hepatitis
  - Autoantibody production
  - Steroid responsiveness
  - Plasma cell-rich necroinflammatory activity
- But differences include
  - Less female preponderance
  - Lower AST/ALT
  - More IgG4+ cells
  - More severe central perivenulitis
  - More centrilobular necrosis and plasma cells

Autoimmunity or rejection?

- Atypical antibodies may be the target: Aguilera described antibodies to GSTT1 which was present in donor liver but not recipient
- Other examples include
  - Antibodies to CK 8/18
  - Carbonic anhydrase
  - BSEP proteins
- Is PCH overlap of auto and alloimmunity

Unanswered questions

- What does CPH represent
- Should it be treated
- If so, how
  - Increased immunosuppression
  - Addition of steroids or others
- What is the role of protocol biopsies
  - Risks and benefits
  - When?

Conclusions

- As survival rates improve, very long term outcomes are becoming increasingly important

- There are many causes of premature graft loss including recurrent disease, de novo disease (viral, immune mediated), alcohol and NAFLD

- Understanding of graft histology is limited by
  - Variable use of histology
  - Confusion in terminology
  - Uncertainty over interpretation of clinical, serological and histological findings