



# C4d in liver allografts

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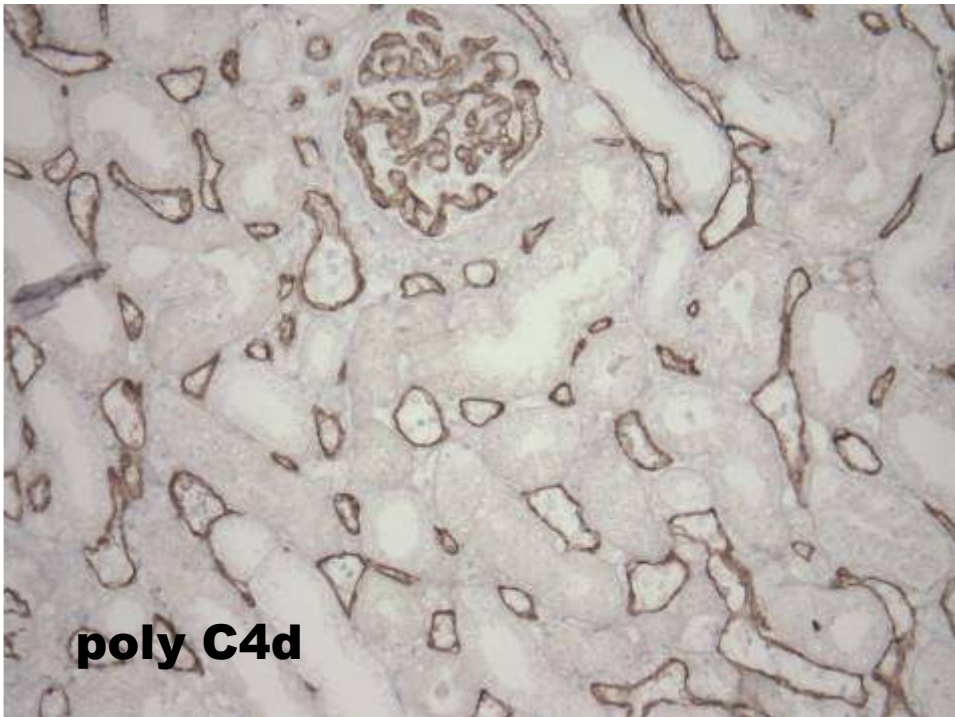
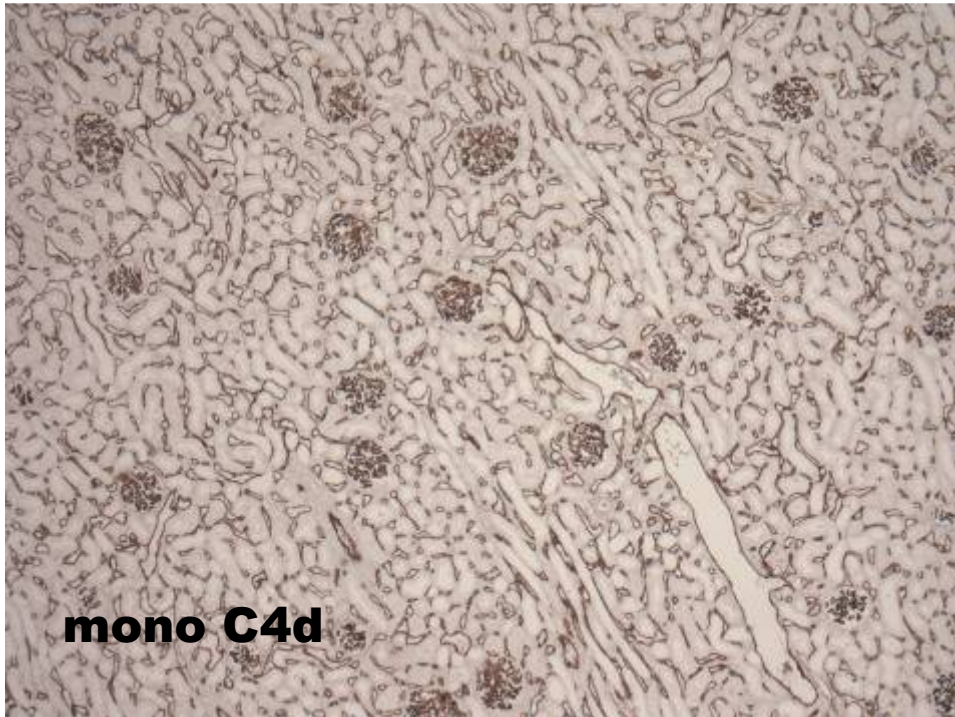
Chris Bellamy

# Background

- Andres, 1972
  - humoral factors ...“less important”
- Hyperacute rejection (ABOI)
  - Demetris 1988, Gordon 1986, Gugenheim 1990
- Preformed antidonor lymphocytotoxic IgG
  - Starzl 1979, Hanto 1978, Bird 1989, Demetris 1992
  - Ratner 1993, Rostron 2005
- C4d deposition
  - Watson 2006, Sakashita 2007
  - Moeller 2005, Krukemeyer 2004, Sawada 2004, Dankof 2005, Schmeding 2006, Jain 2006, Troxell 2006, Lorho 2006, Bu 2006, Soglio 2008, Silva 2009
  - can C4d uncover smouldering humoral rejection?

# C4d

- Marks C'-dependent Ab deposition
  - durable: several days
- ABO-compatible renal allografts
  - capillary C4d ~ Ab-mediated rejection
  - chronic cases are intermittently positive
- Frozen > paraffin
- polyclonal; monoclonal



# Rejection of ABO-compatible grafts: pilot

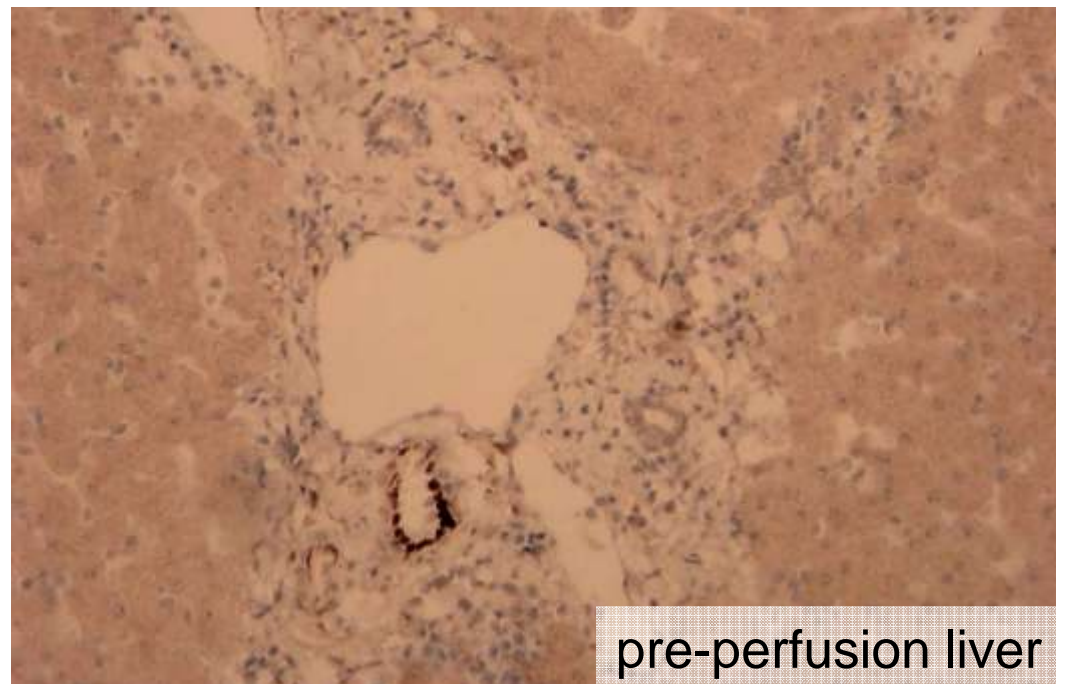
- 129 protocol day 7 biopsies
- 7 suspicious for humoral rejection (H&E)
  - criteria of Demetris 1992 (Hepatology 16:671-81)
    - marked ductular reaction, neutrophilia, oedema, sludging, platelets, endothelial hypertrophy & detachment, portal ectasia with mononuclear\neutrophil accumulation, arterial mural injury.
  - 2 of 7 were C4d-positive

# Study set (ABO compatible)

- 10
  - pre-perfusion
- 10
  - consecutive protocol 7 day bx
- 12
  - T-lymphocytotoxic crossmatch +ve, day 7
- 15
  - primary non-function
- 16
  - mod-severe early acr
  
- 14
  - centrilobular necro-inflammation ( $\bar{x}$  299 d)
- 13
  - chronic rejection hepatectomies ( $\bar{x}$  280 d)
- 11
  - biliary complications (pre-repair) ( $\bar{x}$  565 d)
- 15
  - recurrent PBC ( $\bar{x}$  1438d)
- 9
  - recurrent auto-immune hepatitis

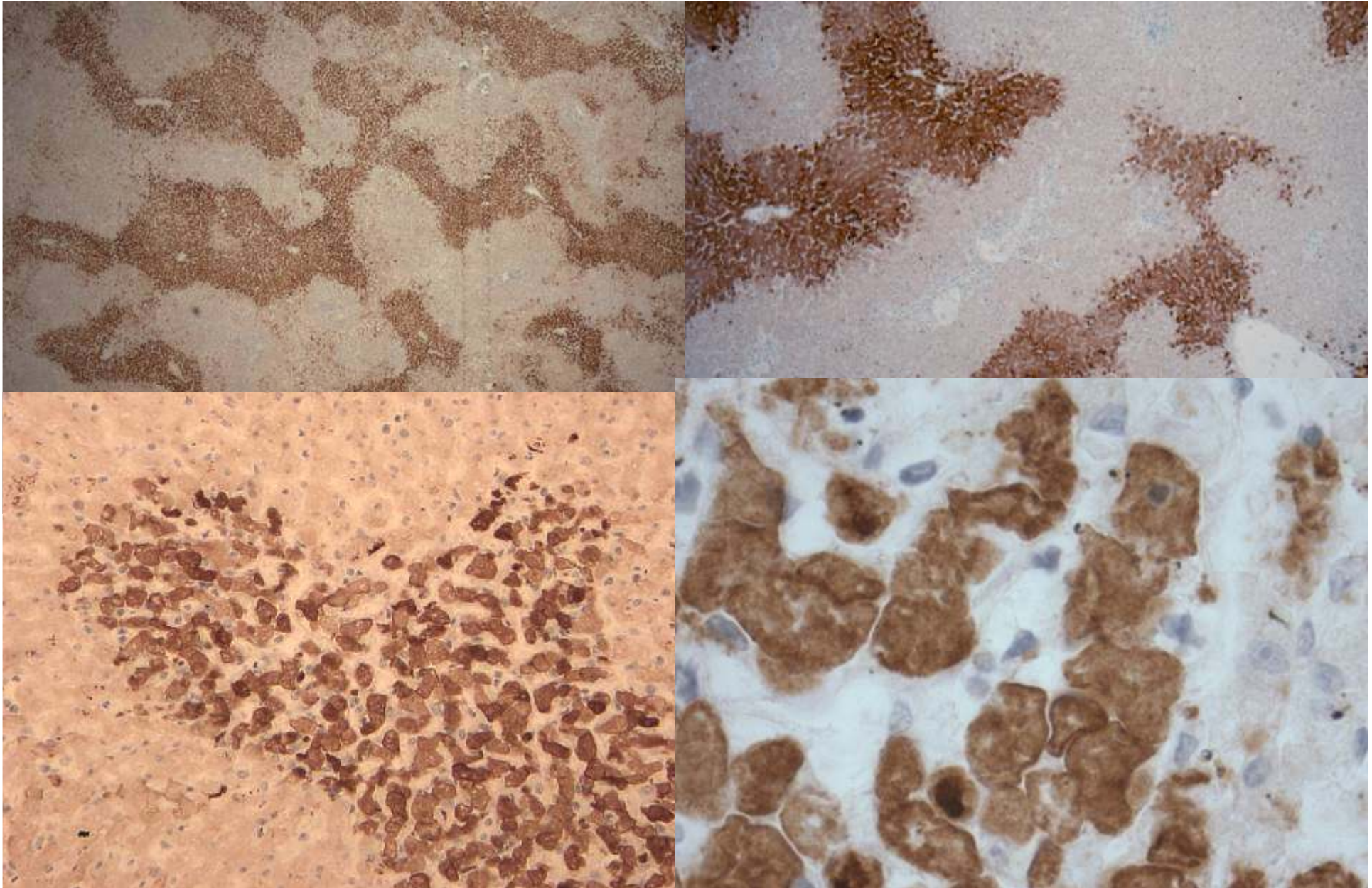
# 3 abnormal staining patterns

- hepatocellular cytoplasmic (not scored +)
- portal venules/capillaries
- lobular sinusoids

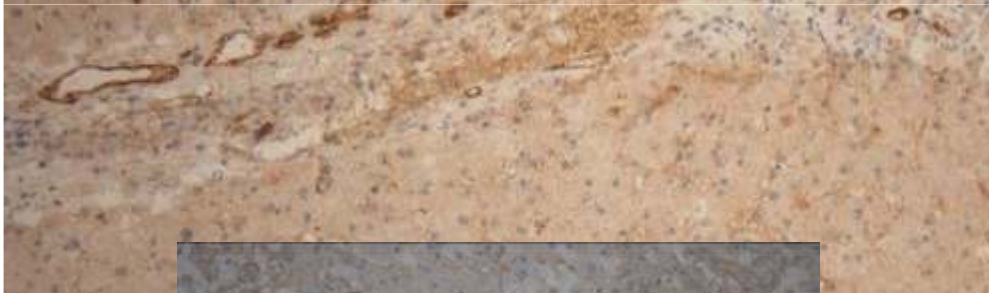
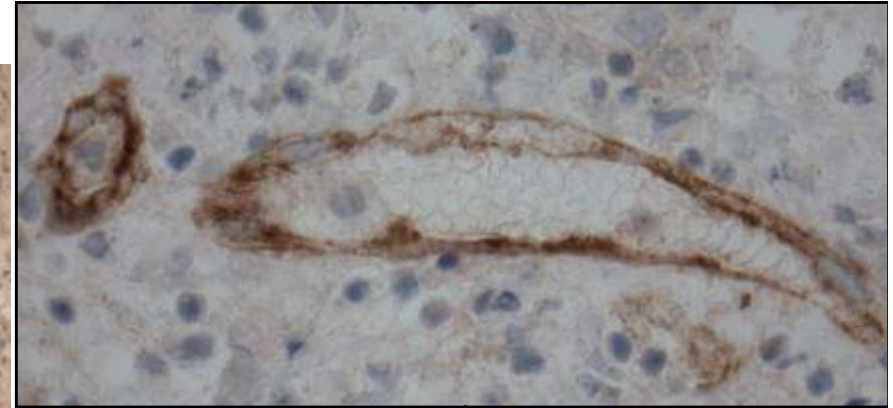
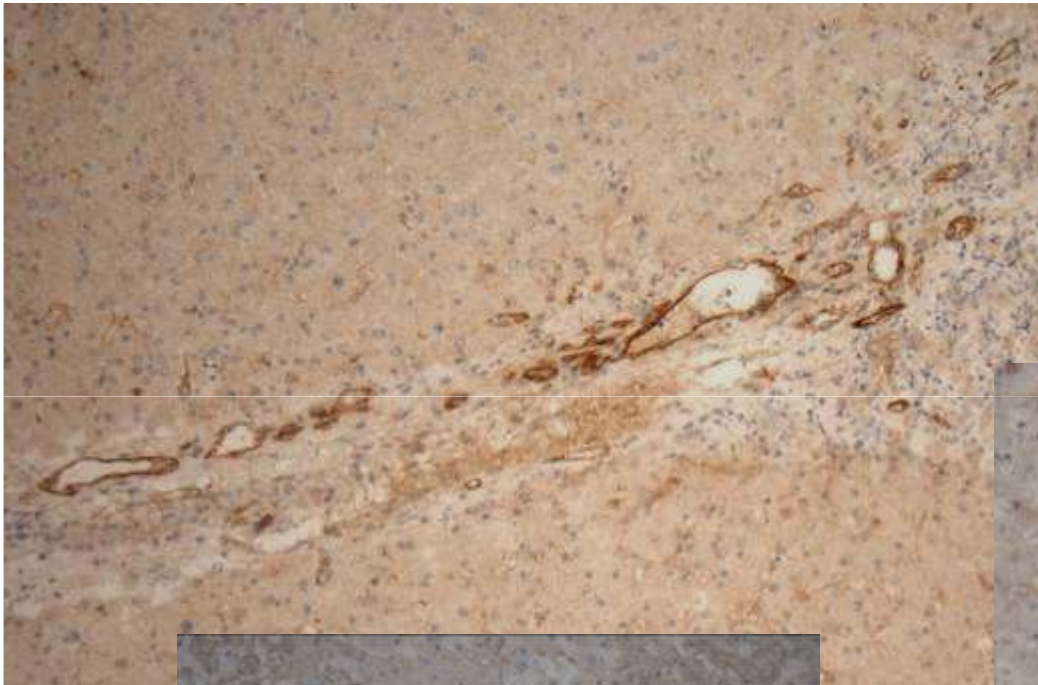


# 1. Hepatocellular staining in PRI

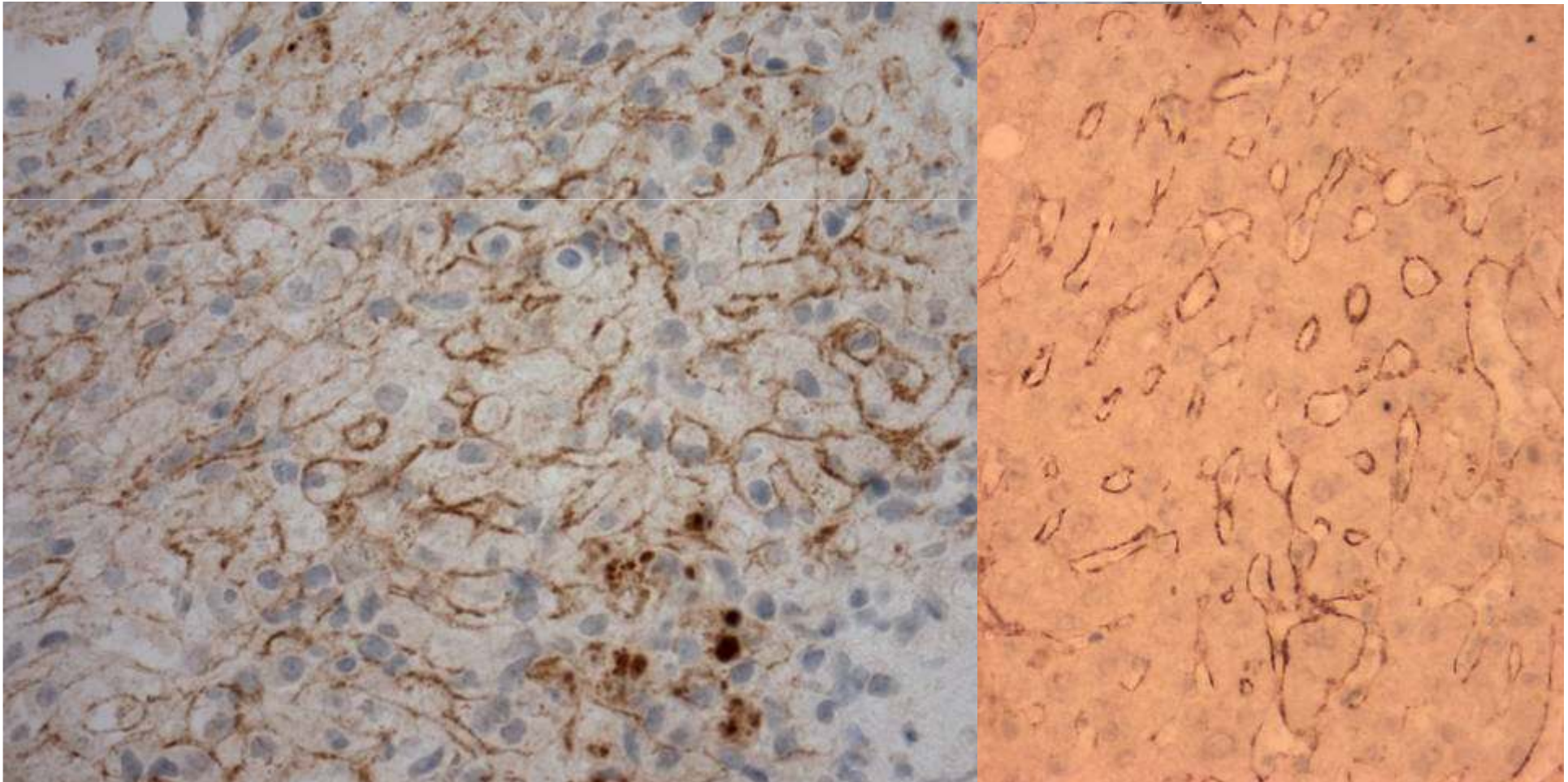
as Straatsburg 2000



## 2. Portal staining



# 3. Sinusoidal staining

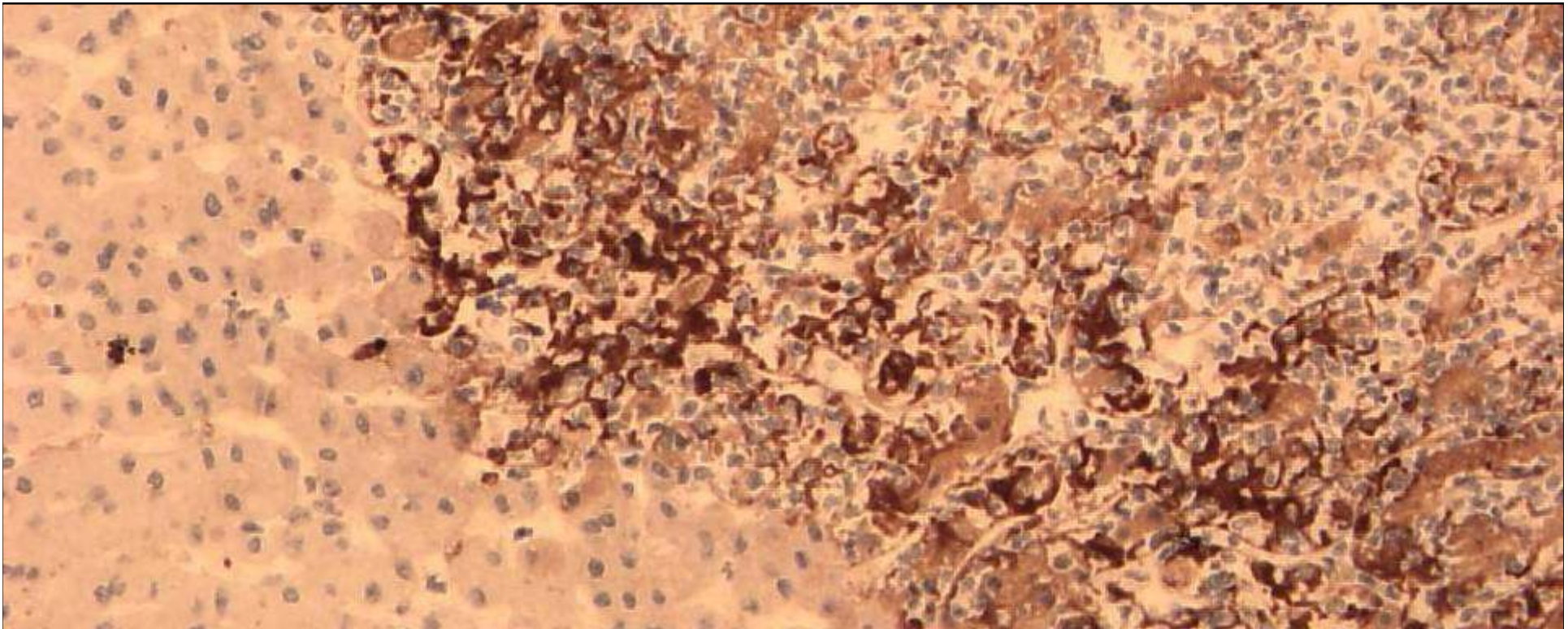


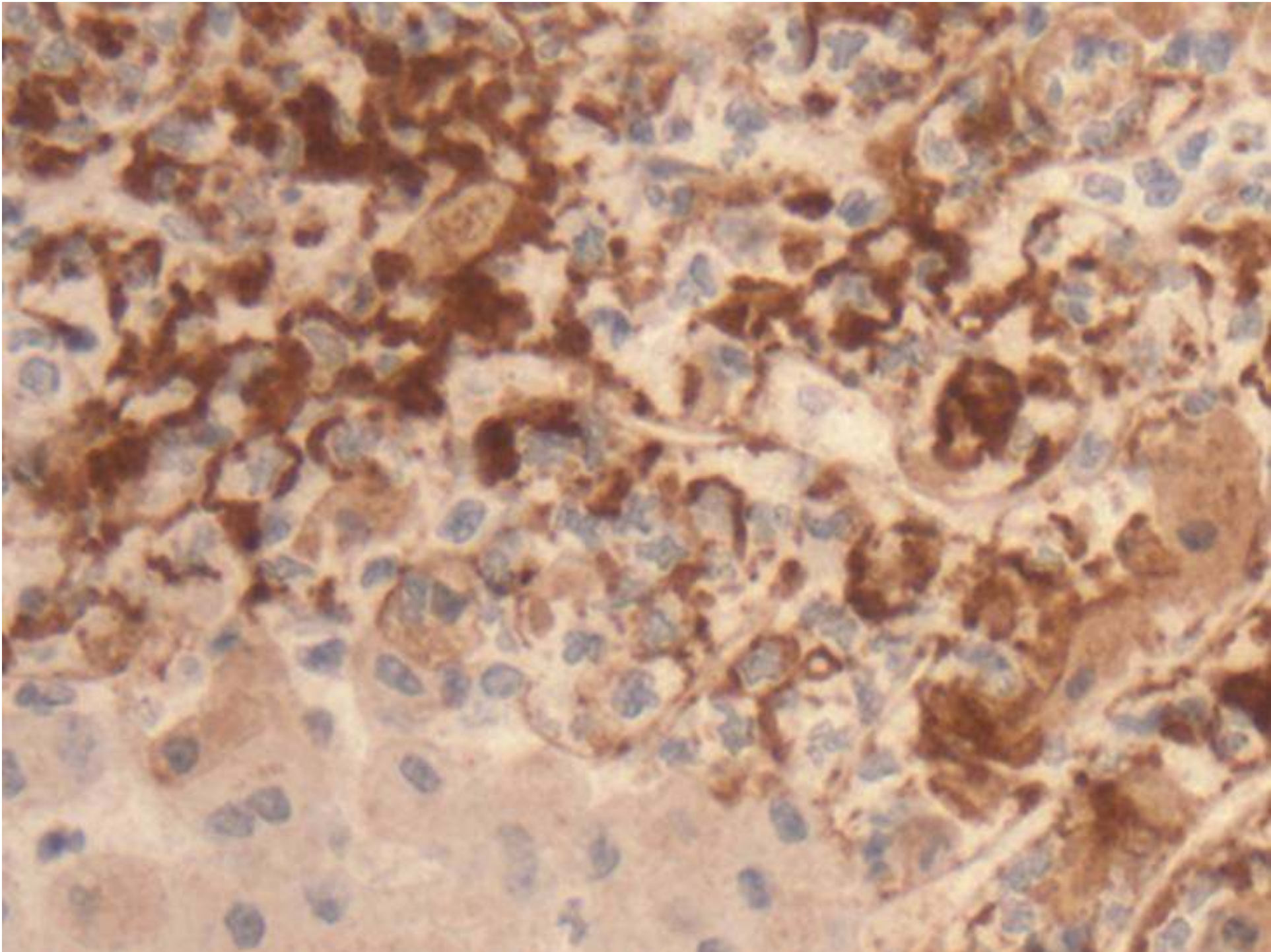
# Negative findings

- pre-perfusion (10)
- consecutive protocol day 7 (15)
  - acr (2 mod, 6 mild)
  - preservation injury (2 sev, 1 mod, 3 mild)
  - minimal abnormality (1)
- recurrent PBC (15)
- recurrent AIH (9)
  
- So, C4d positivity is uncommon...

# Primary non-function

- 1 of 10 C4d-positive
  - morphology: ? accelerated rejection (ABO-compatible, T-lymphocytotoxic crossmatch U/A)



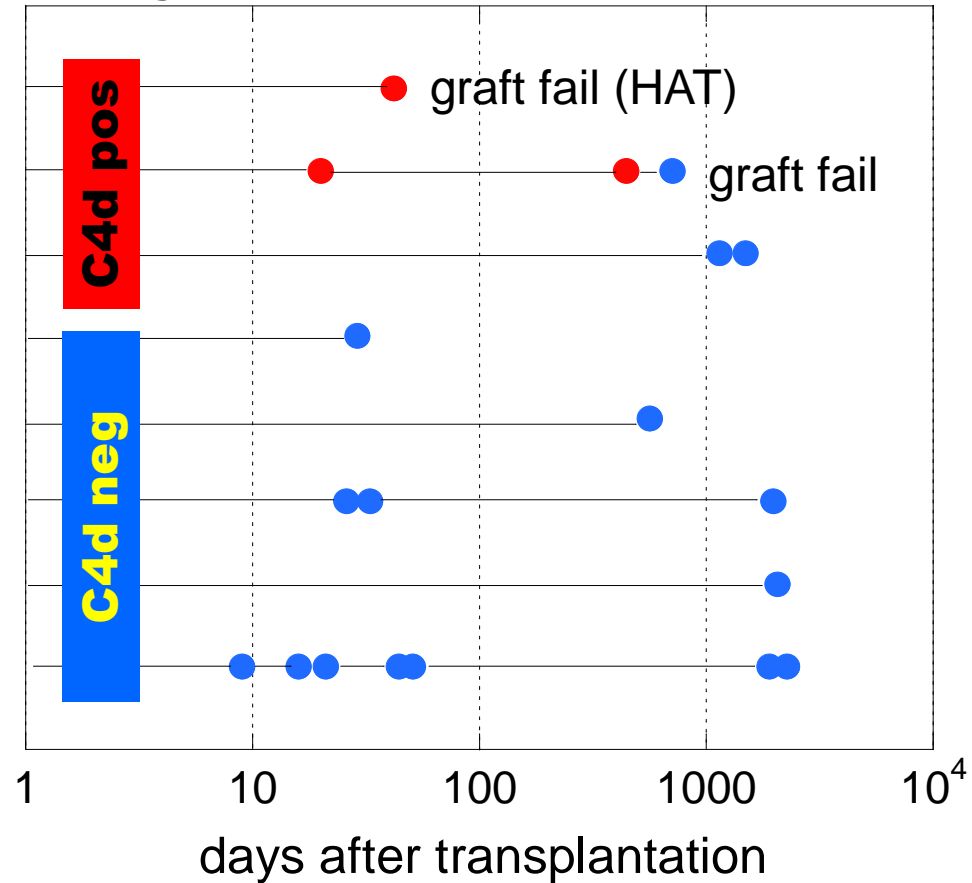


# Crossmatch positive patients

- 3 of 12 (25%) early protocol biopsies +ve
  - All treated for acr (steroids)
  - 2 rebiopsied within 5 weeks: +ve
  - Negatives remained so on repeat biopsies
- So, even in pre-sensitised patients, only 17% had repeated episodes (but had problems), representing <2% OLTx patients

# Crossmatch positive patients: follow-up biopsies

**protocol  
biopsy**

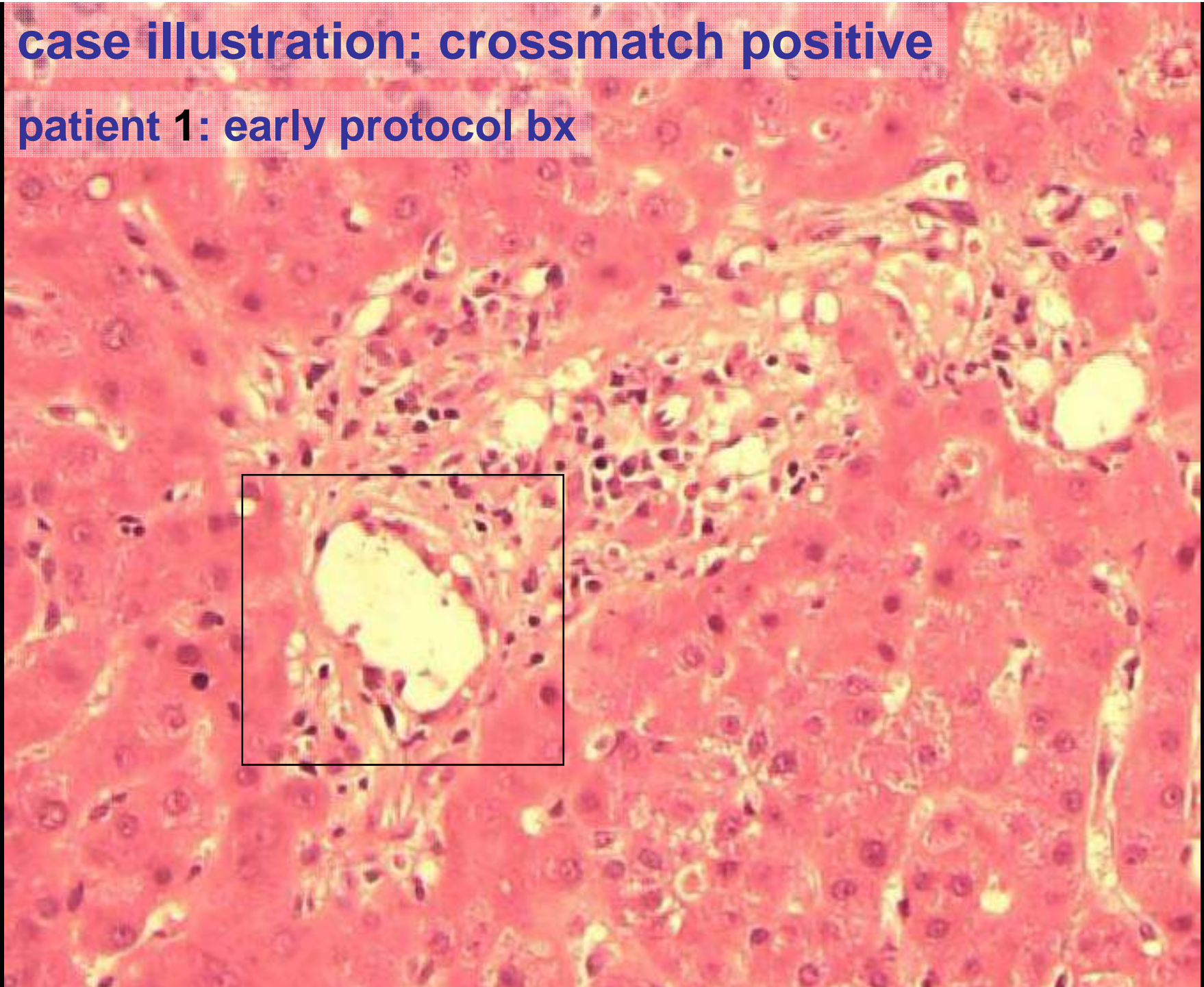


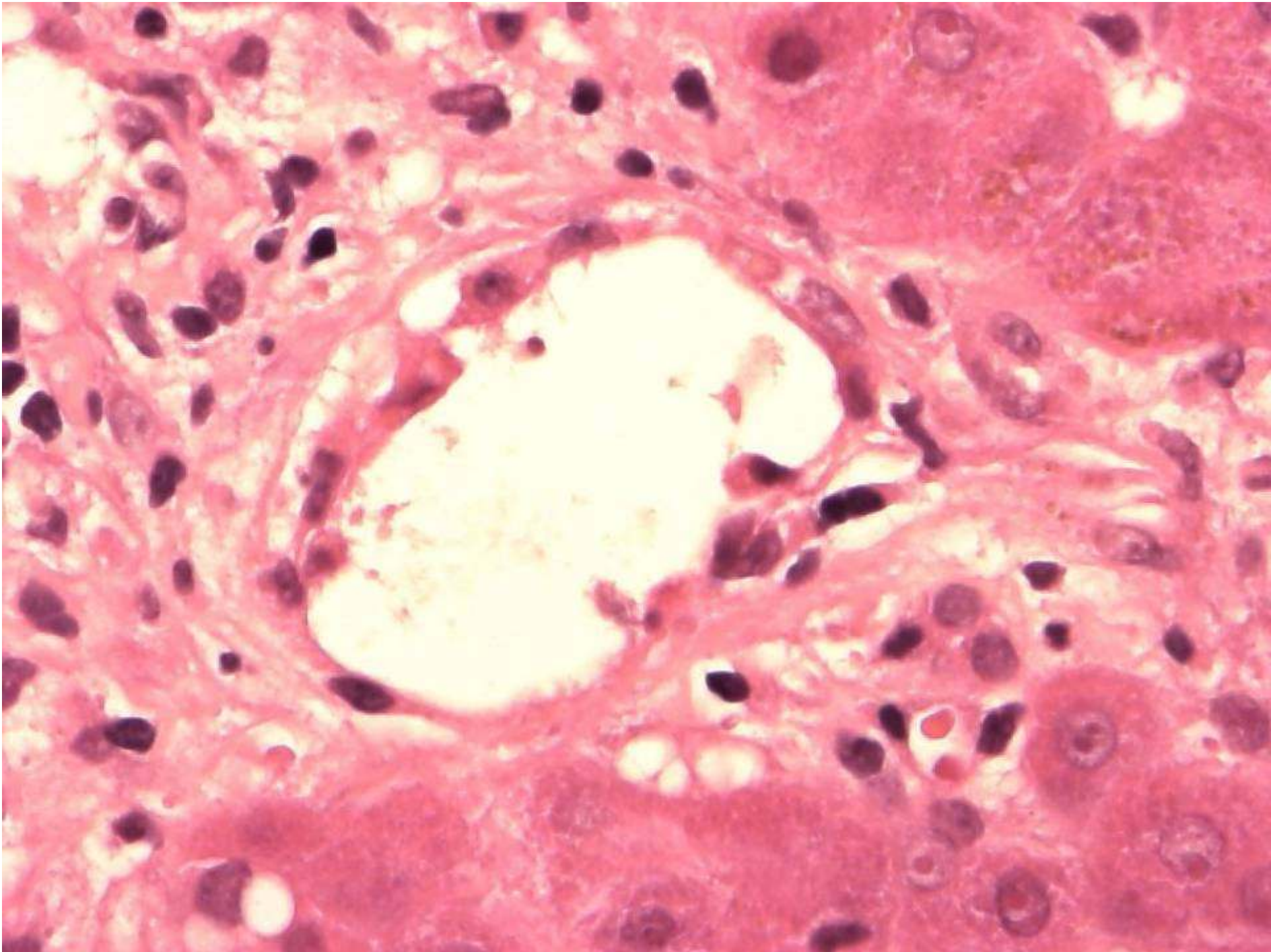
**follow-up biopsies (8 of 12 patients)**

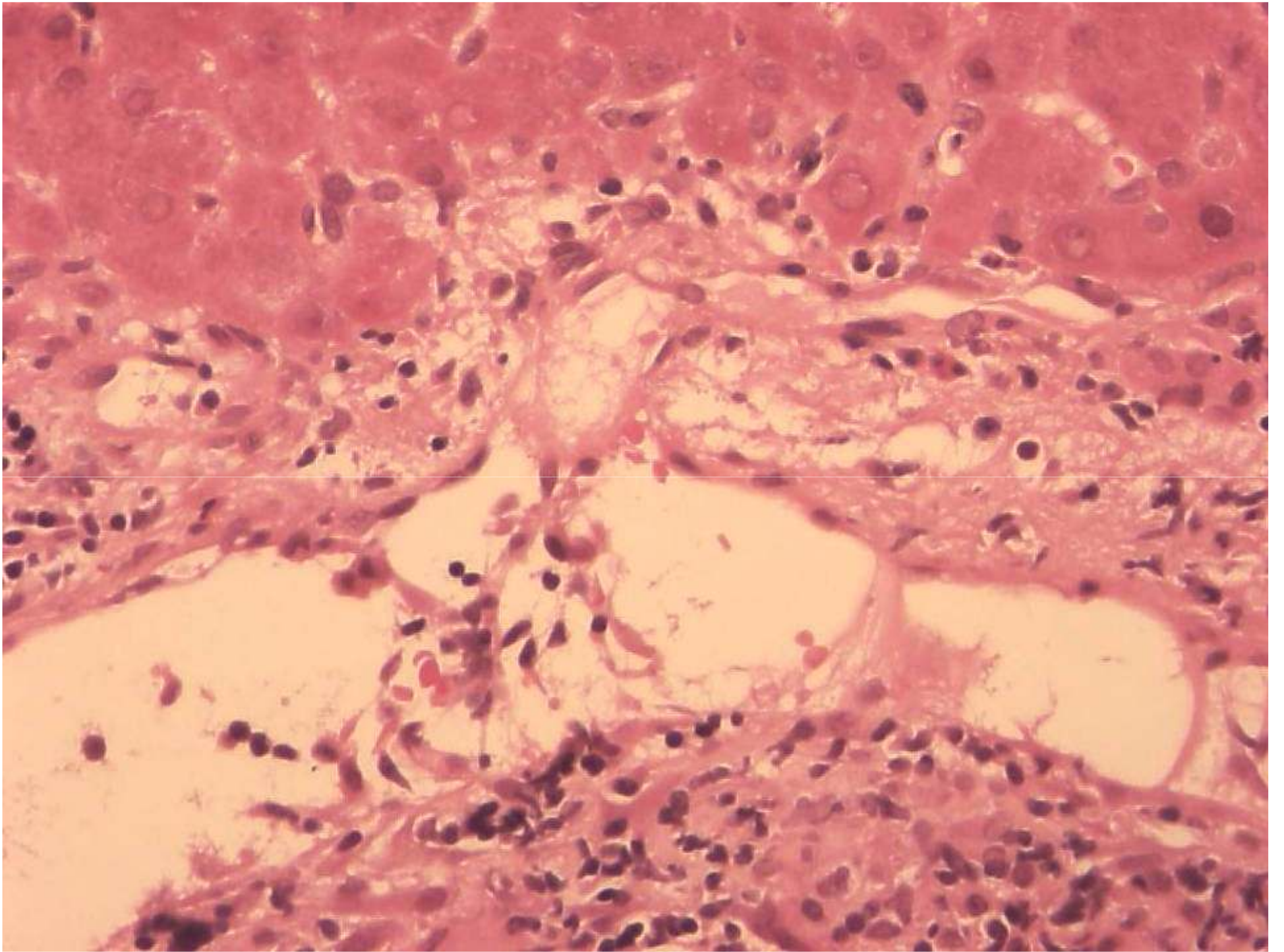
**● = C4d positive**

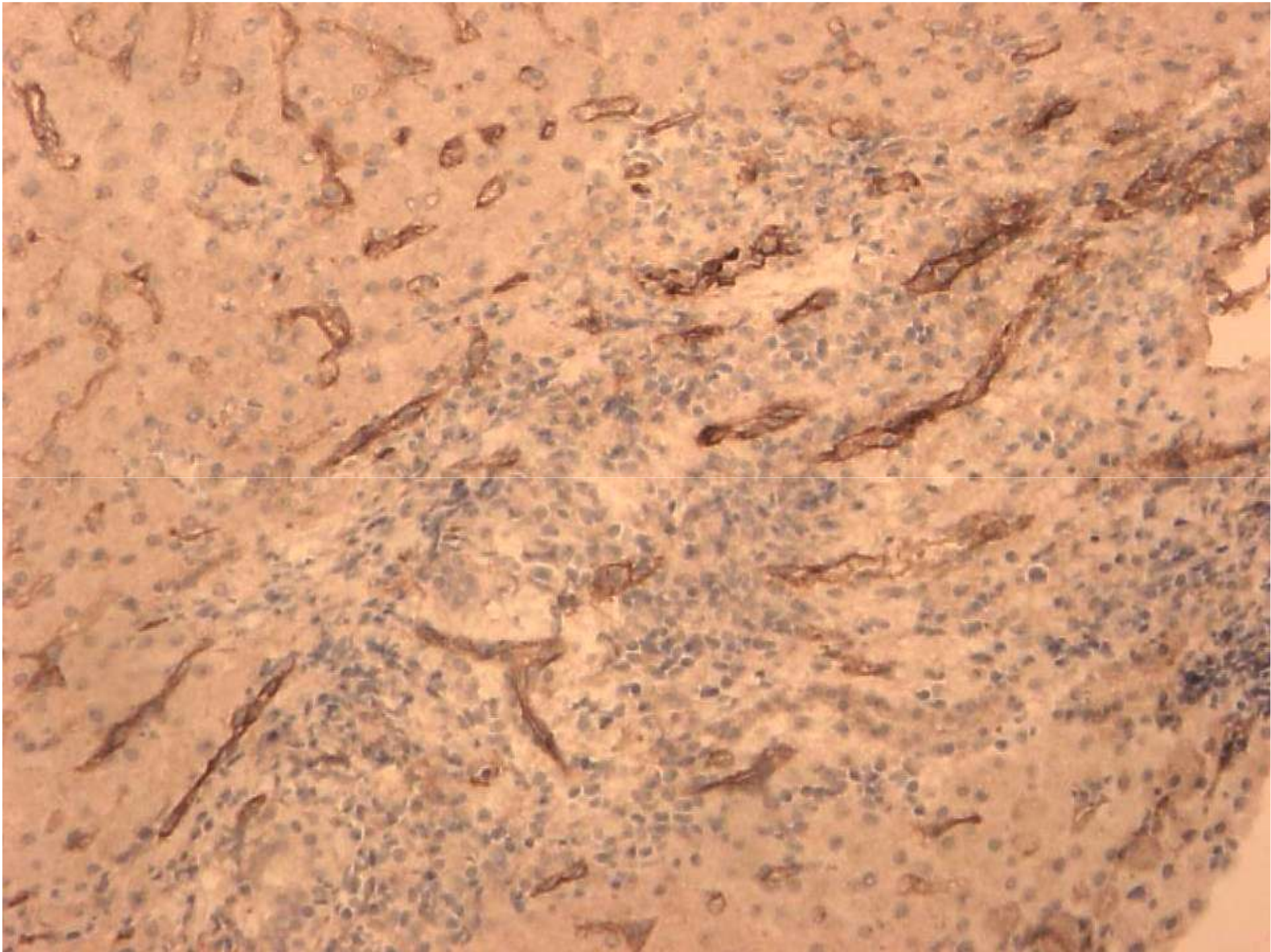
**case illustration: crossmatch positive**

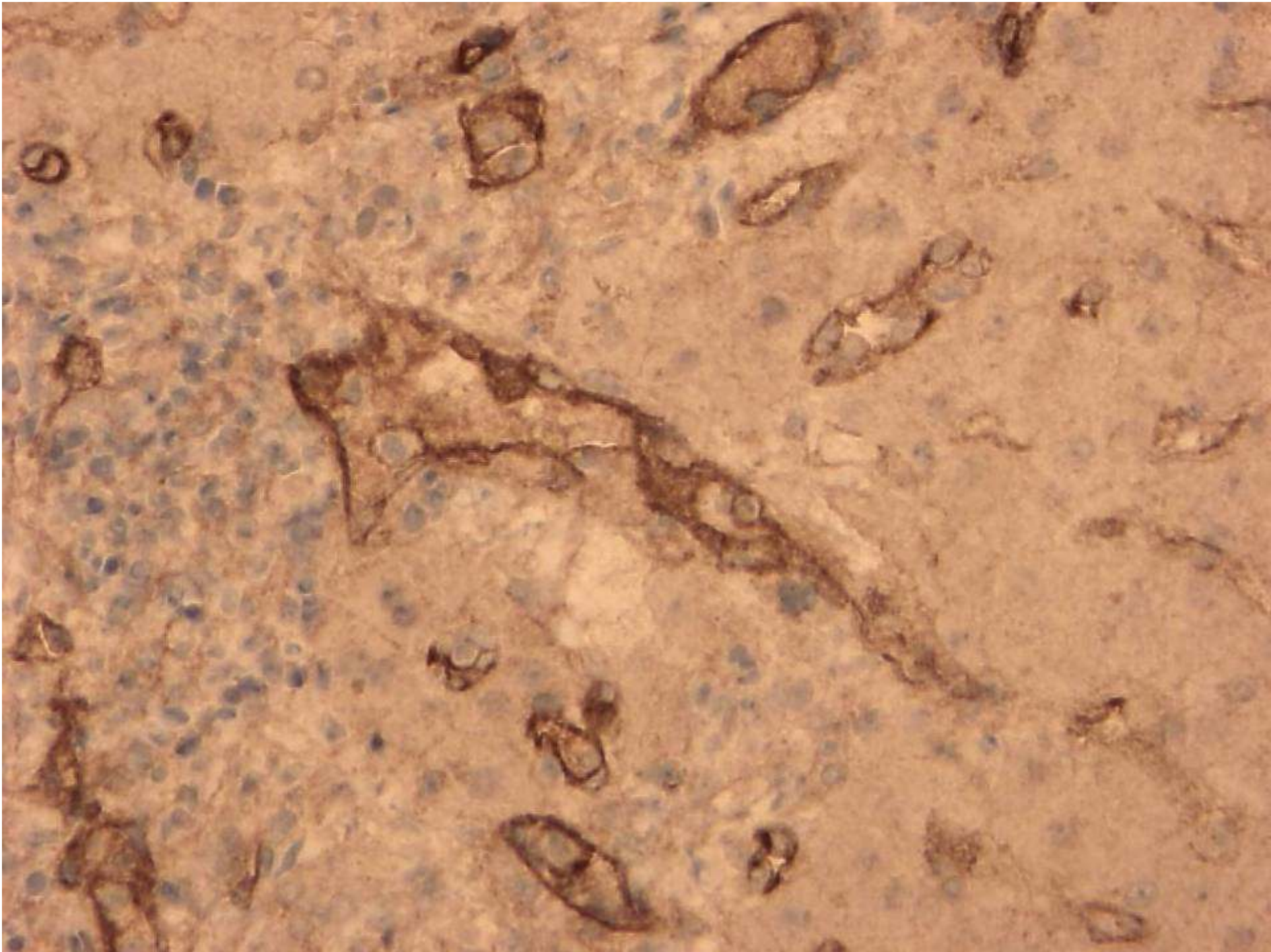
**patient 1: early protocol bx**



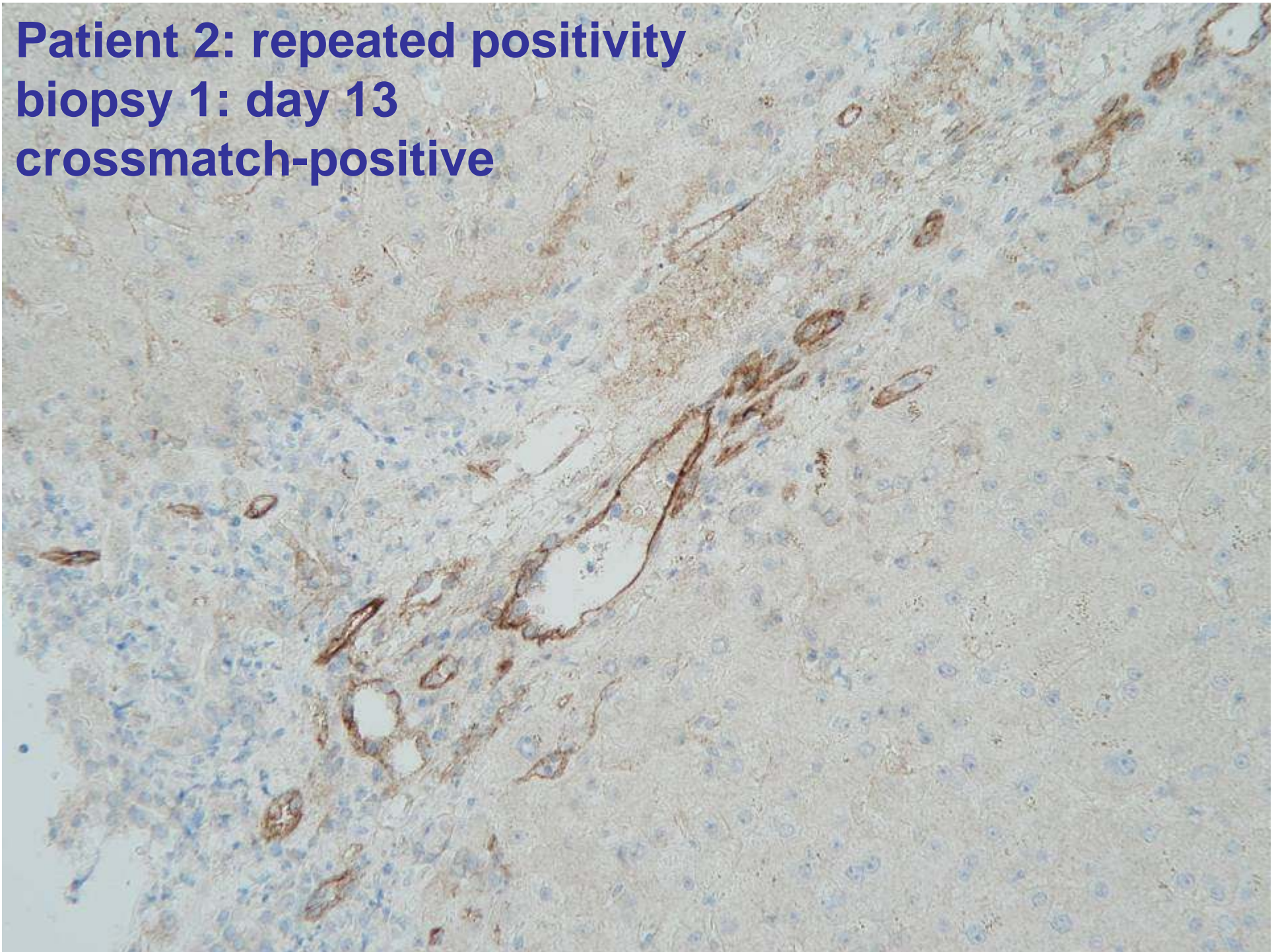


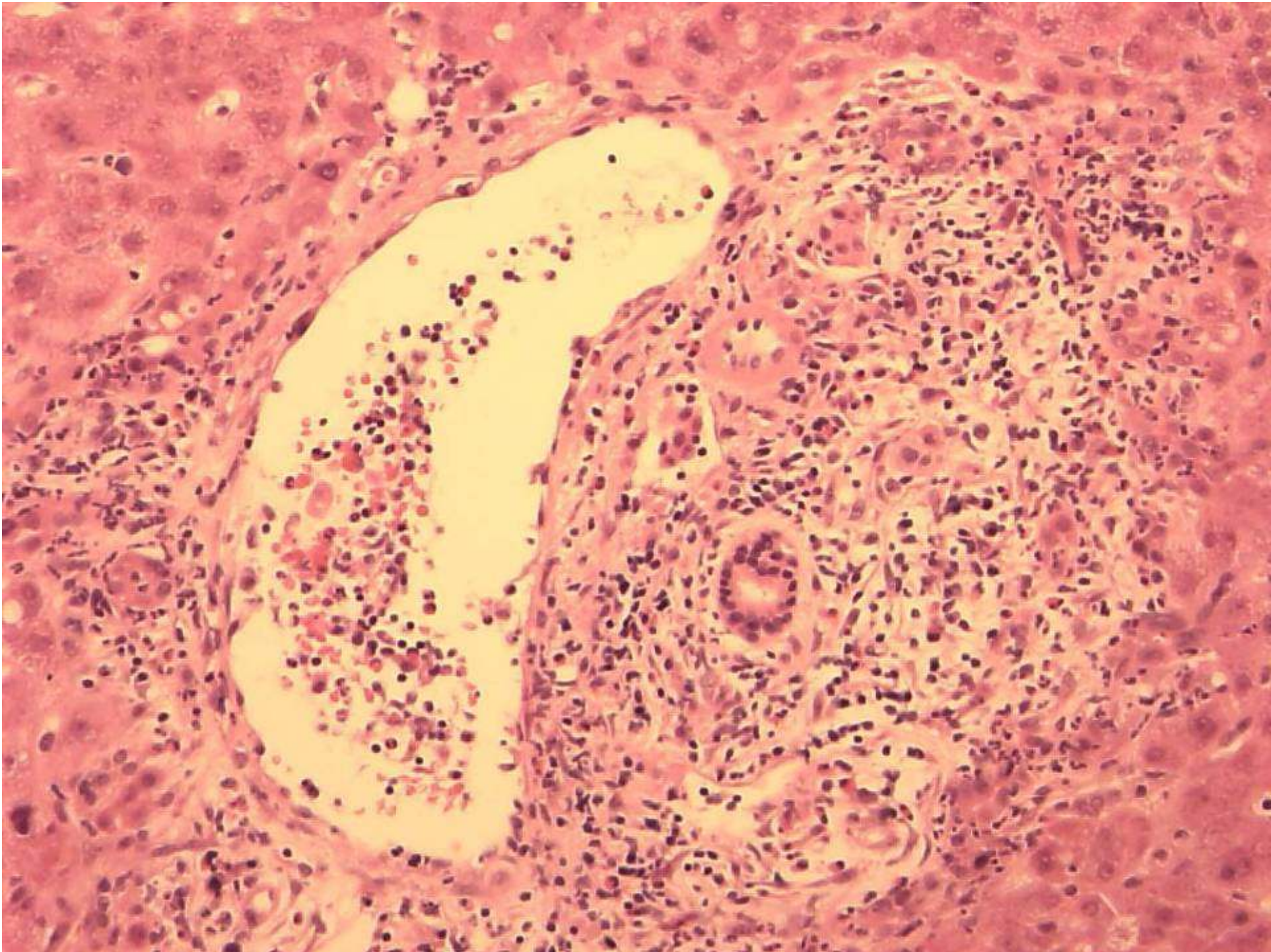


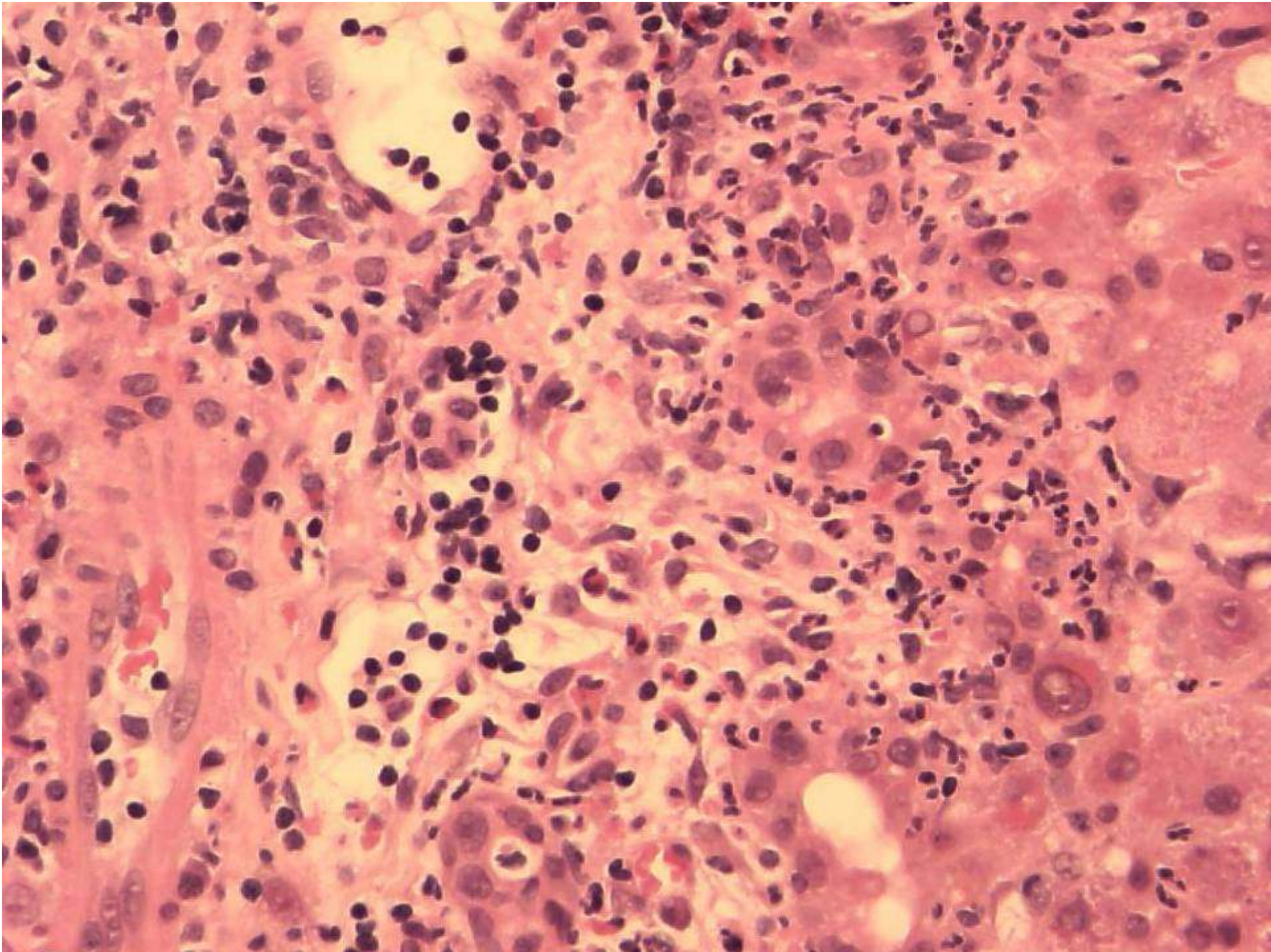




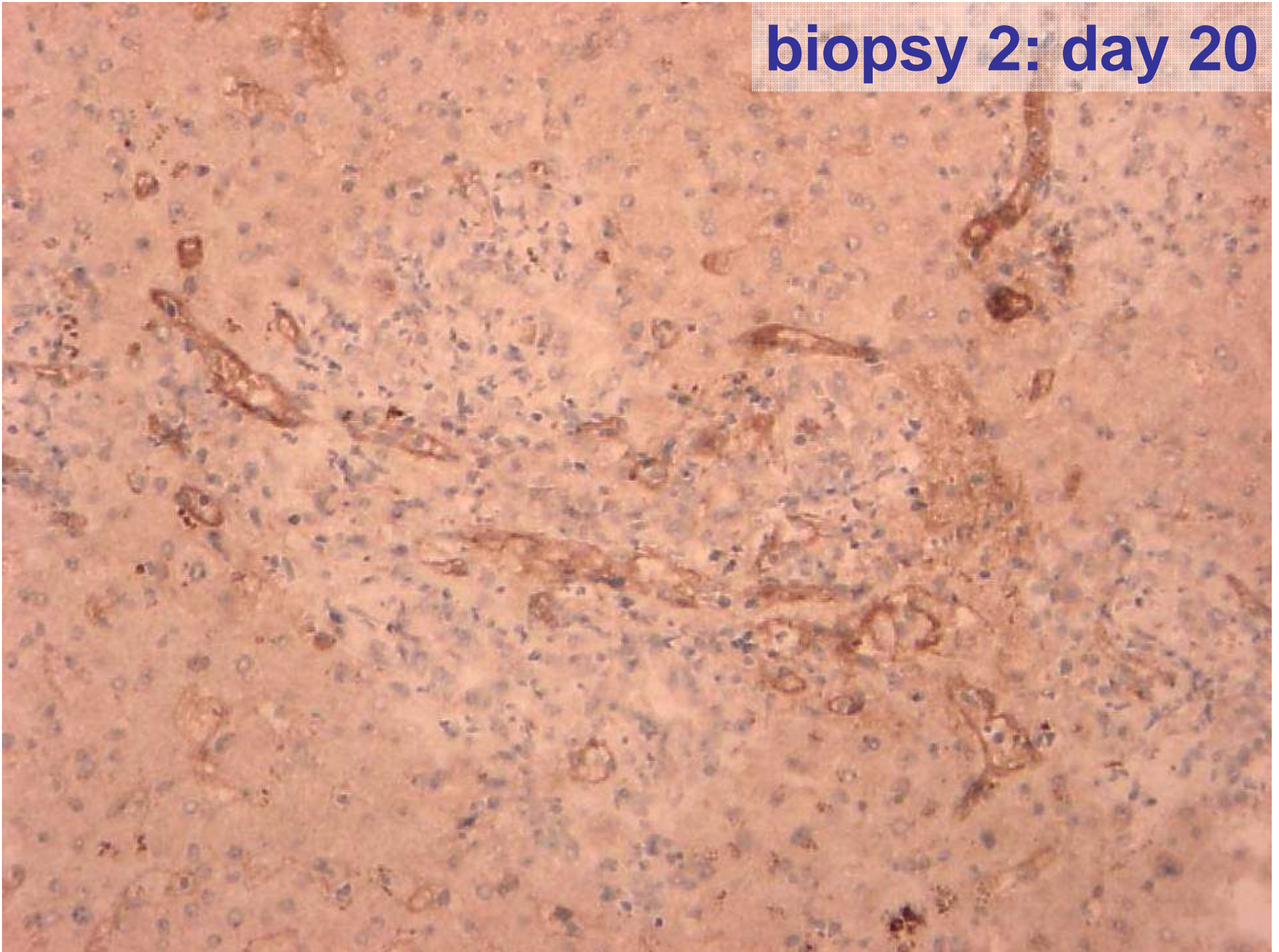
**Patient 2: repeated positivity**  
**biopsy 1: day 13**  
**crossmatch-positive**

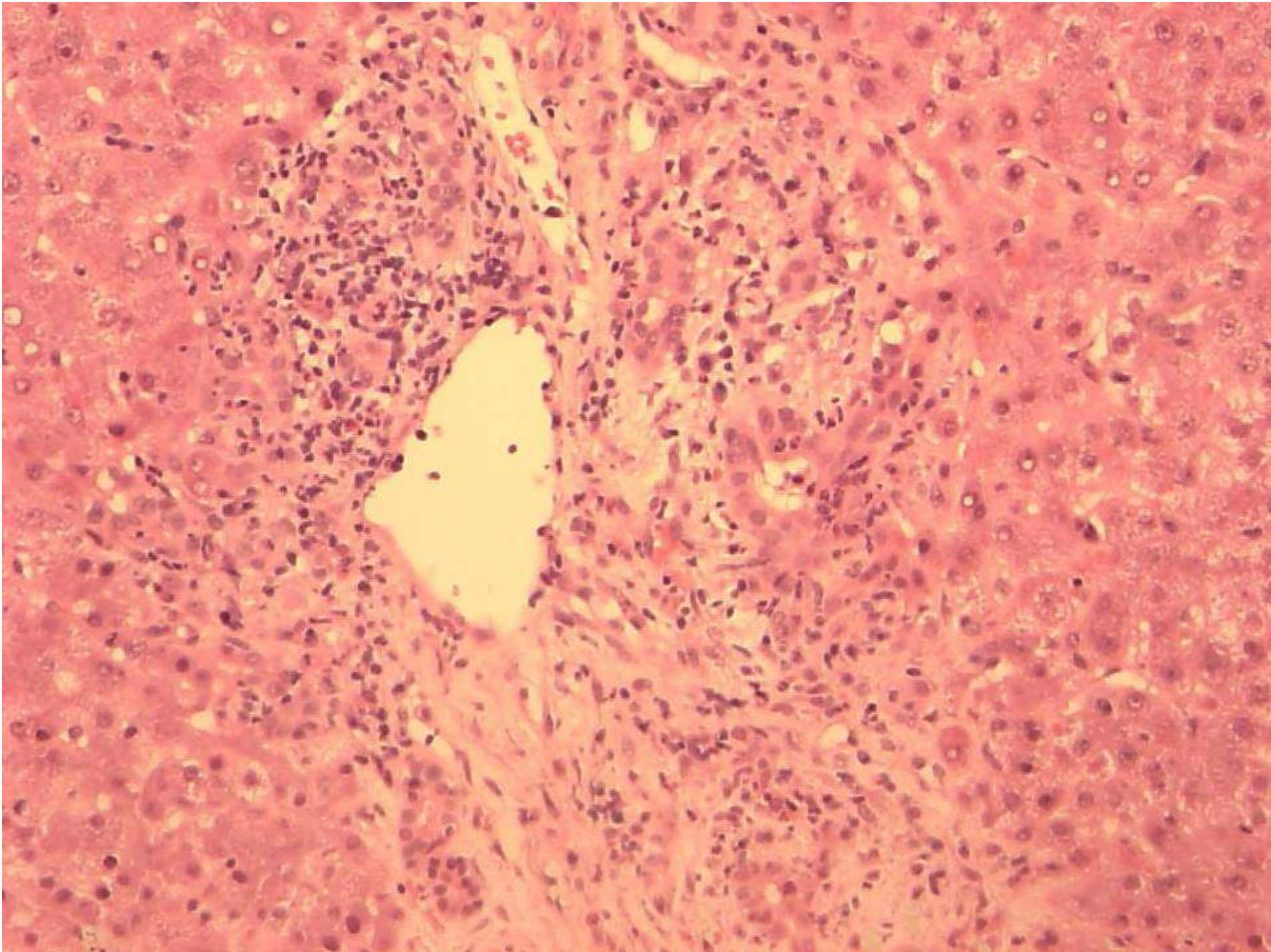


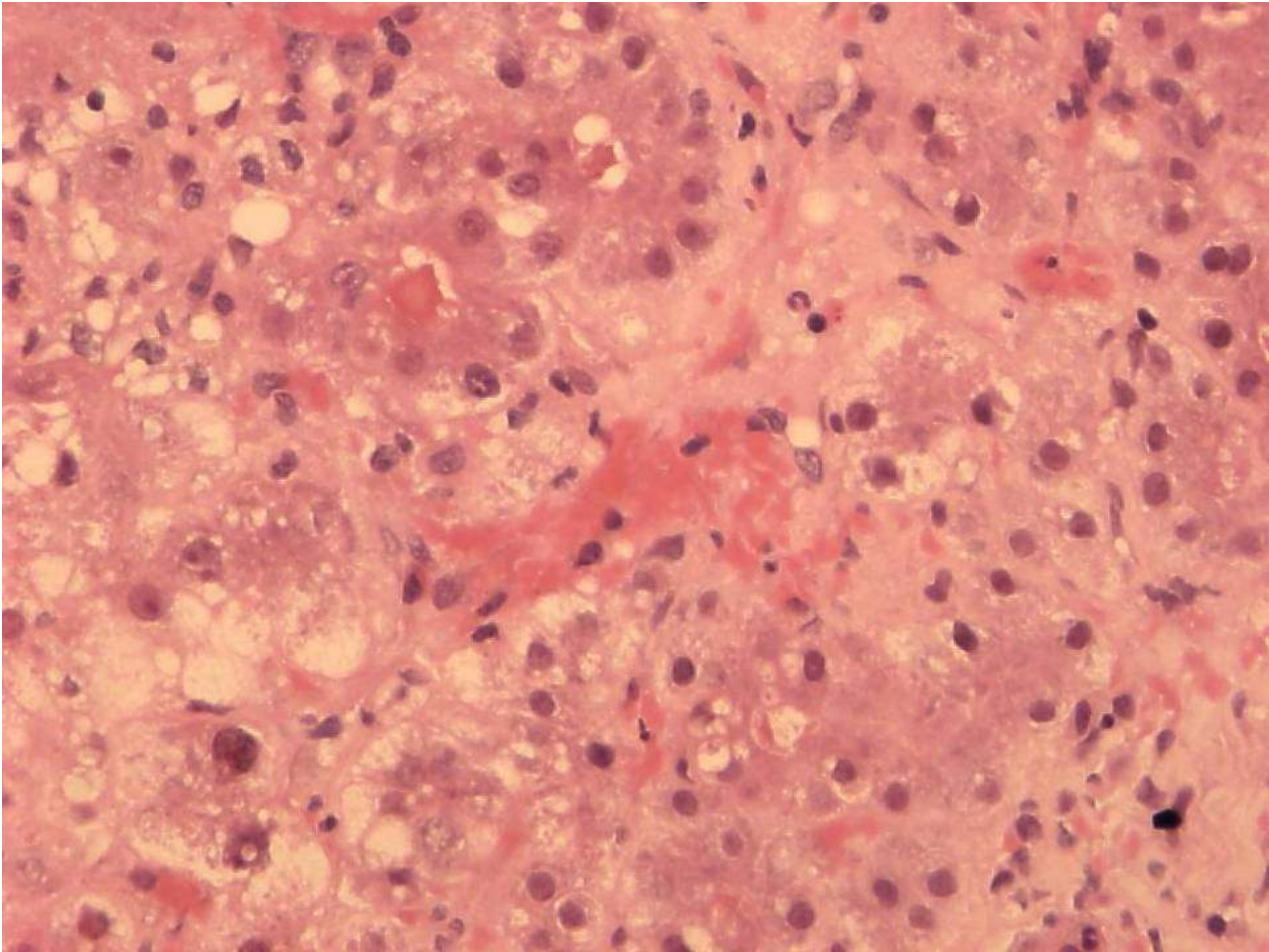




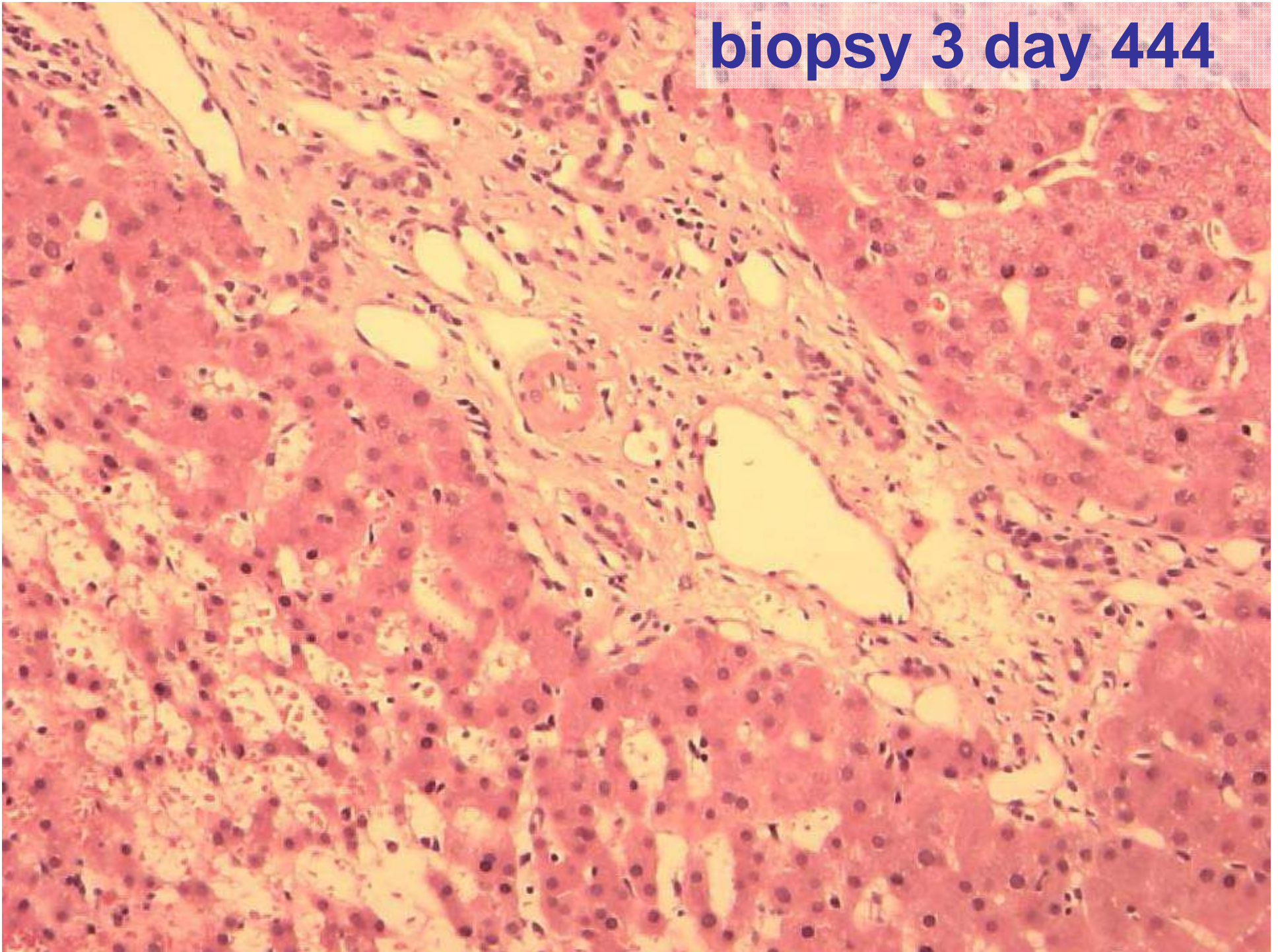
**biopsy 2: day 20**

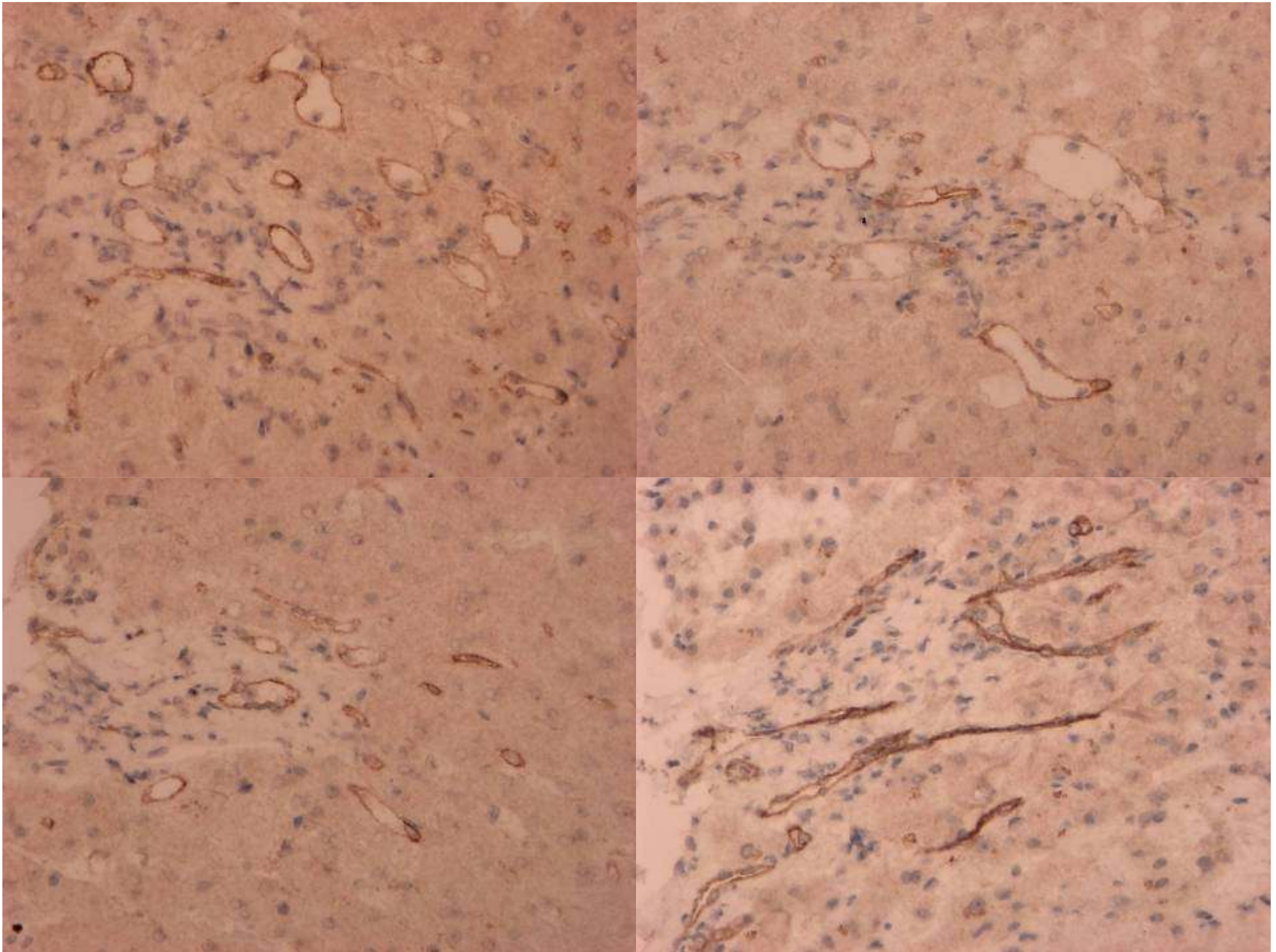






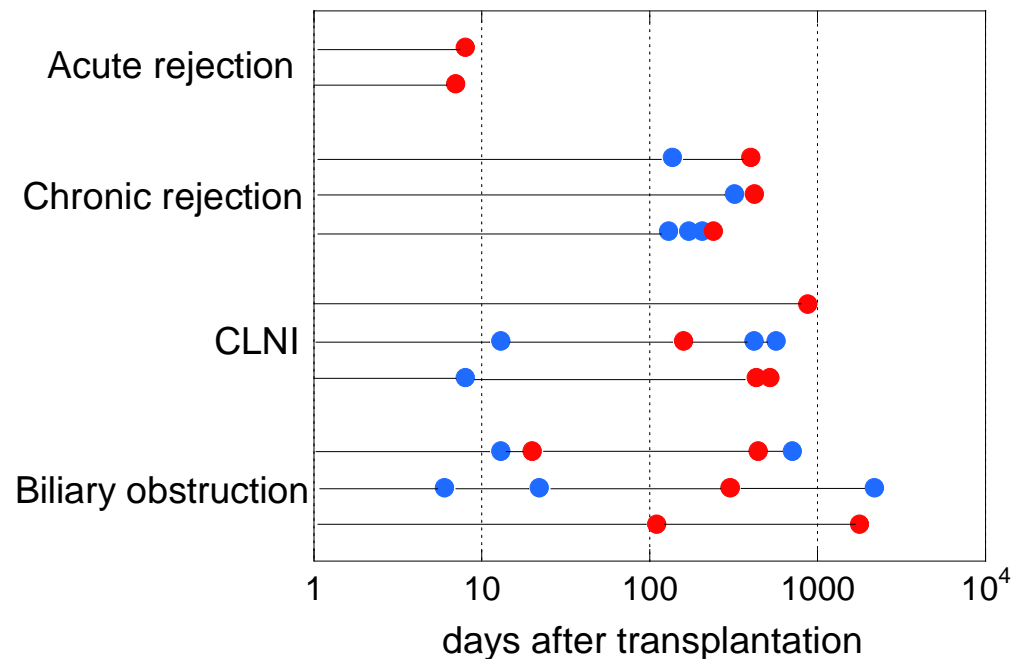
**biopsy 3 day 444**





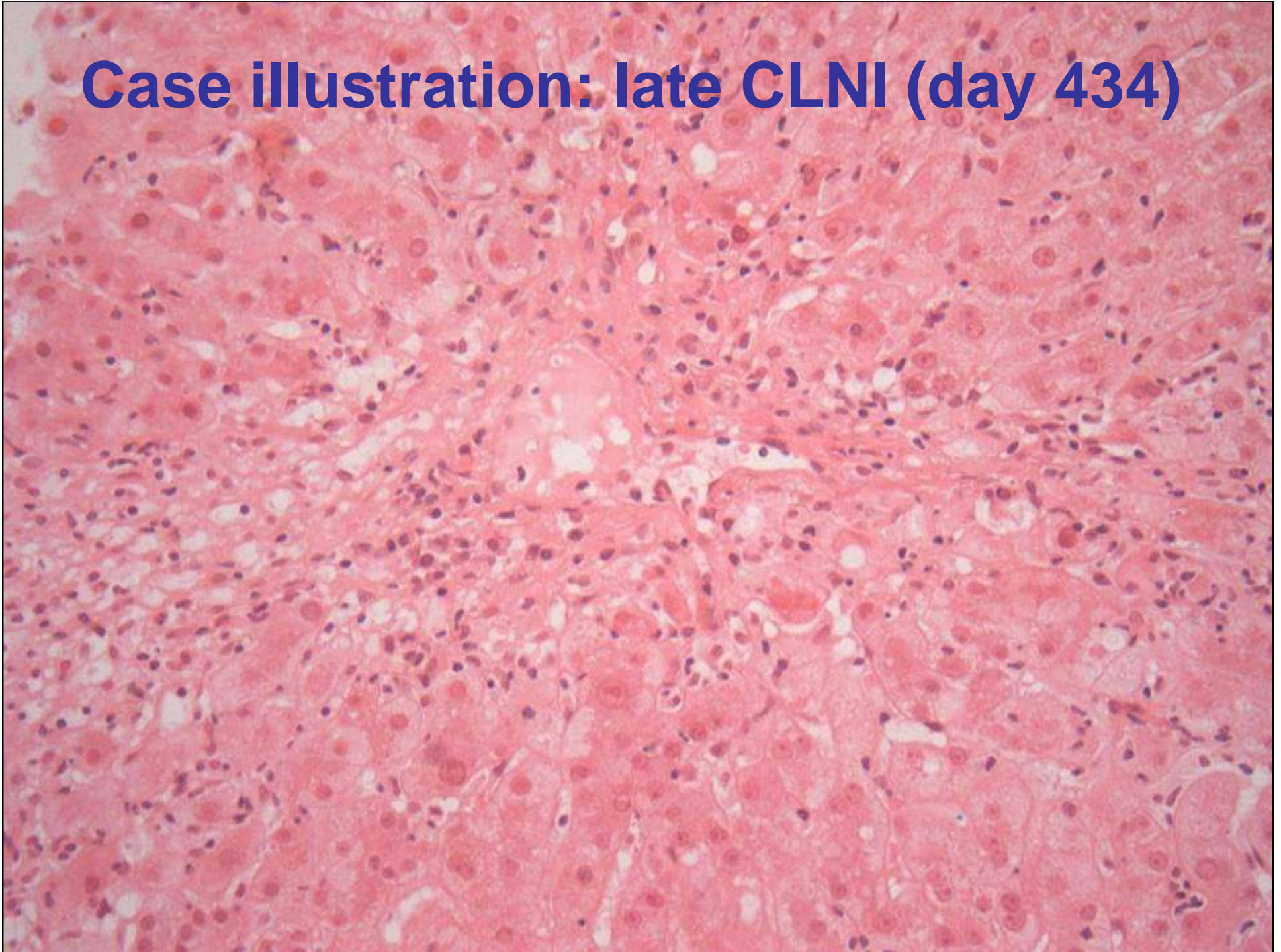
# C4d positivity in disease groups

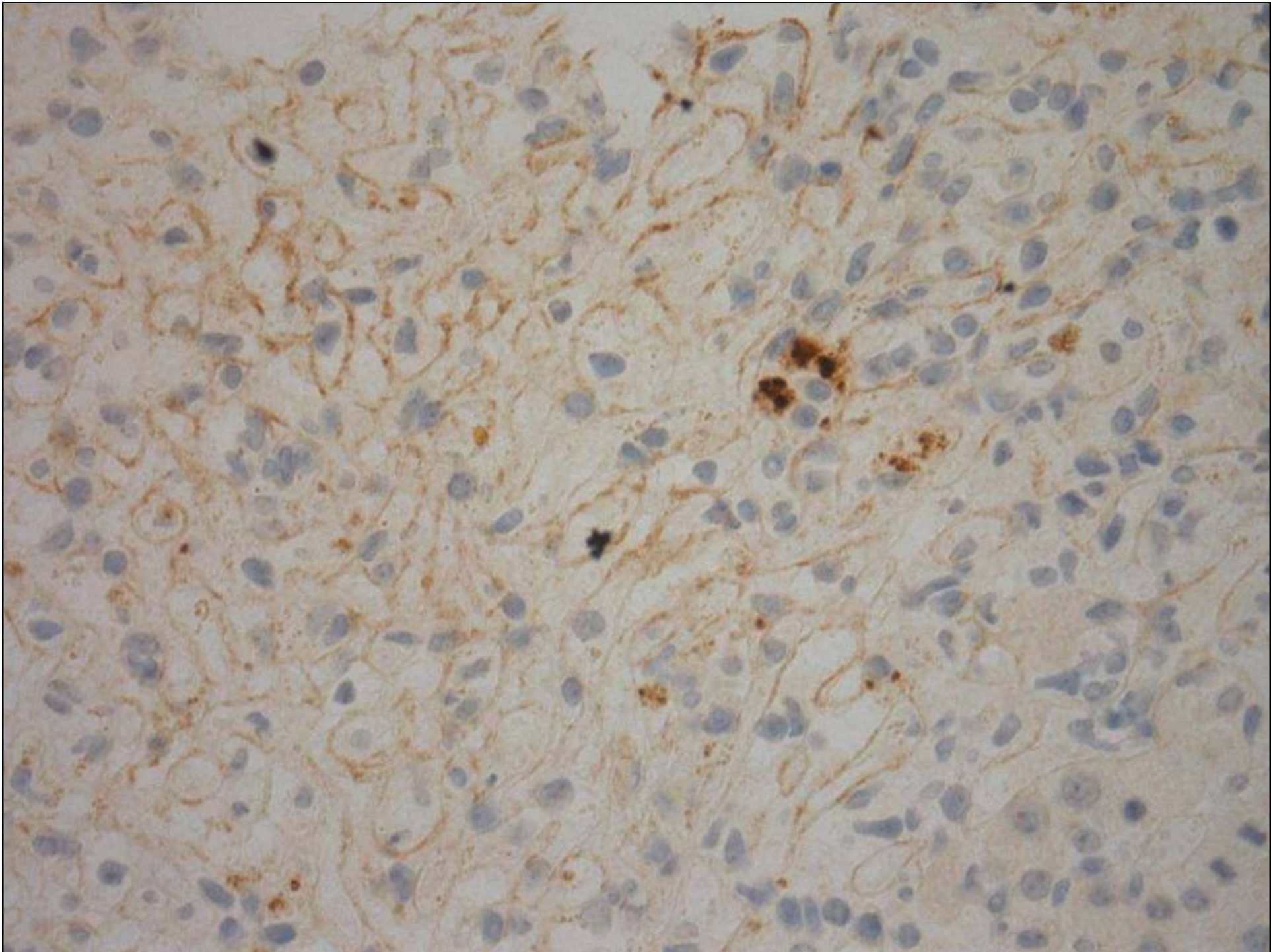
- mod/sev early acute cellular rejection    ■ 2/16 (12%)
- late centrilobular necroinflammation    ■ 3/14 (21%)
- chronic rejection    ■ 3/13 (23%)
- biliary complications    ■ 3/11 (27%)



● = C4d positive

# Case illustration: late CLNI (day 434)

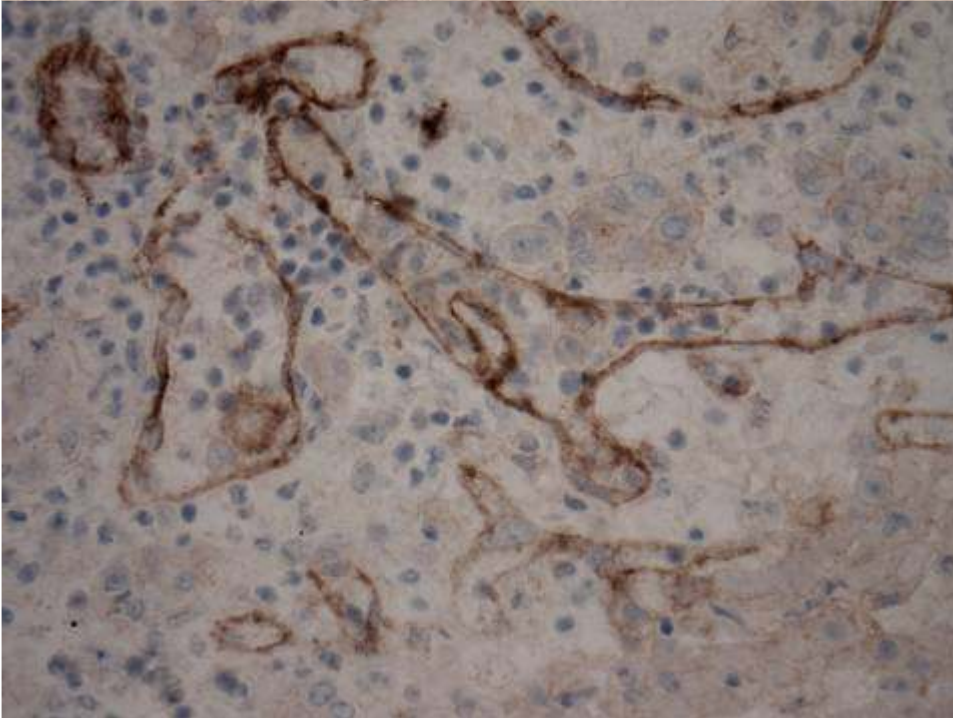
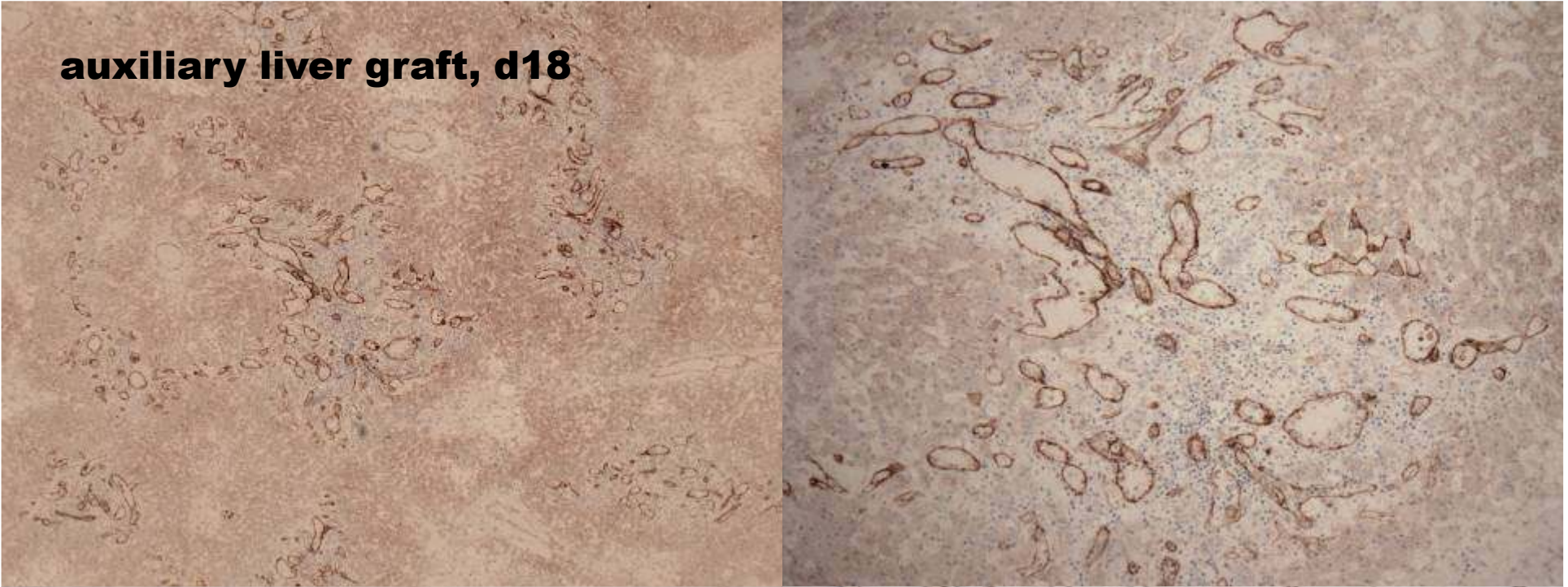




# Patterns of C4d staining

- **Crossmatch-positive patients**
  - All portal at least (6 bx from 3 patients)
  - Portal & sinusoidal staining was crossmatch +ve (?1)
- **Pure sinusoidal staining**
  - was crossmatch negative (incomplete data)
  - associated with CLNI
    - 2/3 late CLNI (?de novo Ab)
    - 1/2 mod/sev early acute cellular rejection (CLNI)
    - 1/1 primary non-function (CLNI)
    - 0/3 chronic rejection explants (no CLNI)
  - **2/3 biliary complication patients**
    - 1/1 crossmatch negative

**auxiliary liver graft, d18**



**kidney graft, d18**

# Conclusion

- C4d-positivity is uncommon
  - even pre-sensitised patients are uncommonly +ve, and negatives stay negative
- Most early positives resolve with steroids
  - small subgroup of persistent humoral injury? (<2%)
- Unresolved: sinusoidal staining (see Watson 2006)
  - different target in crossmatch neg CLNI?
  - relation with biliary complications?
- Utility?
  - Presensitised/morphology/steroid resistant