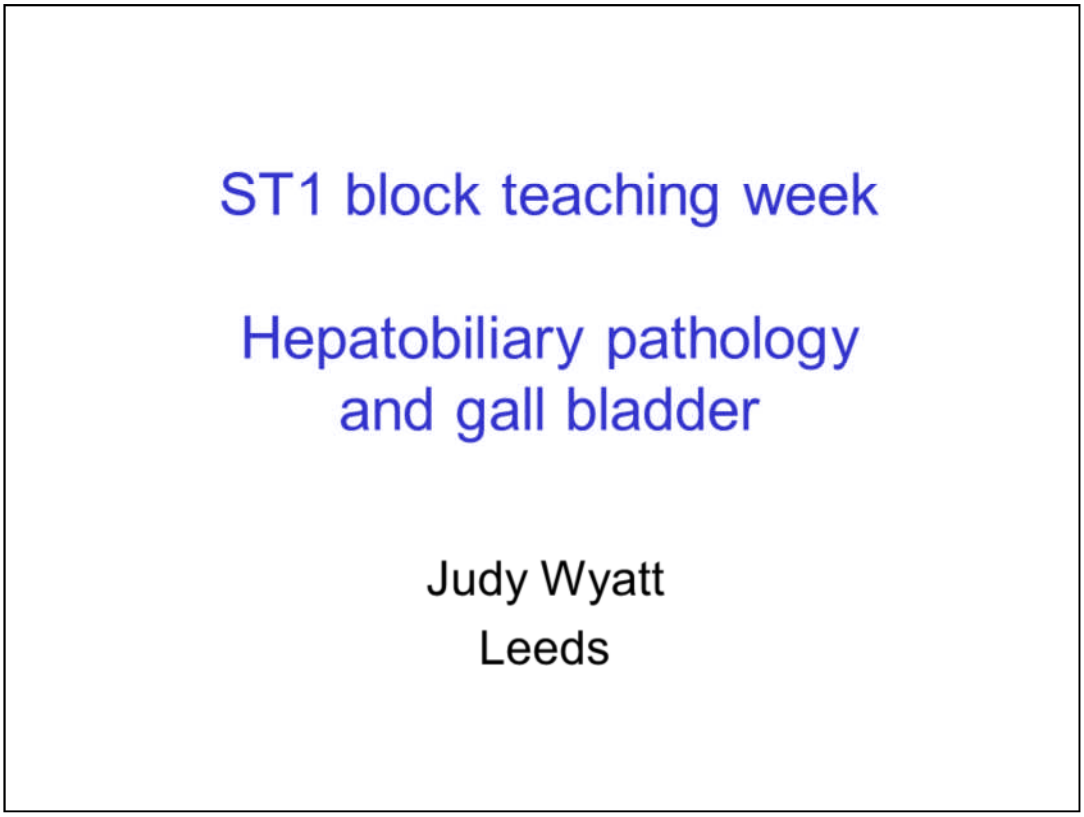
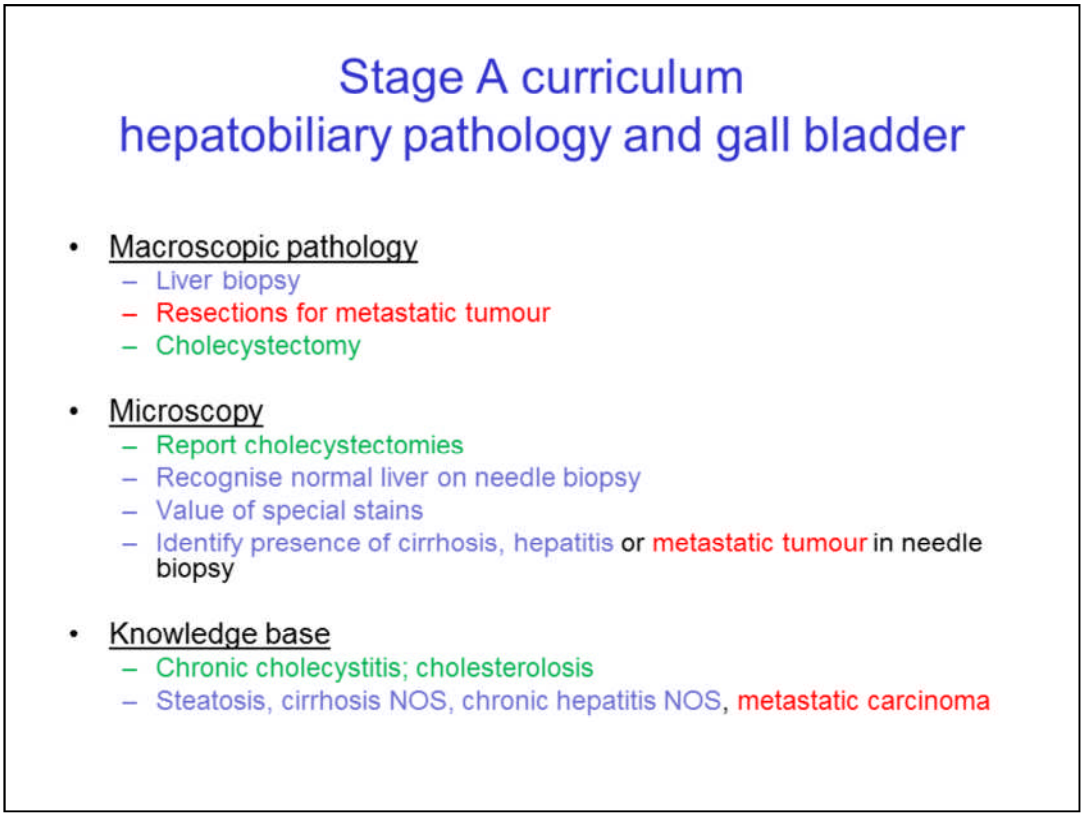


Liver and gall bladder curriculum for ST1, presented at Northern block teaching, January 2017.



The 2015 RCPath Stage A curriculum for hepatobiliary pathology and gall bladder.

Stage A curriculum
hepatobiliary pathology and gall bladder

- Metastatic tumour
  - Macroscopic - Resections for metastatic tumour
  - Microscopy - metastatic tumour in needle biopsy
  - Knowledge base – metastatic carcinoma
- Medical liver biopsies
  - Macroscopic – liver biopsy
  - Microscopy - Recognise normal liver on needle biopsy
  - Value of special stains
  - Identify presence of cirrhosis, hepatitis in needle biopsy
  - Knowledge base - Steatosis, cirrhosis NOS, chronic hepatitis NOS,
- Gall bladders
  - Macroscopic pathology - cholecystectomy
  - Microscopy – report cholecystectomies
  - Knowledge base – chronic cholecystitis; cholesterolosis

The stage A curriculum topics re-arranged – the order followed in this lecture

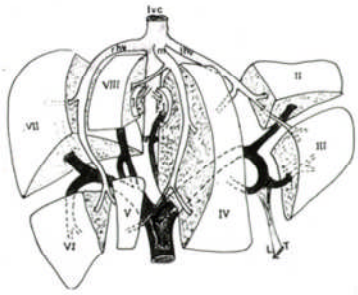
Macroscopic pathology

- Resections for metastatic tumour

Nearly always metastatic colorectal cancer

'metastas ectomy' = small wedge resection

'segment ectomy' = formal resection of liver segments



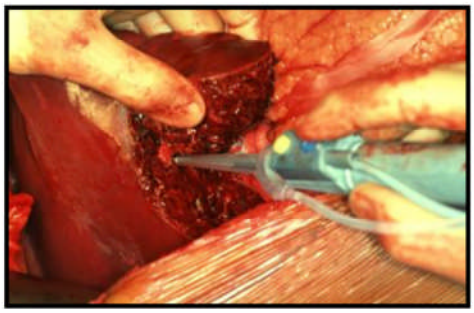
Liver resection for metastatic colorectal cancer is the commonest type of liver resection specimen.

Segmentectomy – can orientate, slice in horizontal plane for comparison with imaging

Metastasectomy – small wedge resection, usually can't orientate, more likely to have a positive resection margin – slice at right angles to margin, especially if you can see tumour close to it.

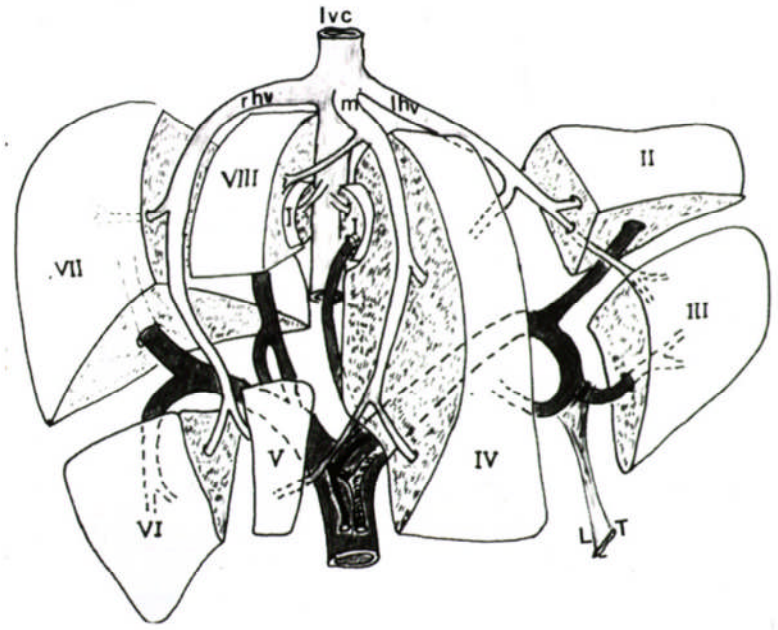
Improved surgical techniques

Liver resections for primary and secondary malignancy



Surgeons use a ‘CUSA’ – an ultrasound devise that vaporises soft parenchymal tissue and leaves tougher structures behind, coagulating the blood. Bigger vessels need metal clips. Paint the surgical surface so that you can see it in tissue sections – I like to use silver nitrate rather than black ink – so that the underlying structures are still visible.

Liver – segmental anatomy

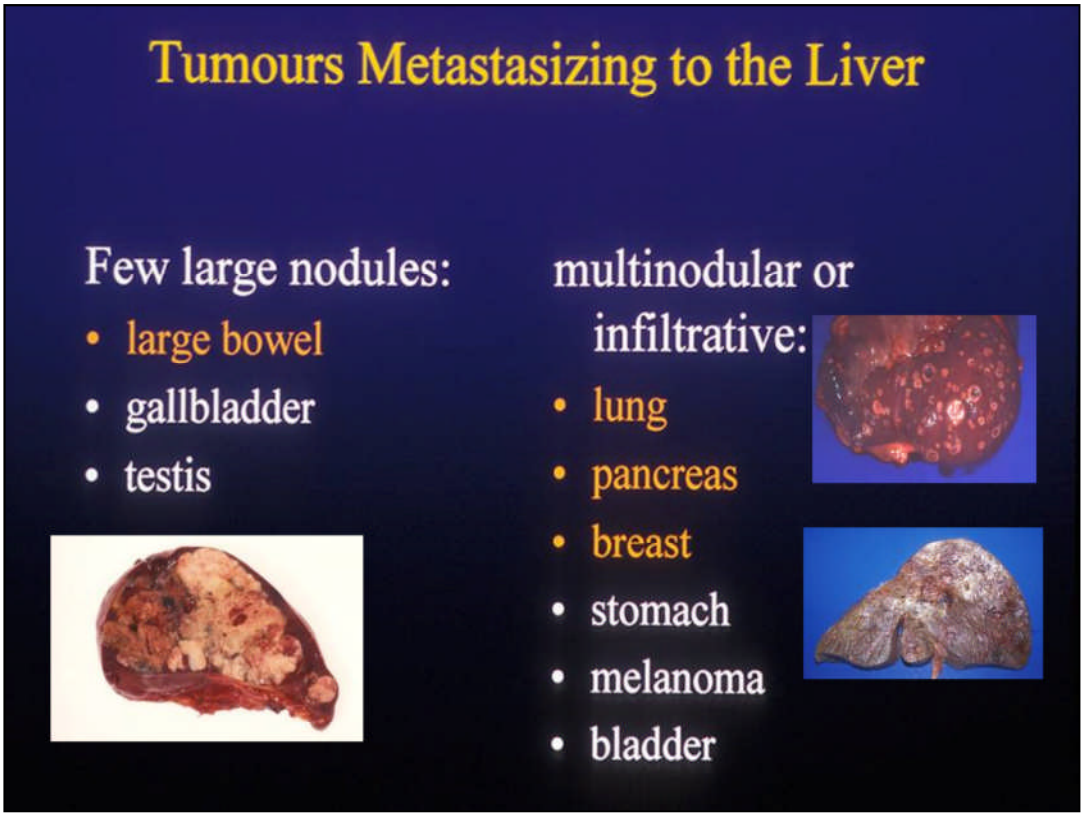


Liver segments are defined by the main portal vessels supplying them (shown in black). Up to second order portal tract divisions – resulting in 8 segments. Most of the boundaries between segments have no liver surface landmark, they are defined during surgery by intra-operative ultrasound. Hepatic veins (white) run in planes that separate segments.

Resection for liver tumours – macroscopic pathology

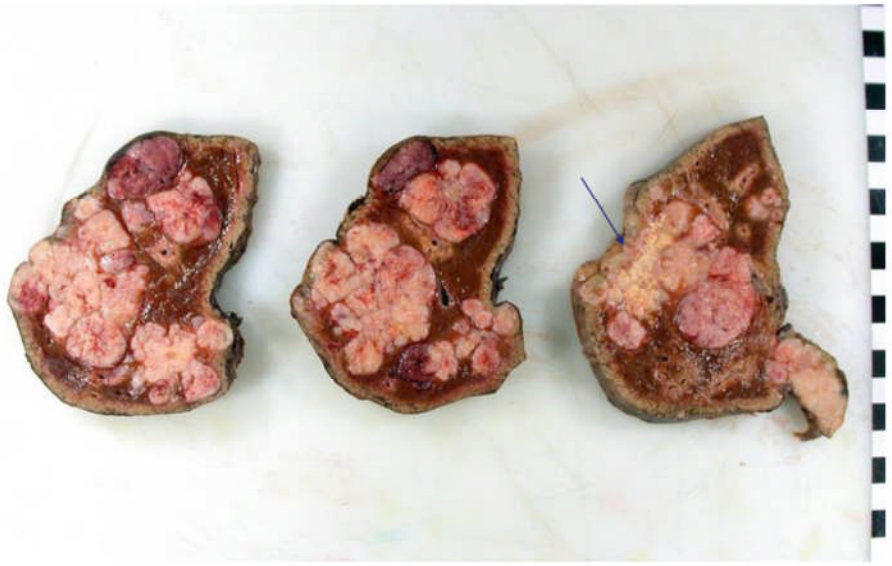
- Specimen segments, weight, dimensions
- Number, size, position, appearance
- Distance from resection margin
- Block taking
  - Each tumour unless very numerous
  - Edge of tumour/liver, especially where irregular
  - Tumour where it's closest to resection margin
  - Background liver

Macroscopic description of liver resection specimen – easy to see where the tumour edge is – so don't need lots of blocks. Liver capsule will not be infiltrated unless there is adherent overlying tissue – so doesn't need a block of visible sub-capsular tumour. Can you see tumour growing inside a tubular structure at the tumour edge? (sometimes grow down vein or bile duct).

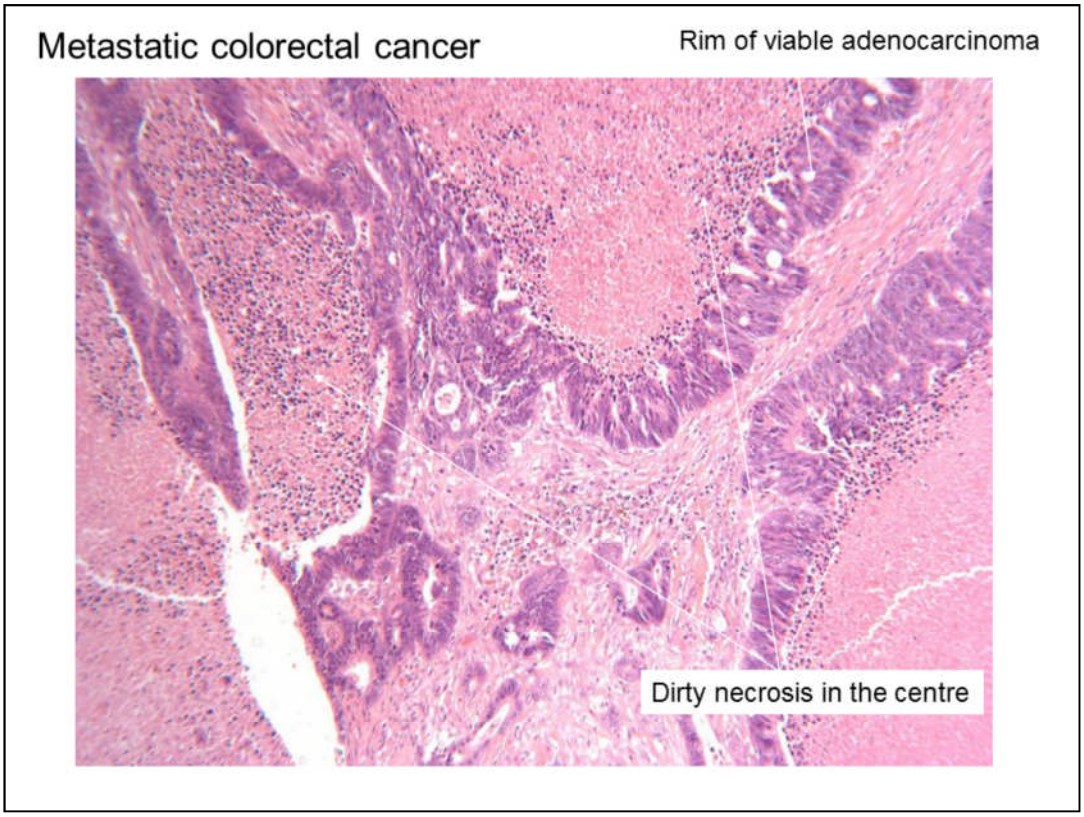


The liver is one of the commonest sites for tumour metastasis, and all malignant tumours can spread to the liver. The ones that do this most commonly are highlighted in the slide. Colorectal cancer often forms a small number of large metastases – and so these can be surgically resected. The 5 year survival is around 40%. Most primary sites metastasize as multiple small nodules, or occasionally diffusely infiltrative and not visible on imaging. These are of course not amenable to surgical management.

47F Dukes A carcinoma splenic flexure Aug 2010,
After chemotherapy had right hepatectomy (segments 5,6,7,8, 900 g)



Right hepatectomy – if you can orientate the specimen, slice in horizontal plane for comparison with imaging. Early stage primary, but still developed bulky liver metastases. Liver mets are haematogenous spread – there is always vascular invasion in the primary. Some of the tumour has previously responded to chemotherapy (yellowish necrosis, depressed capsule indicating tissue loss (arrow)). Most of the tumour is actively expanding – raised capsule, rounded outline.

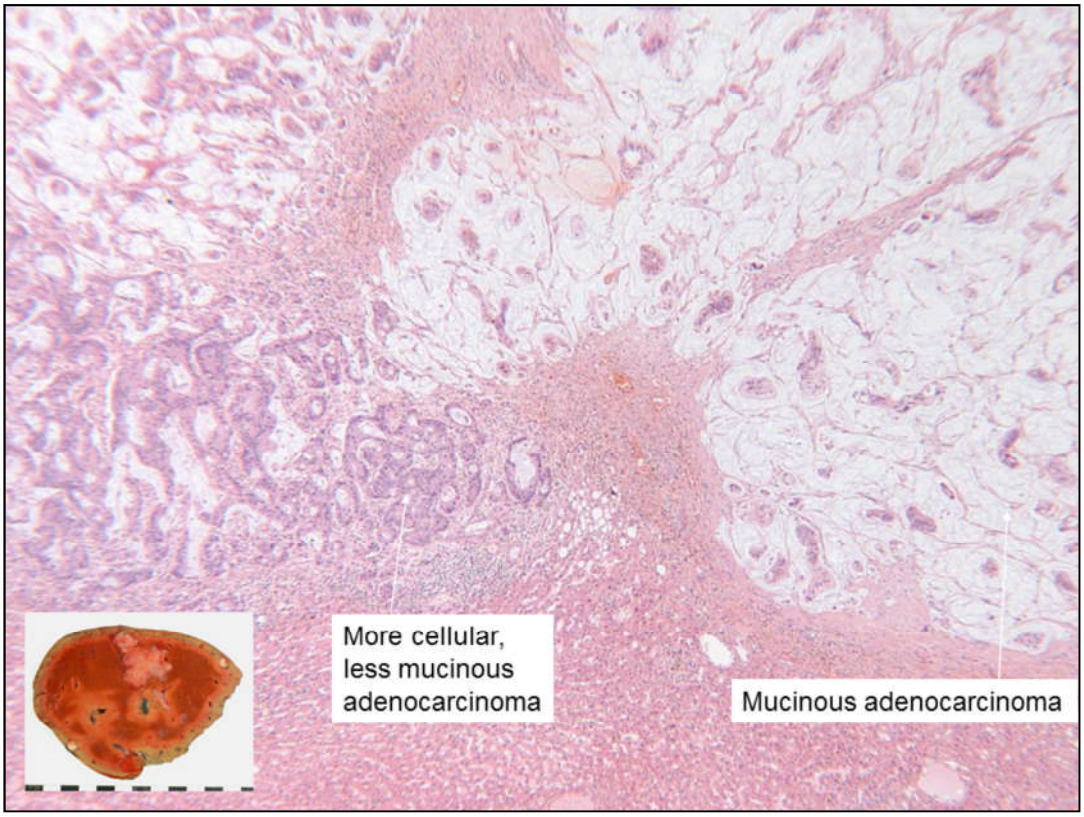


Metastatic colorectal cancer – characteristic microscopic appearance without chemotherapy. A garland of adenocarcinoma surrounding central ‘dirty’ necrosis with cell debris. After chemotherapy, if there has been a response this is recognised by finding less necrotic debris, more fibrosis throughout the tumour, and foamy macrophages or sometimes mucin lakes.

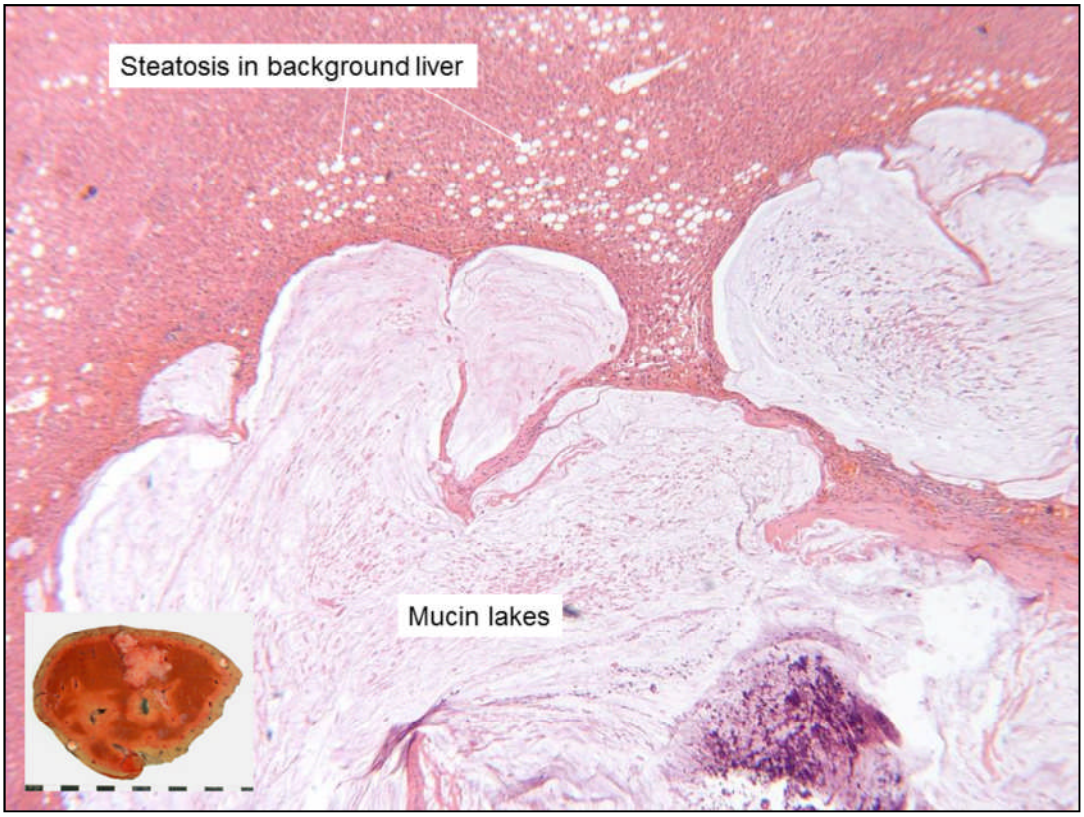
63M lymphoma 2010, carcinoma of caecum found incidentally 2011and resected, T3N1

Multiple lesions in liver, one is growing.

Another case of metastatic colorectal cancer. The main tumour is mucinous – you can see this from the macroscopic cut surface. The two small white nodules surrounded by fibrosis have responded to chemotherapy.



Microscopy of the mucinous adenocarcinoma - lakes of mucin with adenocarcinoma cells floating inside. Part of the adenocarcinoma is less mucinous.



This part of the tumour has responded to chemotherapy, so that only lakes of acellular mucin remain.

There is some steatosis in the background liver.

Microscopy

- metastatic tumour in needle biopsy

Metastatic tumour in biopsy

Lab protocol – 2 shallow levels H&E and spares for immunos

- Is there liver?
- Is there tumour?
  - If no – deeper levels

- What is it? – what does it look like?
  is there any history?

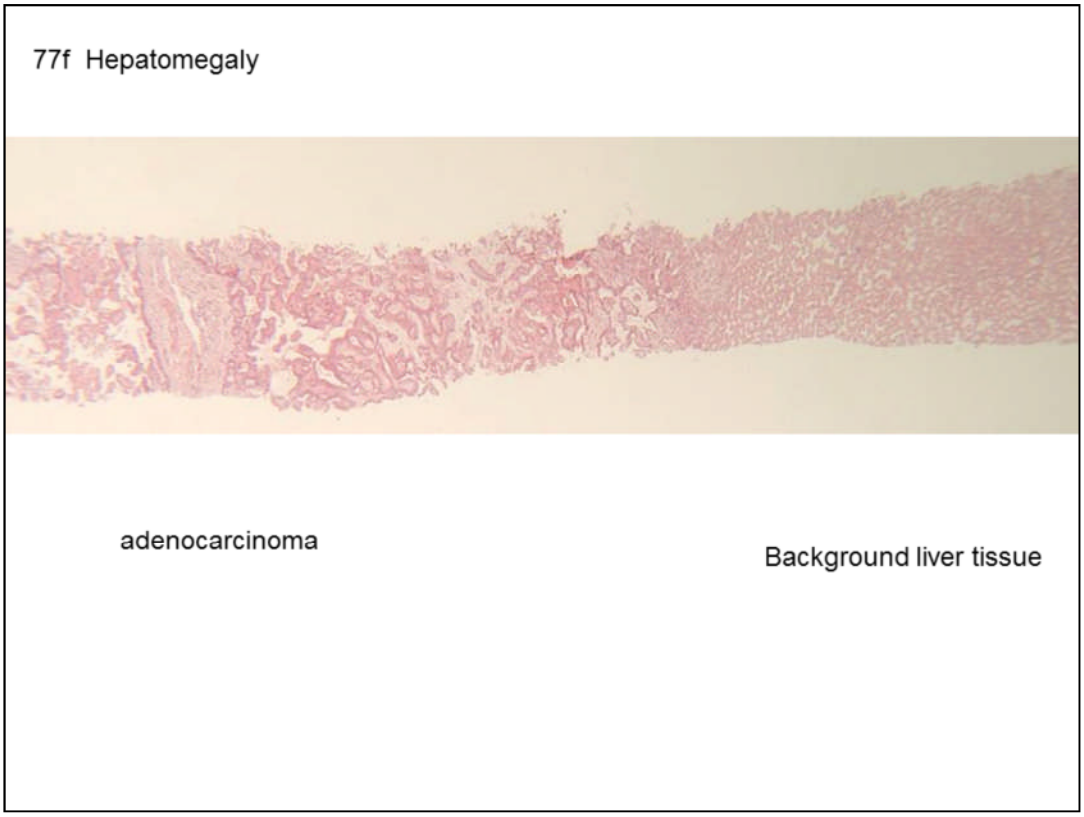
Often need immunos – limited tissue,
plan carefully *'tissue economics'*

There are two different types of needle biopsy of liver – targeted biopsy from a focal lesion, and random biopsy to diagnose diffuse medical liver disease.

They need to be handled differently in the lab.

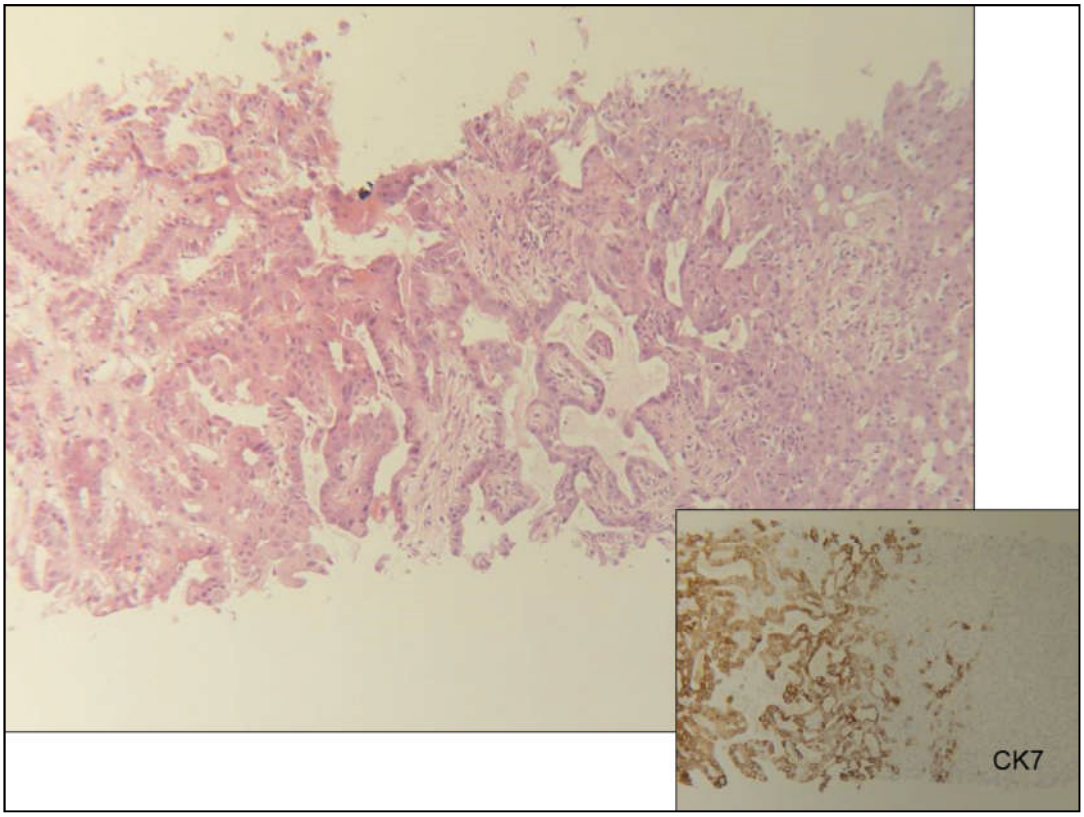
For targeted biopsies – initial shallow levels so that there is still tissue in the block for other investigations. About 80% of our tumour biopsies have immunohistochemistry.

Tumour biopsies are usually taken with 18 gauge needles, average biopsy diameter around 0.5mm – there is more risk of bleeding following tumour biopsies.



In patients with metastatic disease, liver biopsy is often the best opportunity for obtaining a tissue diagnosis. This can be used for immunohistochemistry and for molecular diagnosis.

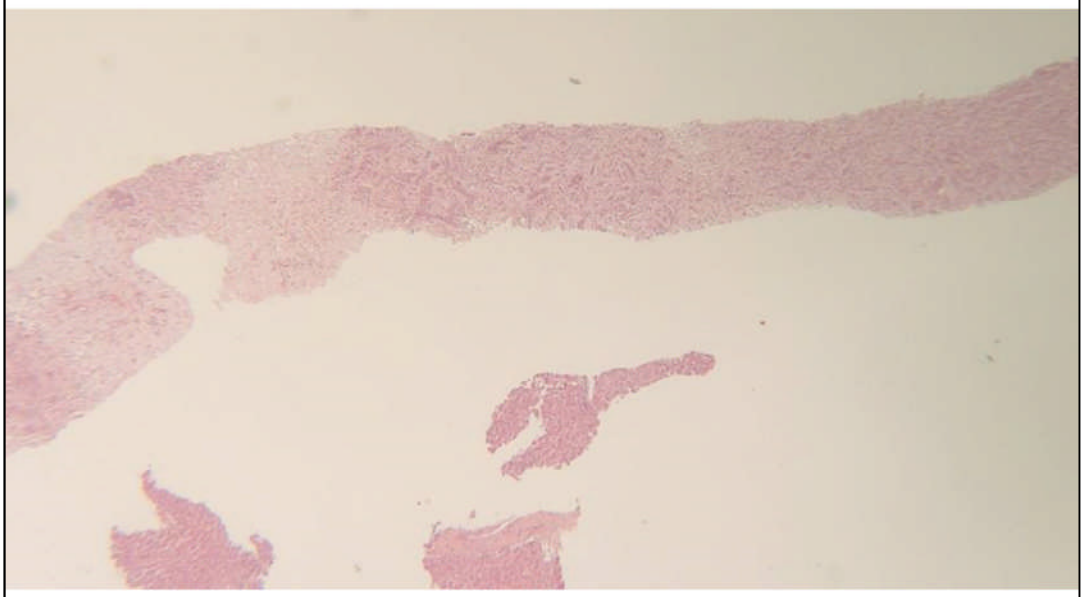
Liver biopsy should be avoided though if there is a possibility of surgical resection – there is a risk of needle track seeding that leads to post-operative recurrence.



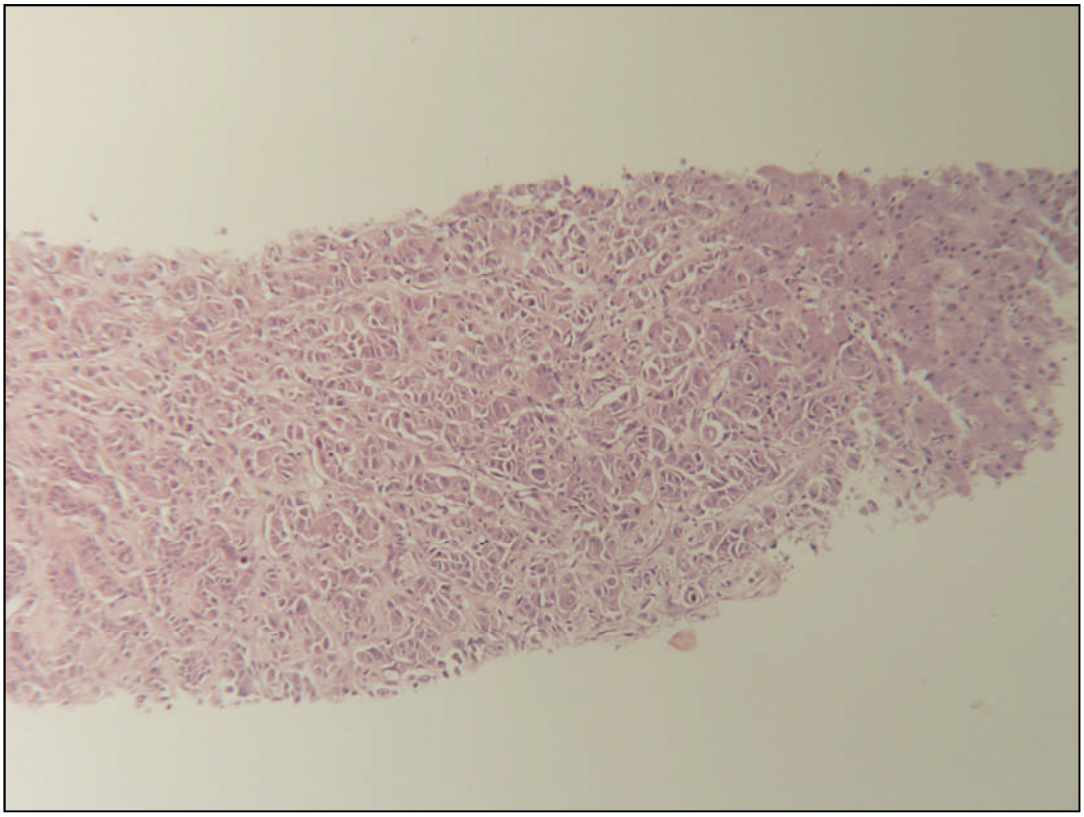
This is adenocarcinoma – it doesn't really need CK7, but may be part of a panel for cancer of unknown primary.

There is a NICE guideline for immunohistochemistry investigation of metastatic adenocarcinoma of unknown primary site – include CK7 and CK20, TTF1 (lung), ER (breast), PSA (prostate), PLAP (germ cell).

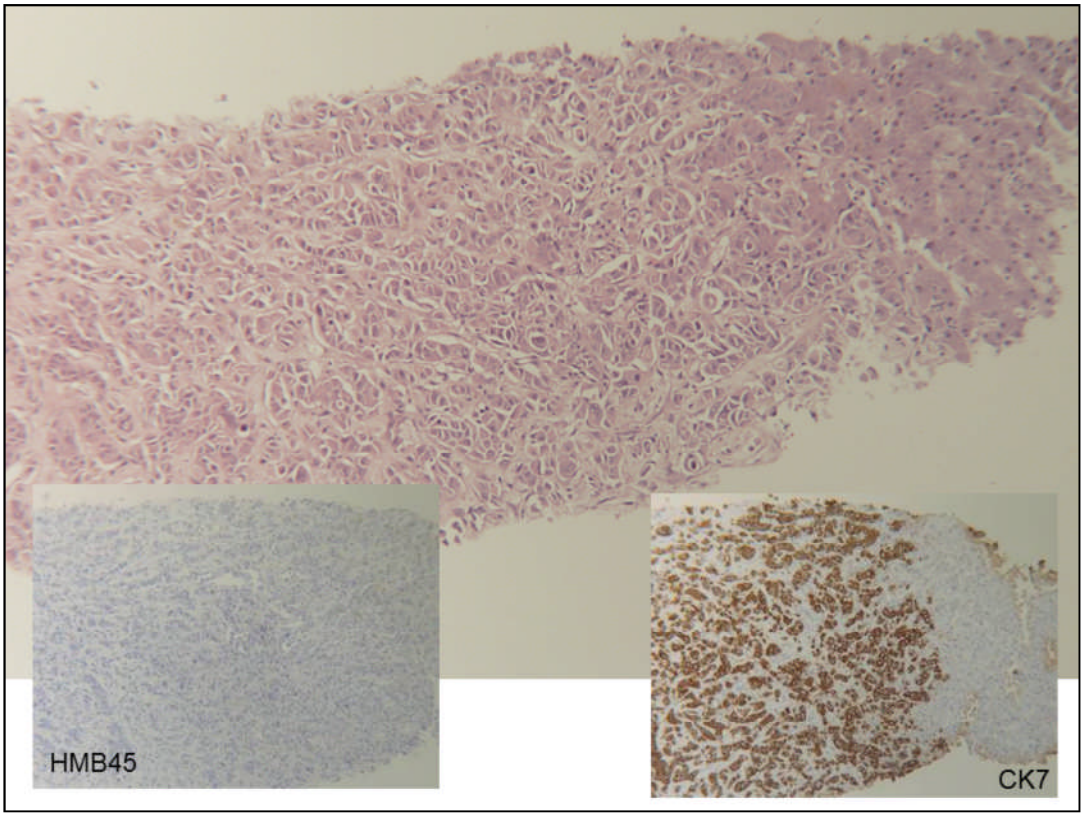
61M central liver tumour,
previous history malignant melanoma 2000



The biopsy contains metastatic tumour.

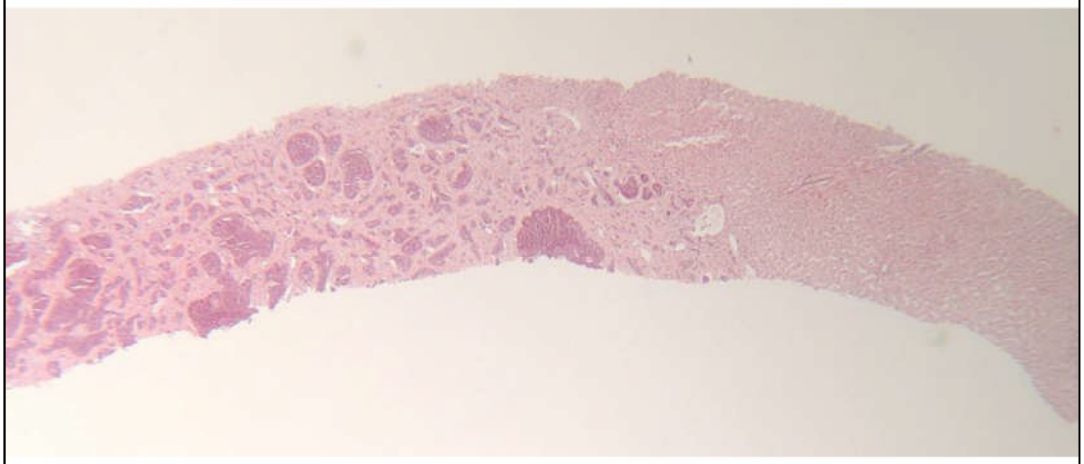


Morphology would be consistent with metastatic melanoma. Do immunohistochemistry to make sure.



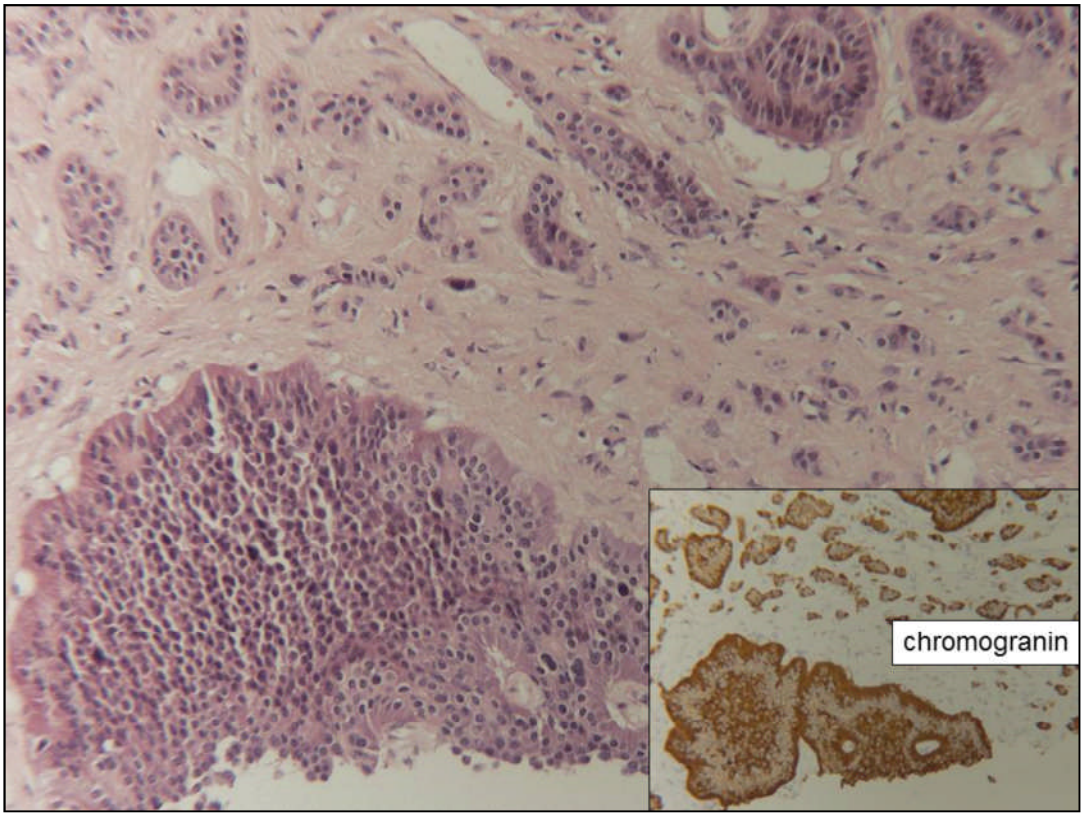
But HMB45 (melanoma marker) is negative. The CK7 is positive – this is metastatic adenocarcinoma.

72F Previous ovarian mucinous cystic neoplasm 2001. Mass in mesentery identified on scan.

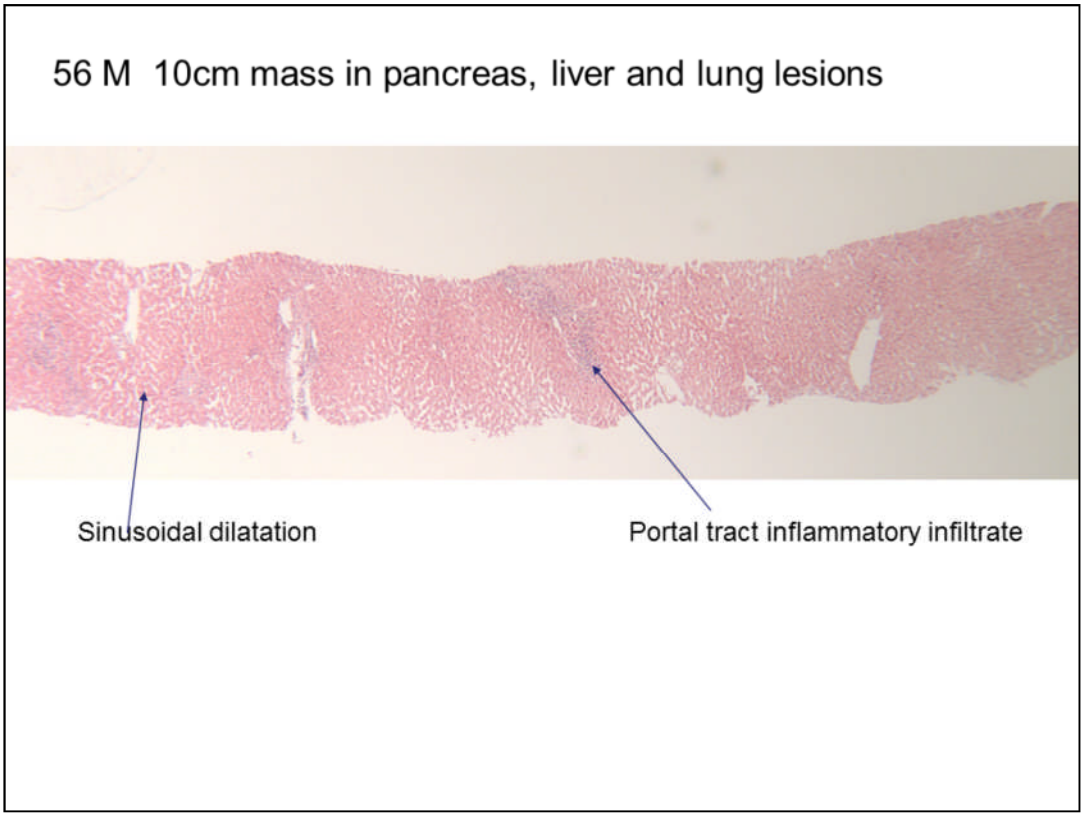


Mass in mesentery – but liver metastasis is more accessible for biopsy.

Liver tissue containing metastatic tumour with a nested pattern.

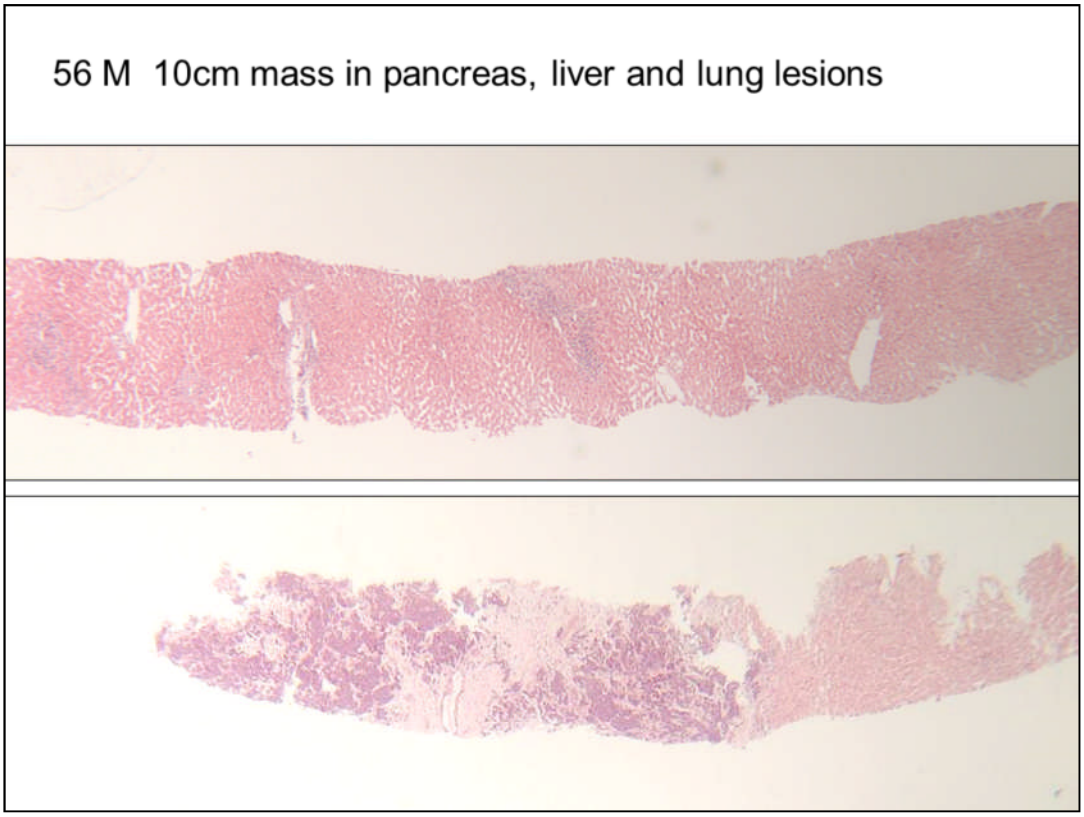


On higher magnification, the morphology is characteristic of a well differentiated neuroendocrine cell tumour – confirmed by positivity for chromogranin which stains the neuroendocrine granules in the tumour cell cytoplasm.

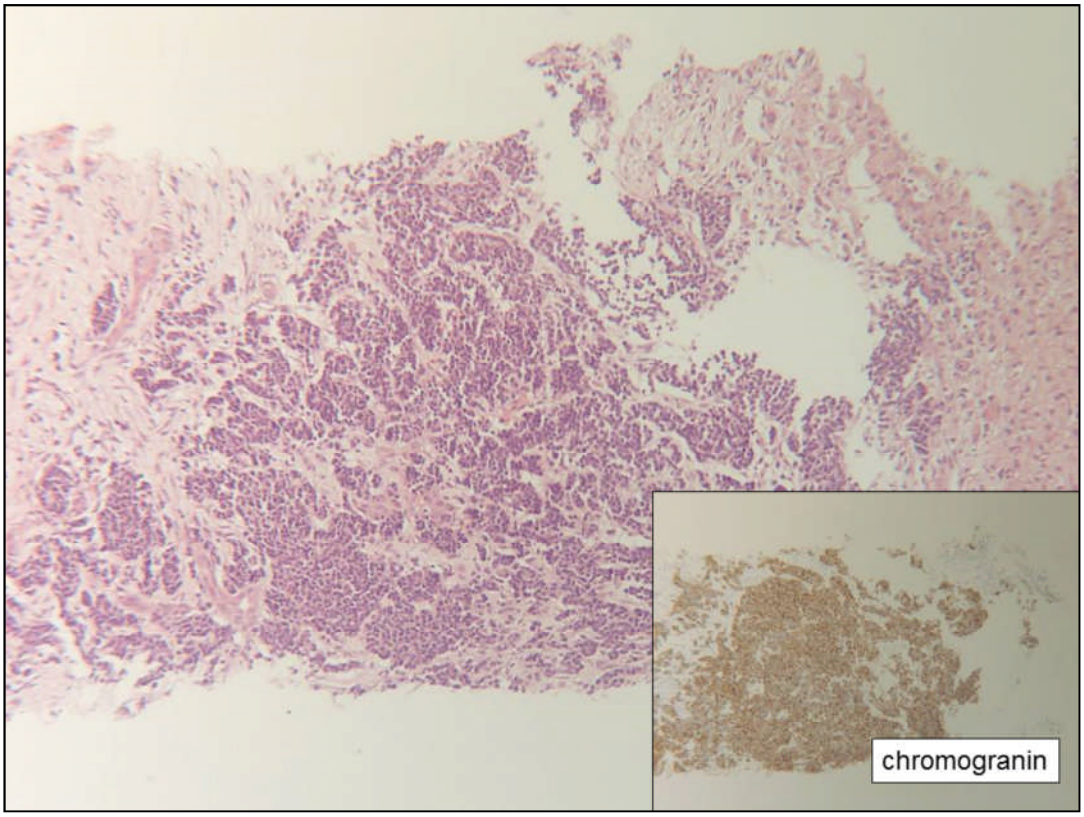


This biopsy shows the features that may be present in liver tissue in the vicinity of a focal lesion – there is non-specific inflammation in portal tracts, and sinusoidal dilatation but no tumour.

If this is all you see in the initial levels, cut deepers before deciding that the biopsy is negative for tumour.



Deeper levels in this biopsy show a focus of malignant tumour.



This has the morphology of small cell carcinoma, and is confirmed as poorly differentiated neuroendocrine carcinoma by the immunopositivity for chromogranin. This may have originated in pancreas or lung.

Knowledge base

- metastatic carcinoma

Targeted liver biopsy for tumour

How to approach targeted liver biopsies: 3 questions

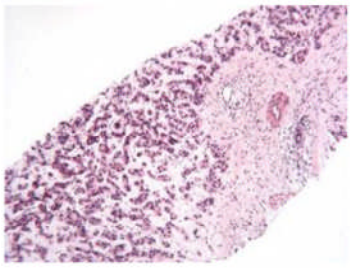
- Is it from liver?
- Does it contain the lesion?
- What is it?

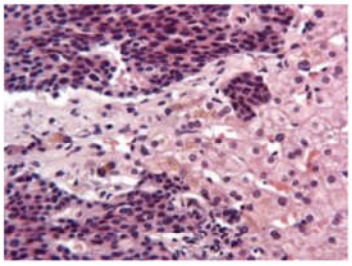
Is there background liver present? (may be a tumour near the liver)

Is there tissue from the lesion? (sometimes primary hepatocyte tumours look very like background liver)

Metastatic carcinoma
growth pattern – adenocarcinoma or not?

- Glands, mucin
  - GI tract upper
  - GI tract lower
  - Pancreas
  - lung
  - Breast
- No glands, no mucin
  - Melanoma
  - Neuroendocrine carcinoma
  - Renal cell carcinoma
  - Poorly differentiated SCC





This slide lists common tumours metastasizing to the liver.

On H&E morphology – decide if it is adenocarcinoma or other sort of carcinoma, sarcoma, melanoma, lymphoma

Metastatic tumour in needle biopsy

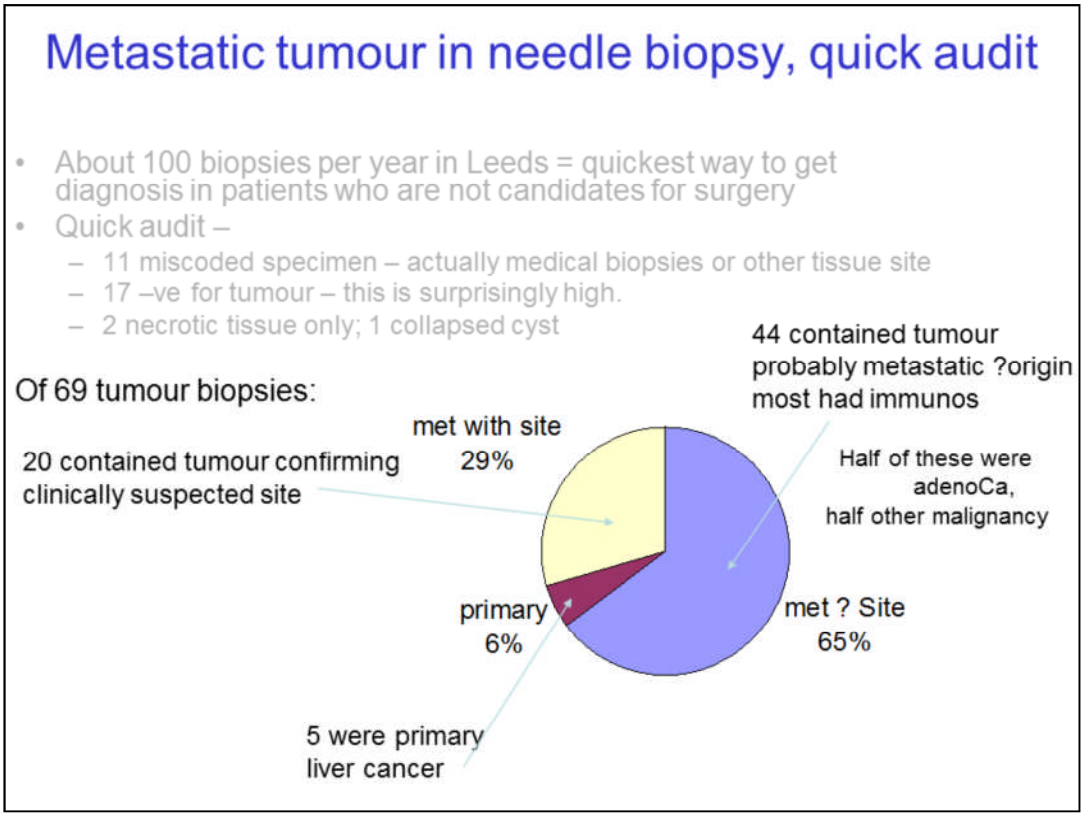
- About 100 biopsies per year in Leeds = quickest way to get diagnosis in patients who are not candidates for surgery
- Quick audit of 100 liver tumour biopsies in 2012 –
  - 11 miscoded – these were not tumour biopsies.
  - 17 –ve for tumour
  - 2 necrotic tissue only; 1 collapsed cyst

  = 69/89 with tumour – 79% which is rather low.

Miscoded – specimen coded as tumour biopsy on booking in to the lab, but actually medical liver biopsies.

Liver biopsy to get tumour tissue for diagnosis but should be avoided in patients who may be suitable for resection, as taking the biopsy perforates the liver capsule and can result in needle track spread with chest wall recurrence.

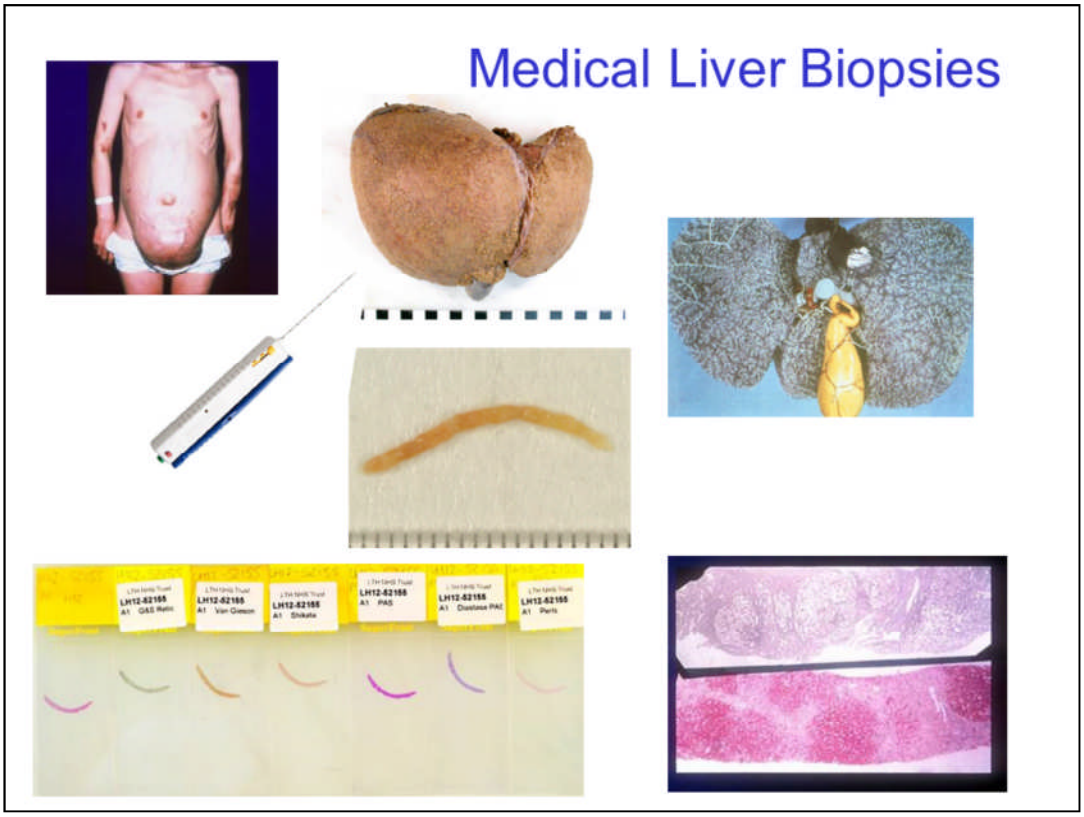
I did a quick audit and was surprised that the yield of tumour was so low.



The audit was to find out more about these tumour biopsies – this pie chart shows that about 30% confirmed the suspected primary site, 65% the primary site was not previously known, and 6% were from a primary liver lesion – hepatocellular carcinoma, cholangiocarcinoma, or hepatocellular adenoma.

Macroscopic pathology

- Liver biopsy



Histological sections of a liver biopsy represent a tiny proportion of the liver. We are extrapolating from the microscope view (bottom right) to try to explain the clinical signs and symptoms of the whole patient with liver disease – late stage disease illustrated top left. There are assumptions along the way – that the disease is sufficiently uniform in the liver that the pattern in the section – in this case micronodular cirrhosis – is representative of the rest of the liver. There is not much variation from place to place and therefore not much sampling error. Fortunately this is true for most liver disease, although less so when caused by some biliary or vascular disorders. In those, the distribution varies if one of the main bile ducts or hepatic veins is affected more than others.

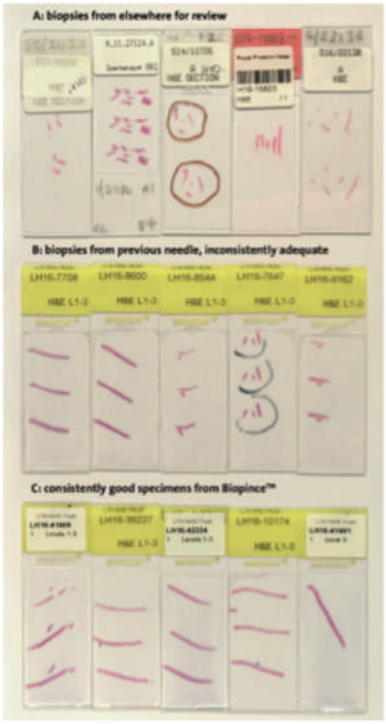
Macroscopic pathology

- Liver biopsy

Liver biopsy adequacy and biopsy needle type

Letter, College Bulletin Jan 2017

Fig 1 Recent liver biopsies in Leeds



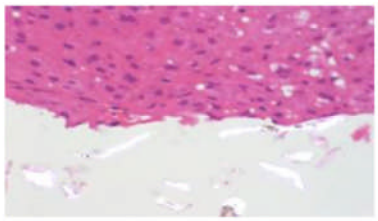
The quality of the needle biopsy sample is important. Until 1990's biopsies were usually taken on the ward by clinicians using Mengini type needles which produced a long intact core. Since then, the trend is for biopsies to be taken in radiology under ultrasound guidance. The type and gauge of needle affects the size of the biopsy – there is evidence that to avoid sampling error in staging, and biopsy should be with the 16gauge needle and be more than 20mm long.

Percutaneous liver biopsy

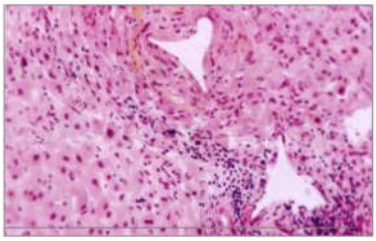
- Essential not to loose information during handling / processing of these small specimens

Handling the biopsy specimen

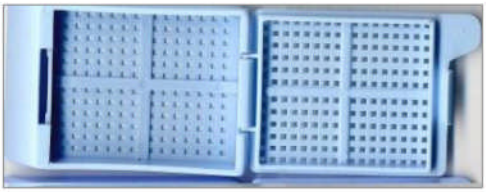
Liver biopsy specimens are best left floating in the fixative solution
⇨ **No blotting paper**



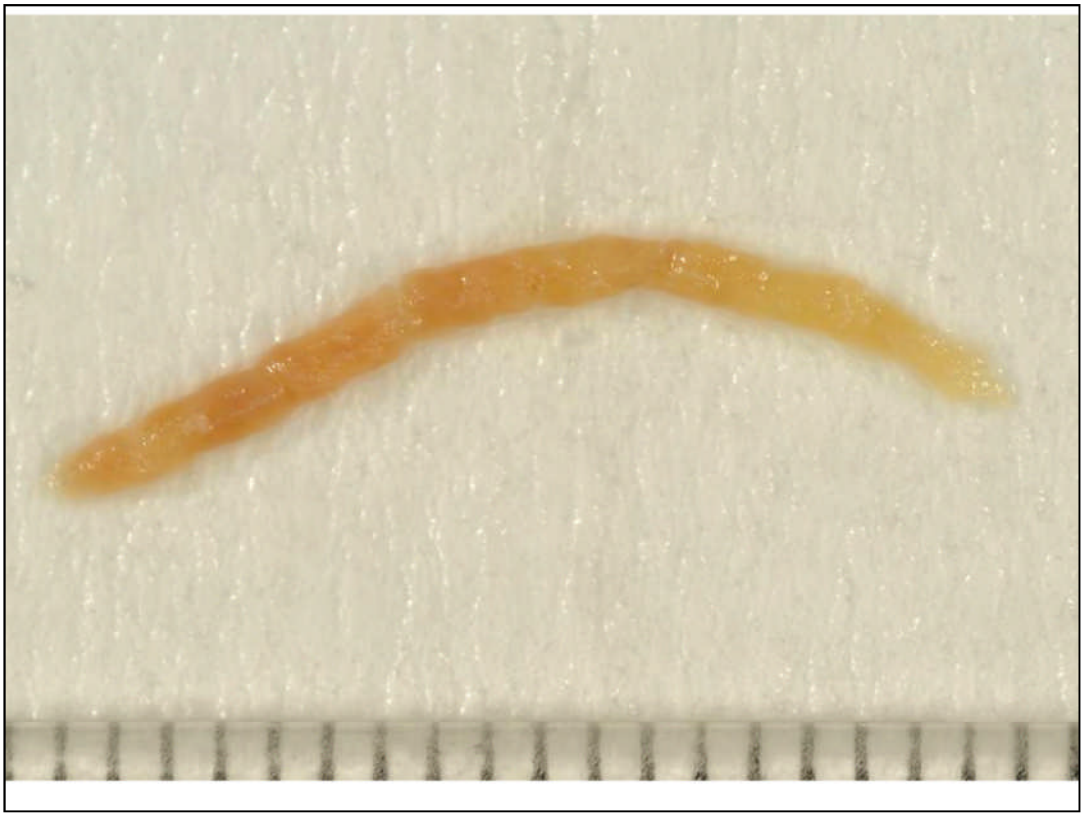
Avoid rough packing between foam sponge



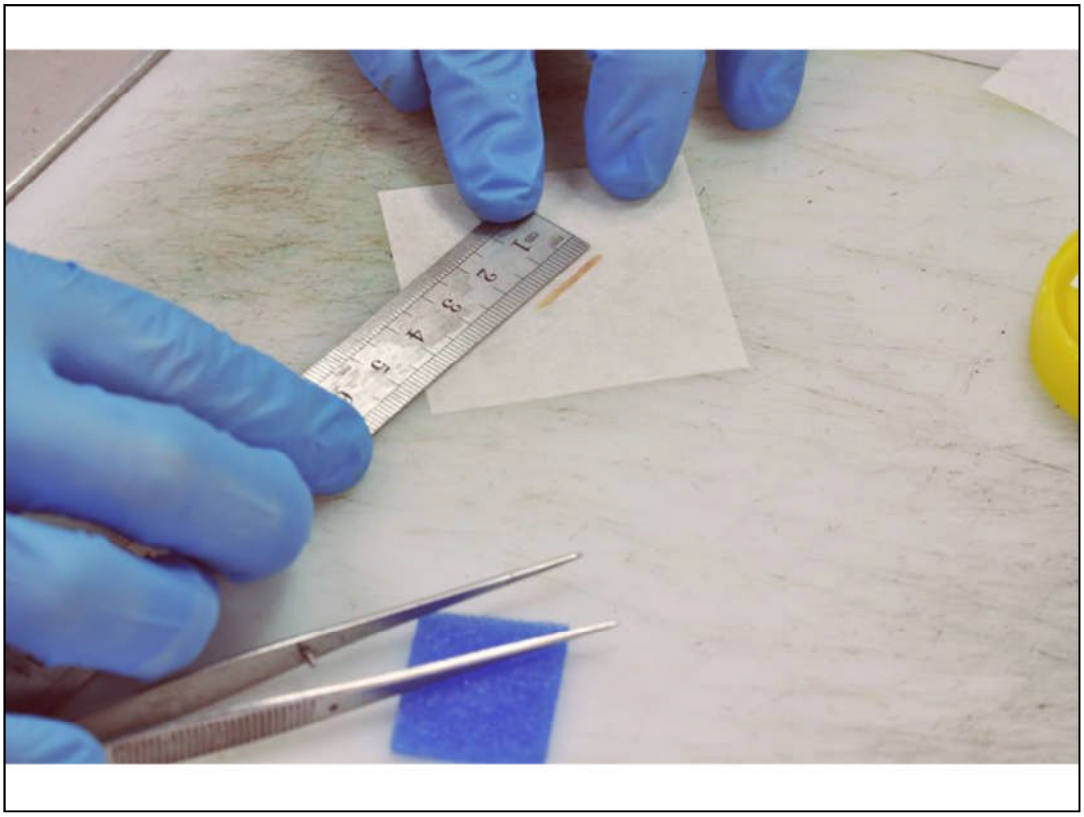
⇨ **Use fine mesh cassettes or lens-paper wrapping**

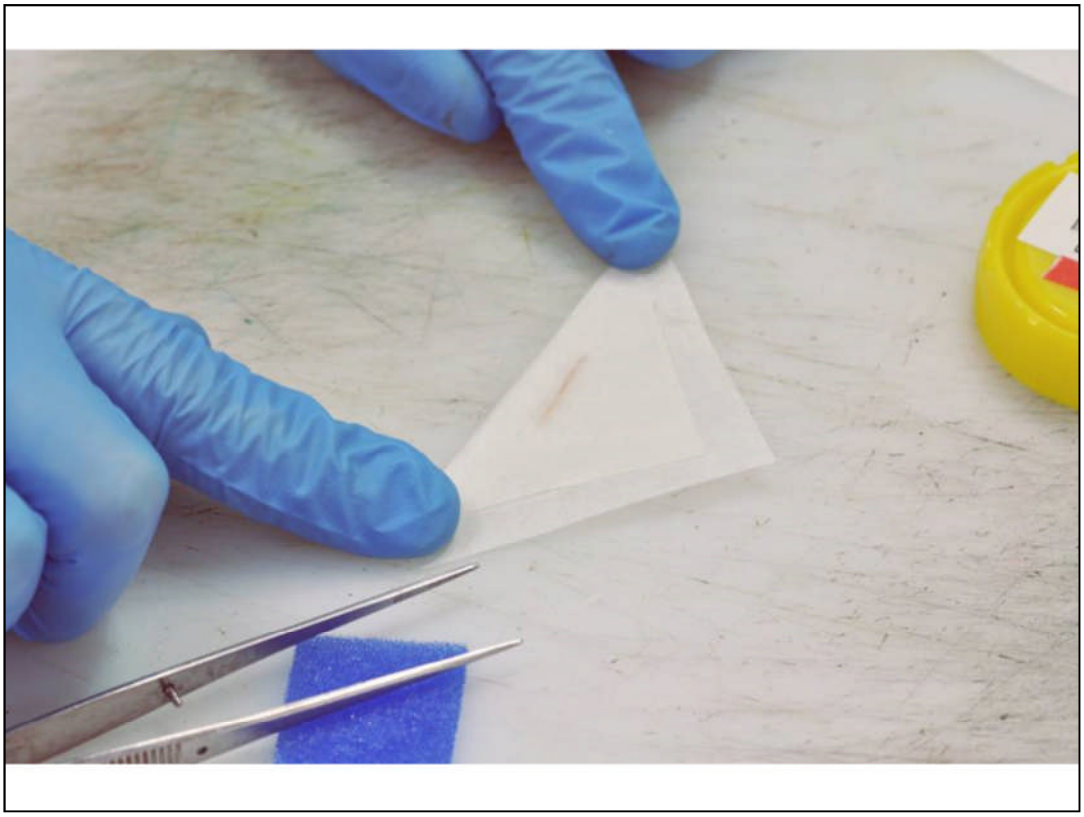


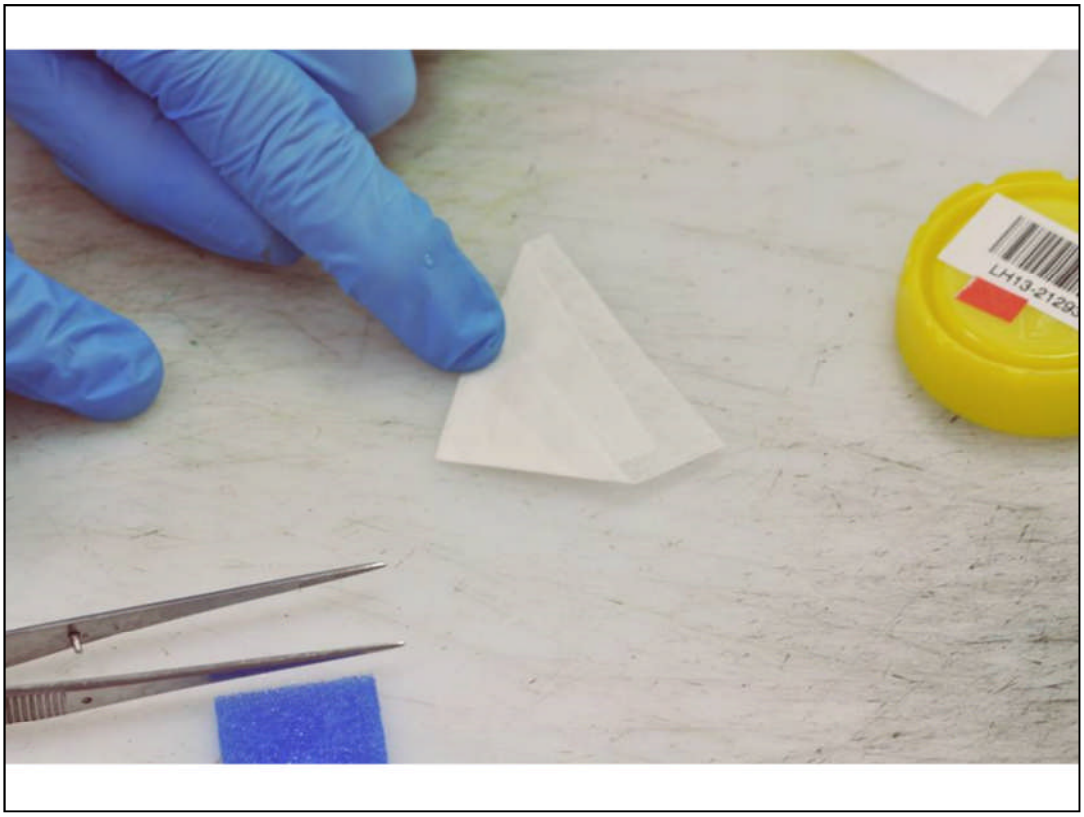
The top photo is of drying artefact in a biopsy placed on blotting paper. The lower photo shows triangular dents in the tissue caused by the ridges on foam sponge used to keep the biopsy in place during processing. This should be avoided, use fine mesh casettes or wrap sample with lens paper.

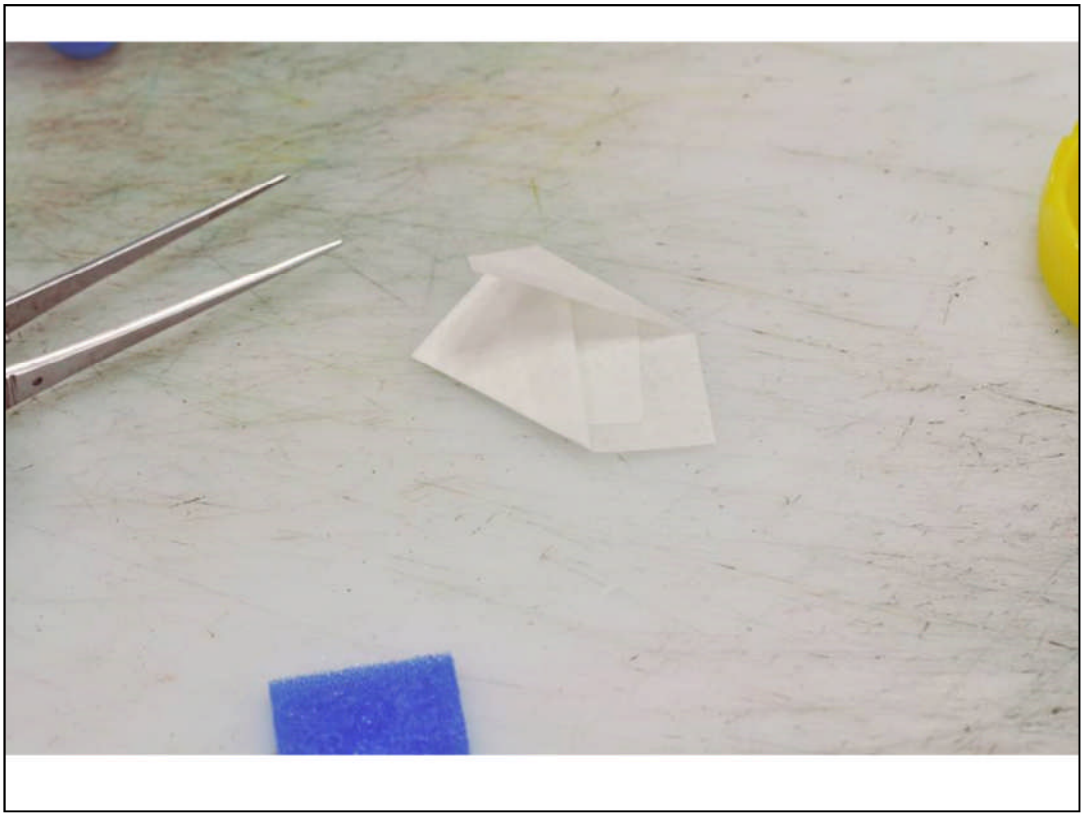


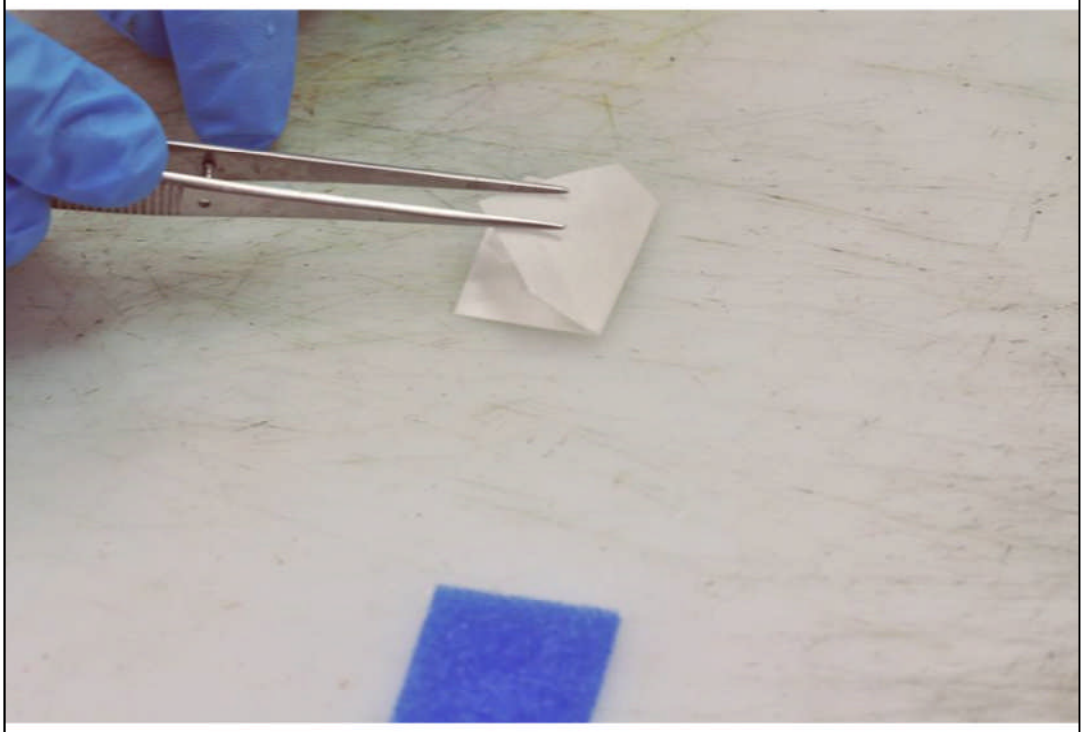
Measure the length of the biopsy core.

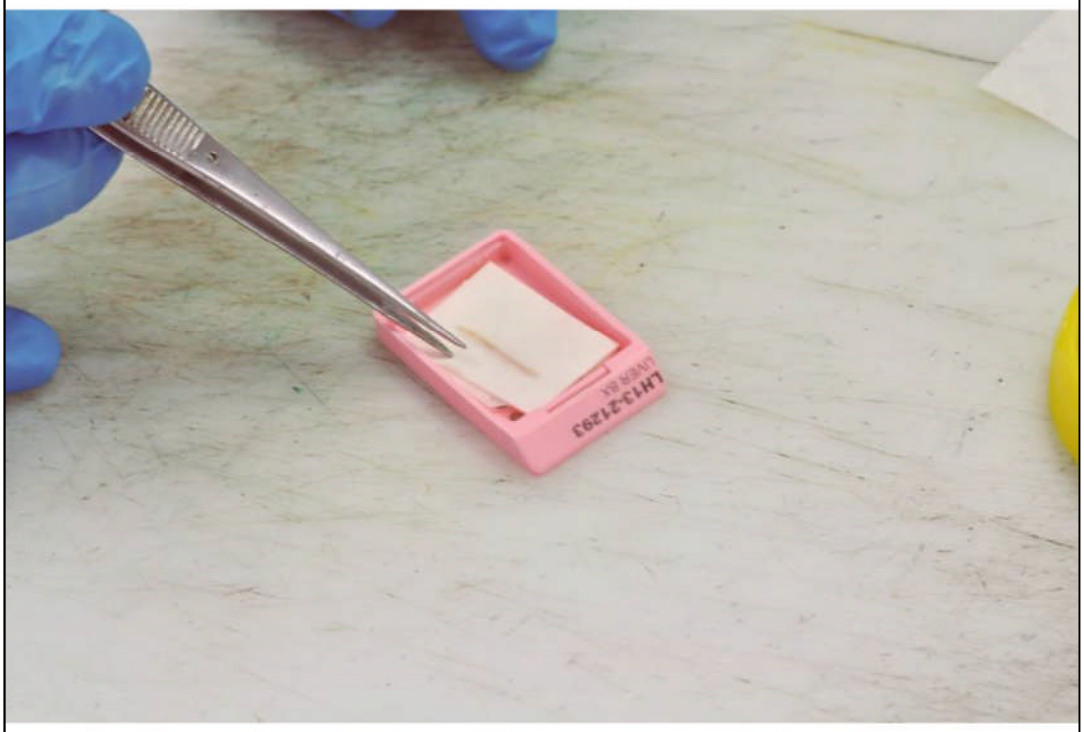








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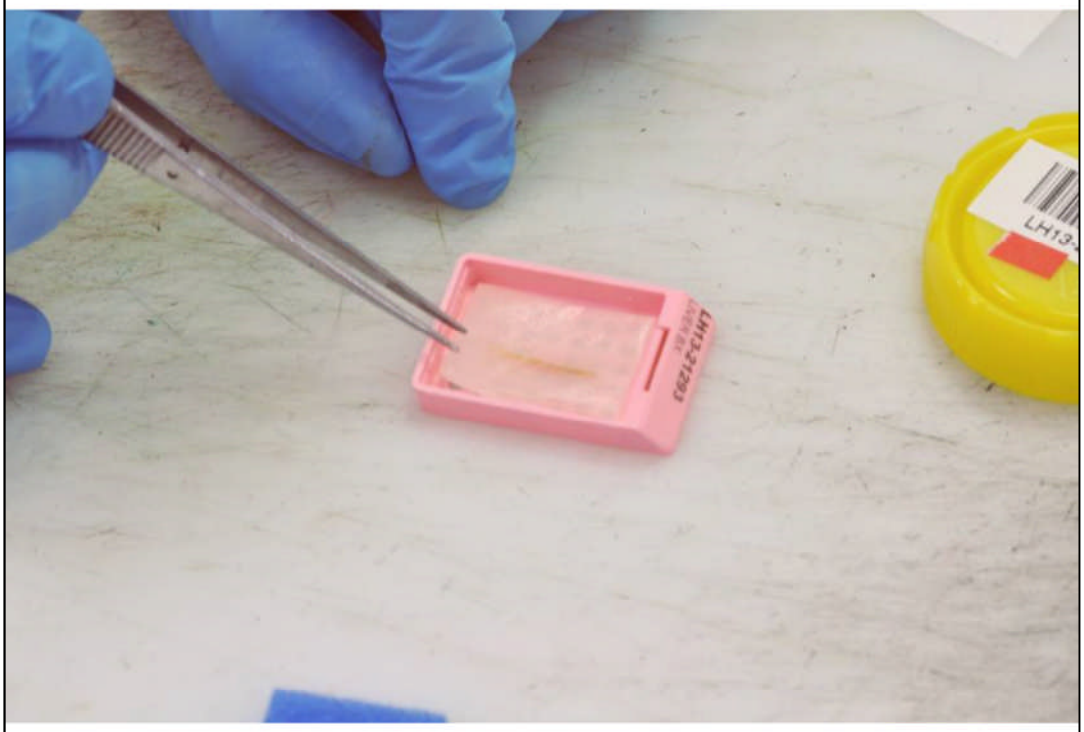


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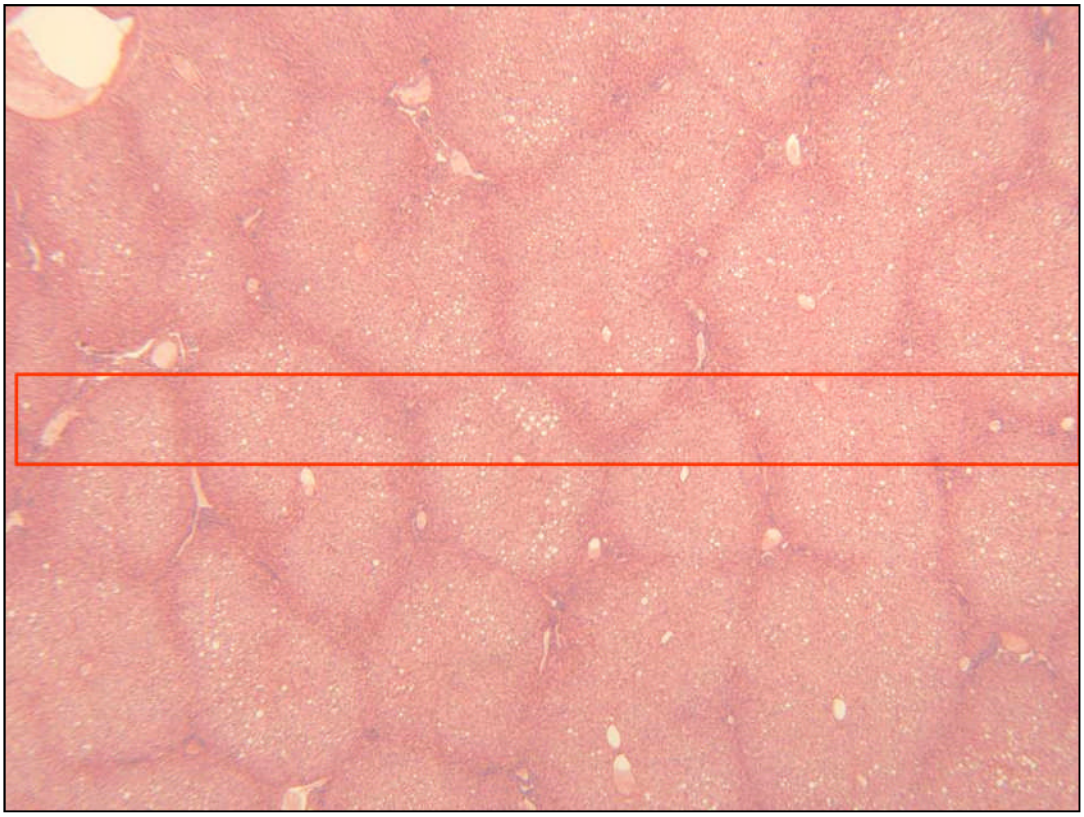
Microscopy

- Report cholecystectomies
- Recognise normal liver on needle biopsy
- Value of special stains
- Identify presence of cirrhosis, hepatitis or metastatic tumour in needle biopsy

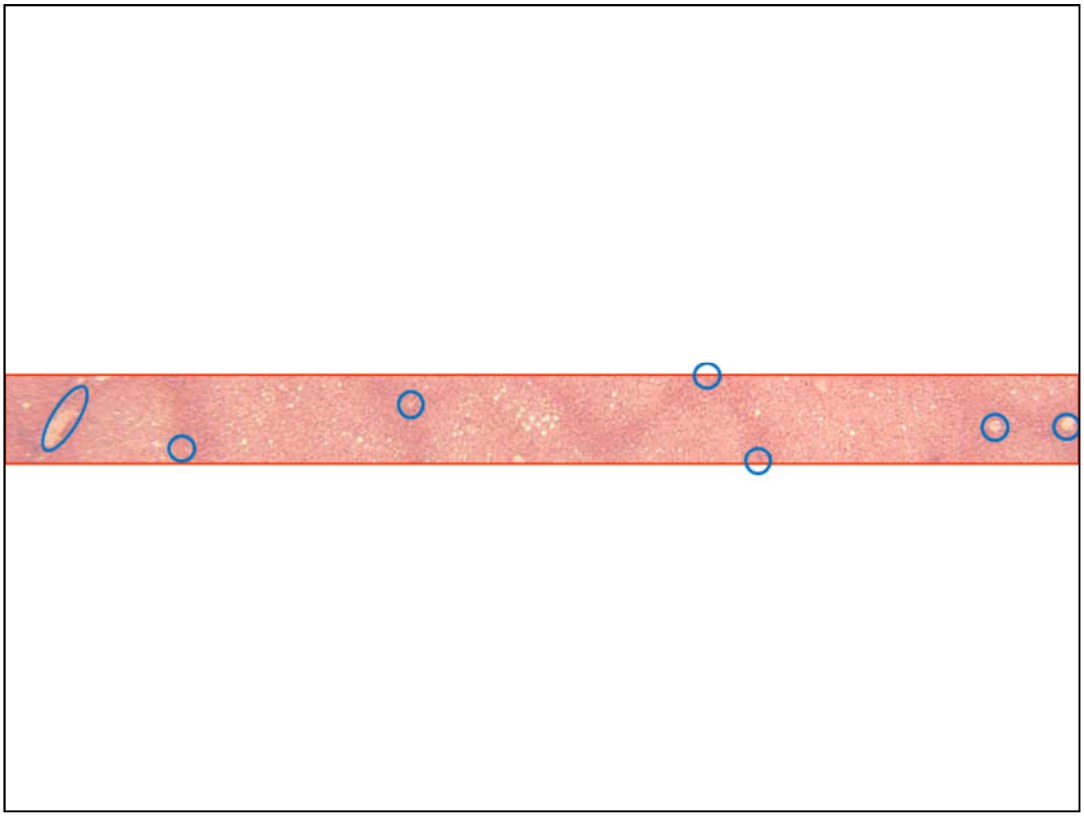
Microscopy

- Recognise normal liver on needle biopsy
  - How to approach liver biopsy
    - Identify portal tracts,
    - hepatic veins (terminal hepatic venules)
    - Everything between = parenchyma (hepatocytes) and sinusoidal cells – endothelium, Kupffer, etc.
  - Identify abnormalities –
    - architecture = distribution of vascular structures and fibrosis
    - Portal tracts
    - Parenchyma
    - Special stains
  - Put it all together in clinical context

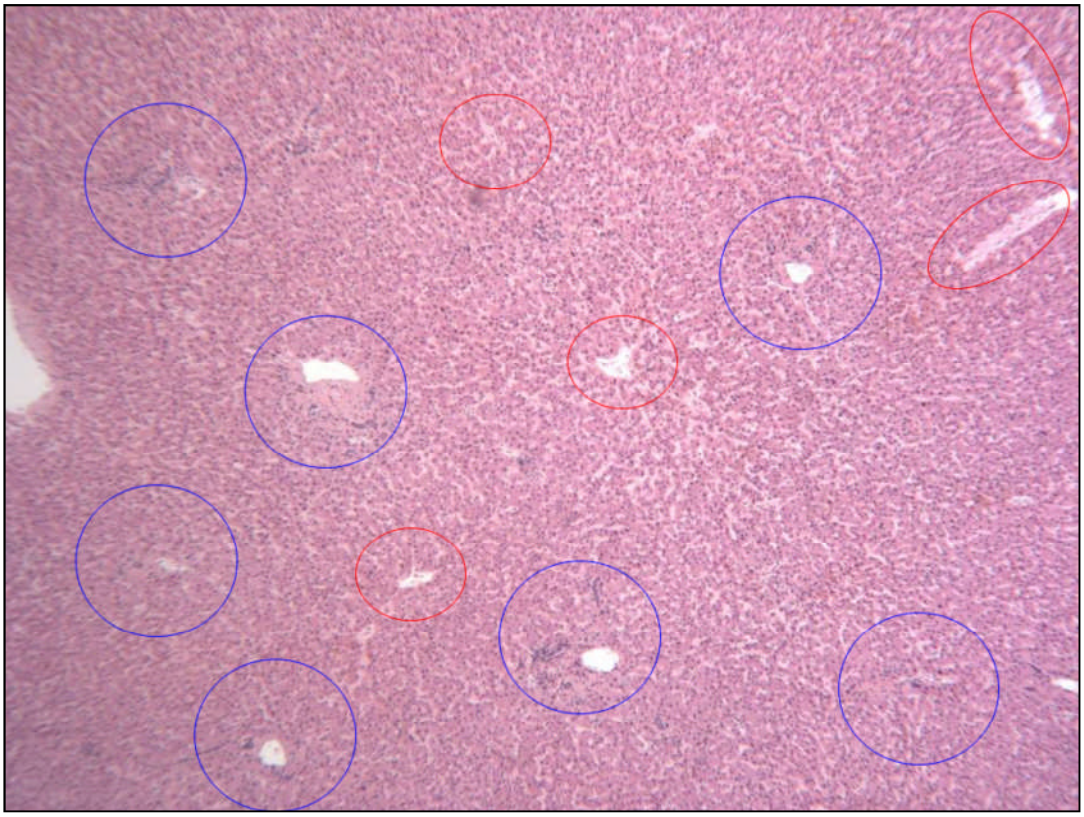
Have a system for looking at biopsy – overall pattern of abnormalities, is there chronic disease (abnormal architecture, increase reticulin) and systematic scrutiny of each of the structures, portal tracts, hepatic veins, parenchyma, special stains.



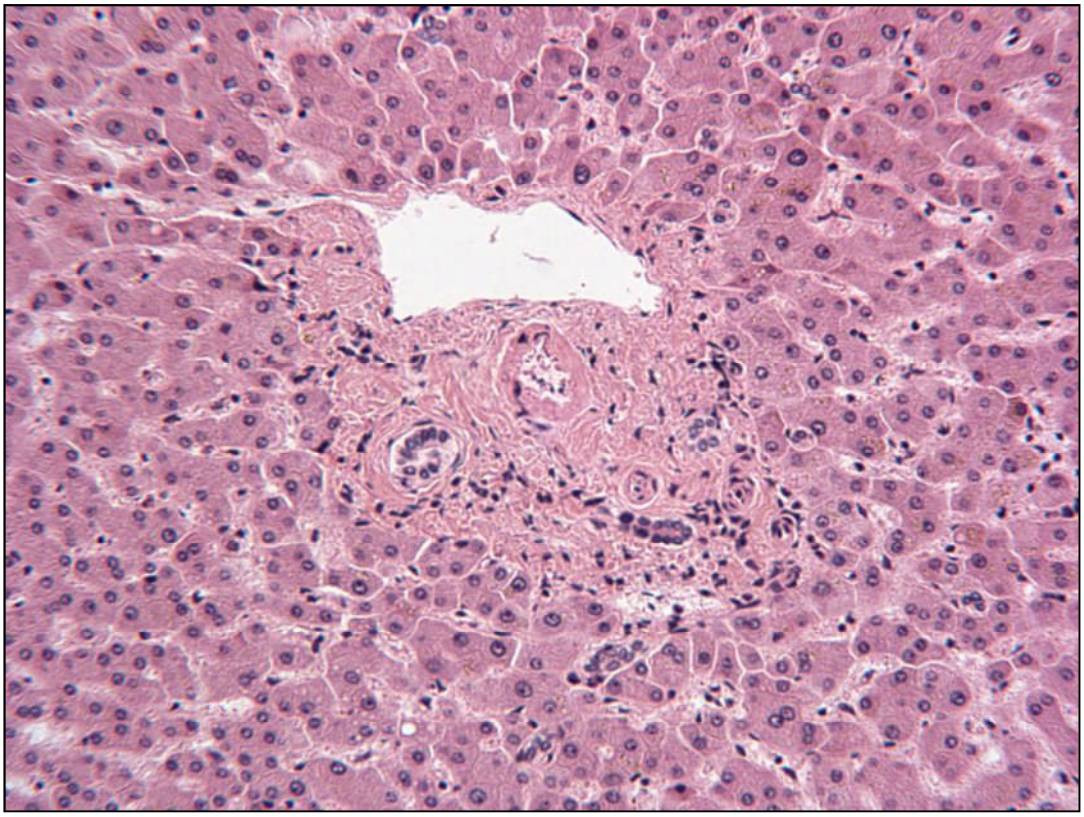
Sometime normal liver has accentuated lobular architecture due to different staining in hepatocytes – like the liver here. The red box is an example of the size of a needle biopsy.



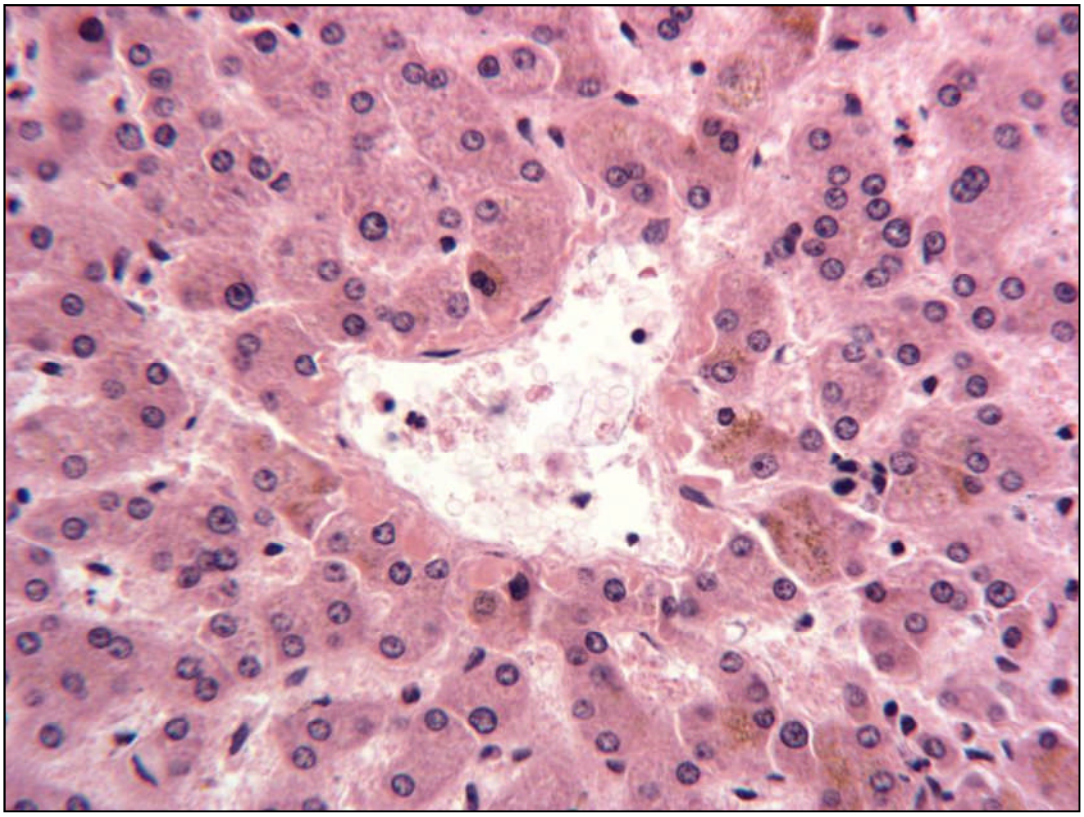
The portal tracts are outlined in blue. In normal liver they are separate but not all evenly distributed – think of branches of a tree.



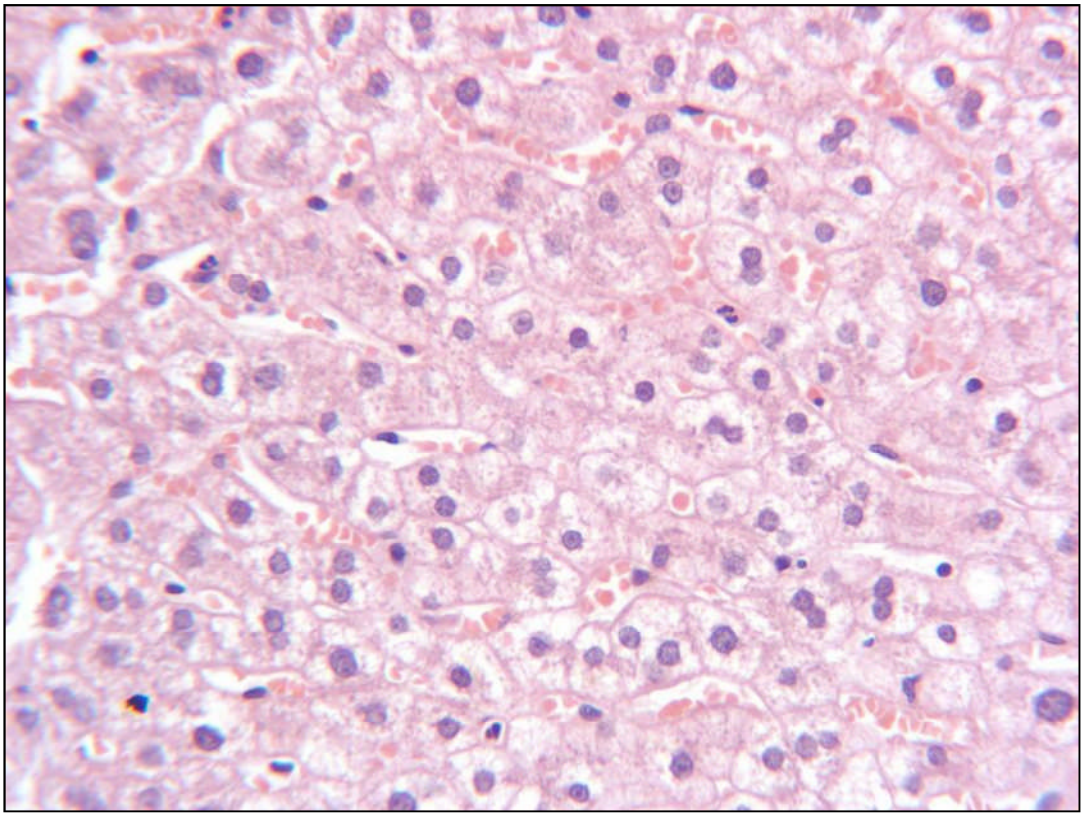
The hepatic (efferent) veins lie between portal tracts, approximately equidistant.



Normal portal tracts contain at least one each of hepatic artery, bile duct and portal vein. The hepatic artery and bile duct are similar in calibre and lie within 3 diameters of each other. The portal vein is about x4 the size of the hepatic artery.



Hepatic veins are single structures, with some collagen in their wall. Surrounding hepatocytes often have some pigment. This can be ceroid pigment if there is little hepatocyte turnouver, or bilirubin if there is cholestatic liver disease with jaundice. These look very similar. If the pigment is bilirubin, this will be convincing because of bile plugs in the tiny canaliculi between adjacent hepatocytes, and/or active Kupffer cells (which are positive on PAS diastase).



Hepatocyes in the parenchyma between the vascular structures. Liver cells lie in cords/plates that are 1-2 cells thick with sinusoids between, them. Hepatocytes are often binucleate. The parenchyma is considered in acinar zones – zone 1 = periportal hepatocytes, zone 3 = perivenular hepatocytes and zone 2 are hepatocytes between. These zones have different functions e.g. drug metabolism is mainly in zone 3 – but they look the same in routine stains.

Microscopy

- Report cholecystectomies
- Recognise normal liver on needle biopsy
- Value of special stains

Usual panel

Architecture:
- Retic
- van Gieson
- Shikata

Hepatocytes:
- PAS, PASD,
- Shikata

Pigment:
- Perl's

This is our panel of routine liver stains. They serve to highlight different aspects of liver histology, like looking through coloured glasses to emphasise different structures.

Usual panel

Architecture:

Retic – liver cell plates, stage of chronic liver disease

van Gieson – mature collagen, acute v. chronic liver disease, hepatic veins

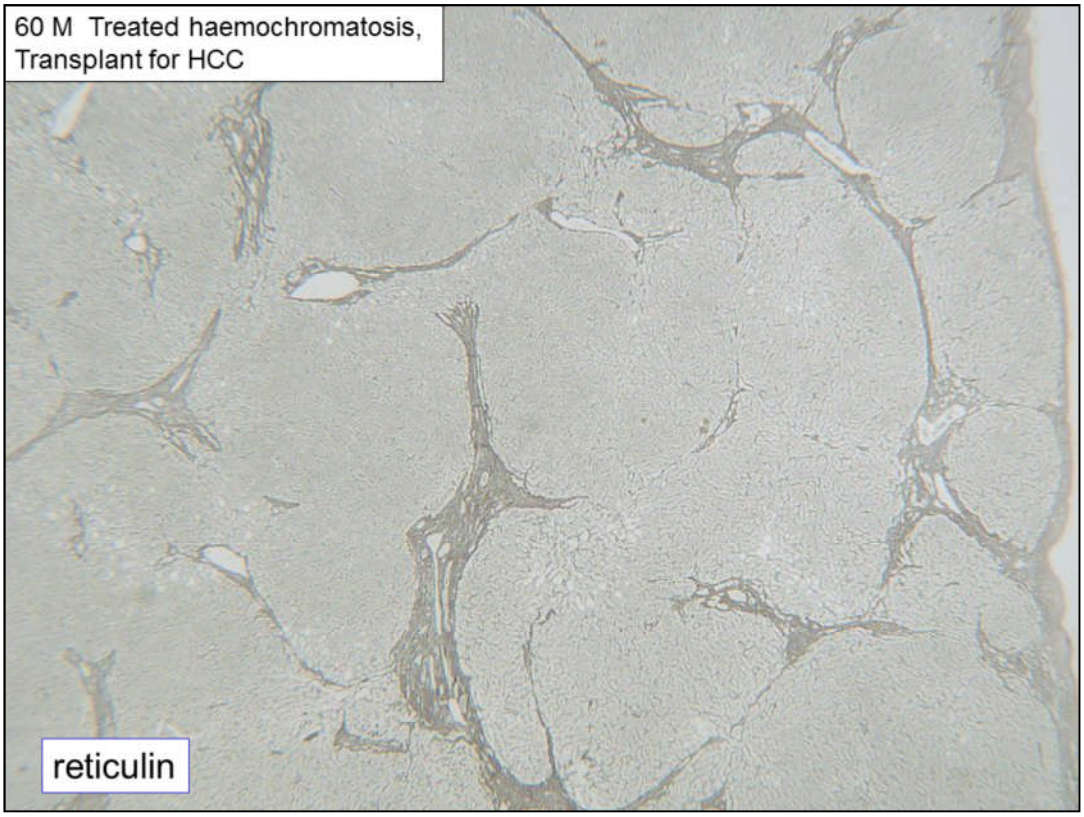
Shikata – elastic, in vessels and long standing fibrosis

Hepatocytes:

PAS, PASD,
Shikata

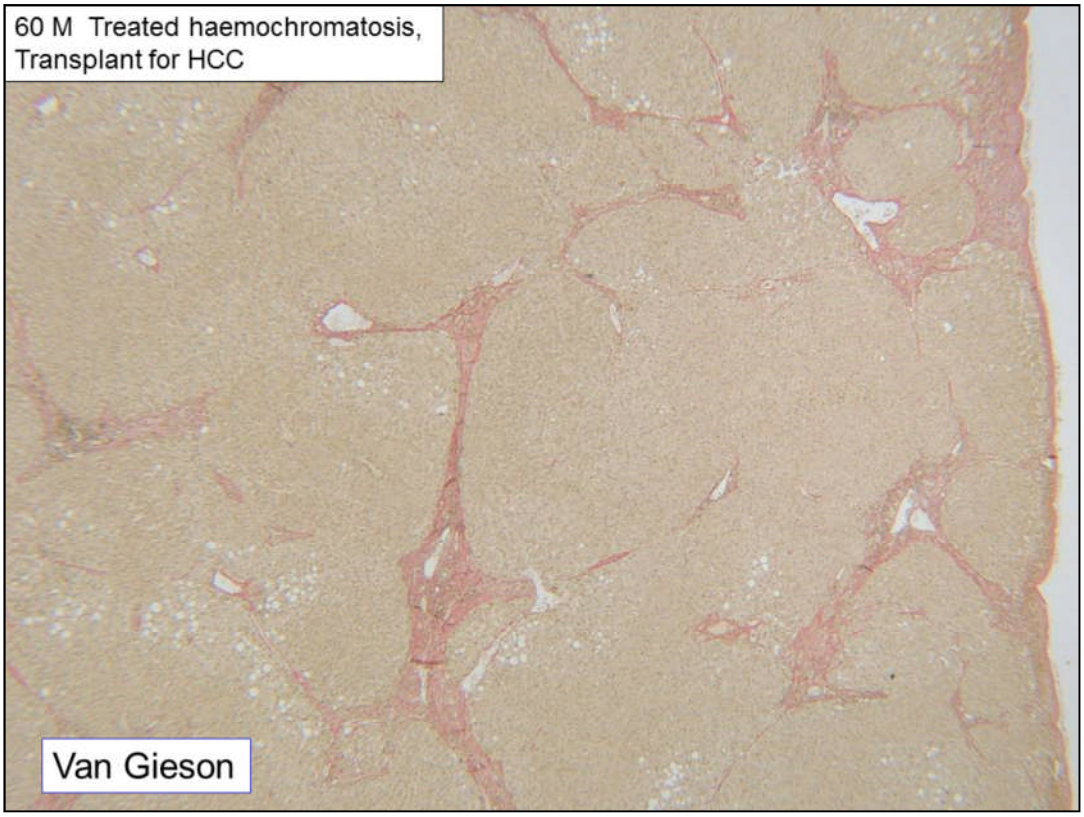
Pigment:

Perl's

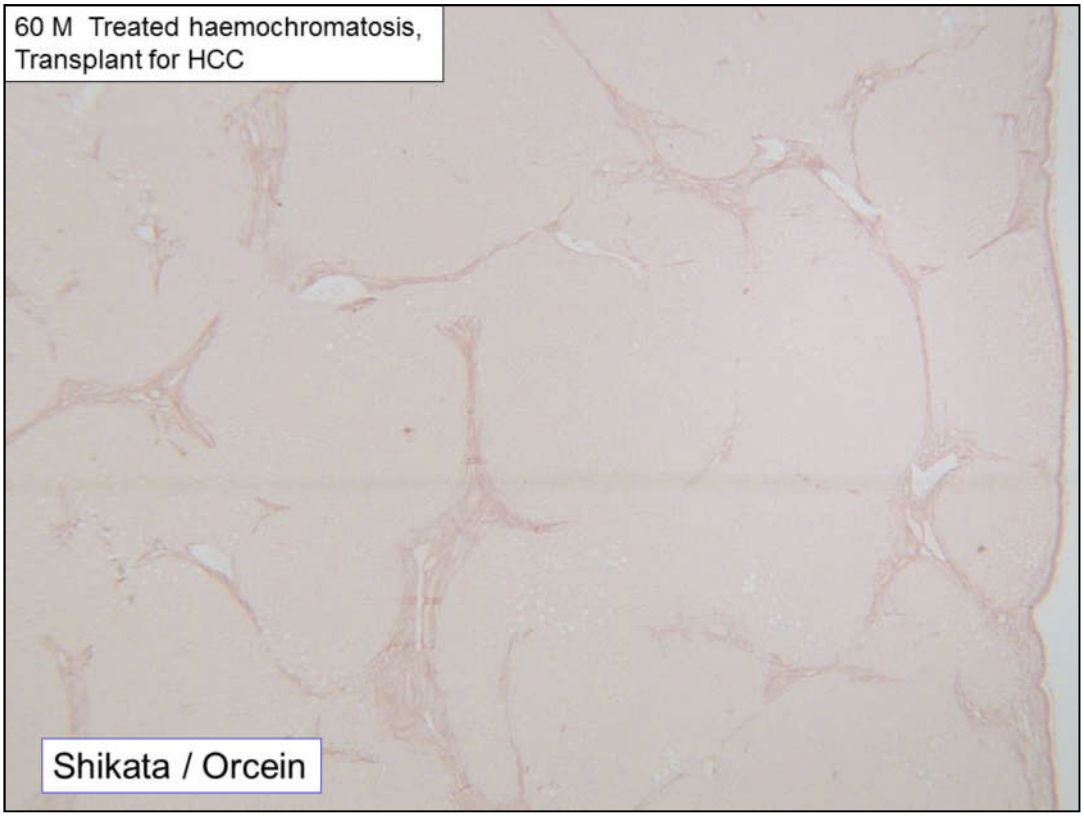


Reticulin is in portal areas, and also outlines liver cell plates. These don't have a basement membrane. In chronic liver disease, septa of fibrosis develop and grow to link vascular structures – in 3d these are curved surfaces of connective tissue, not lines. In this case of treated haemochromatosis, the fibrosis present is long standing, and therefore also shows positivity for elastic fibres on the Shikata stain.

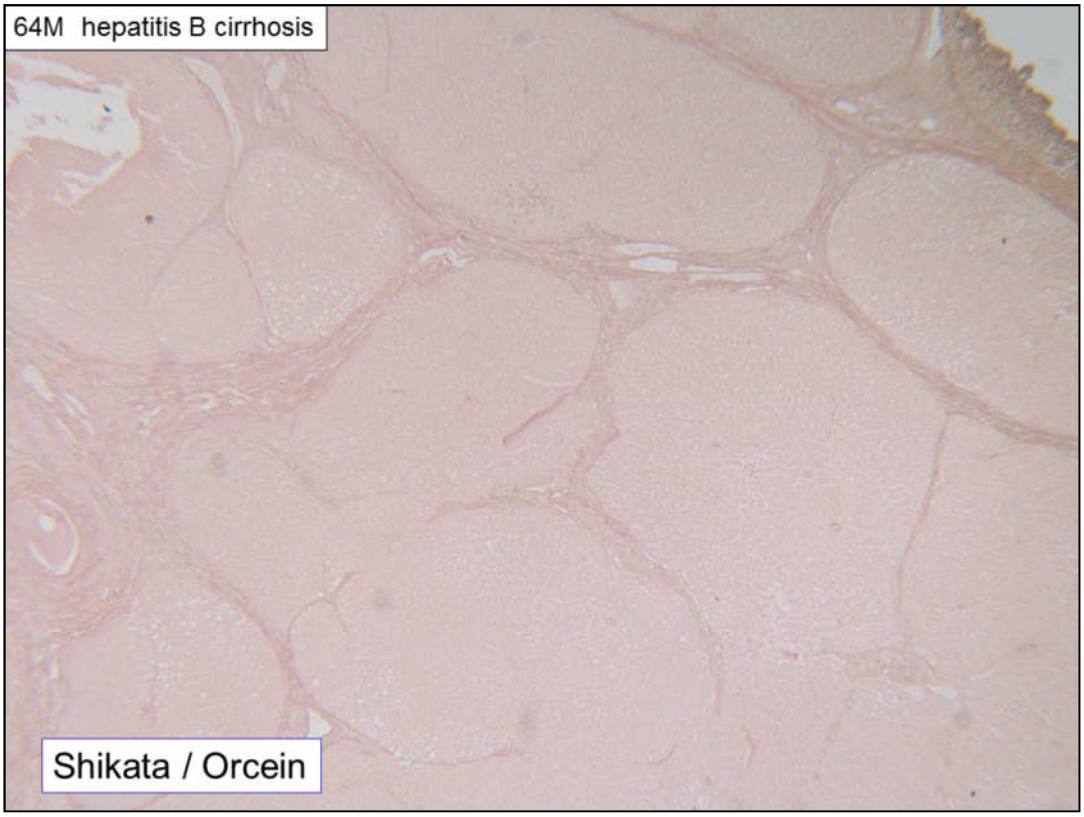
If there has been severe acute liver injury when confluent areas of liver cells undergo necrosis, the reticulin fibres come to lie close together forming apparent areas of fibrosis – but these are 'passive' septa without active scar formation.



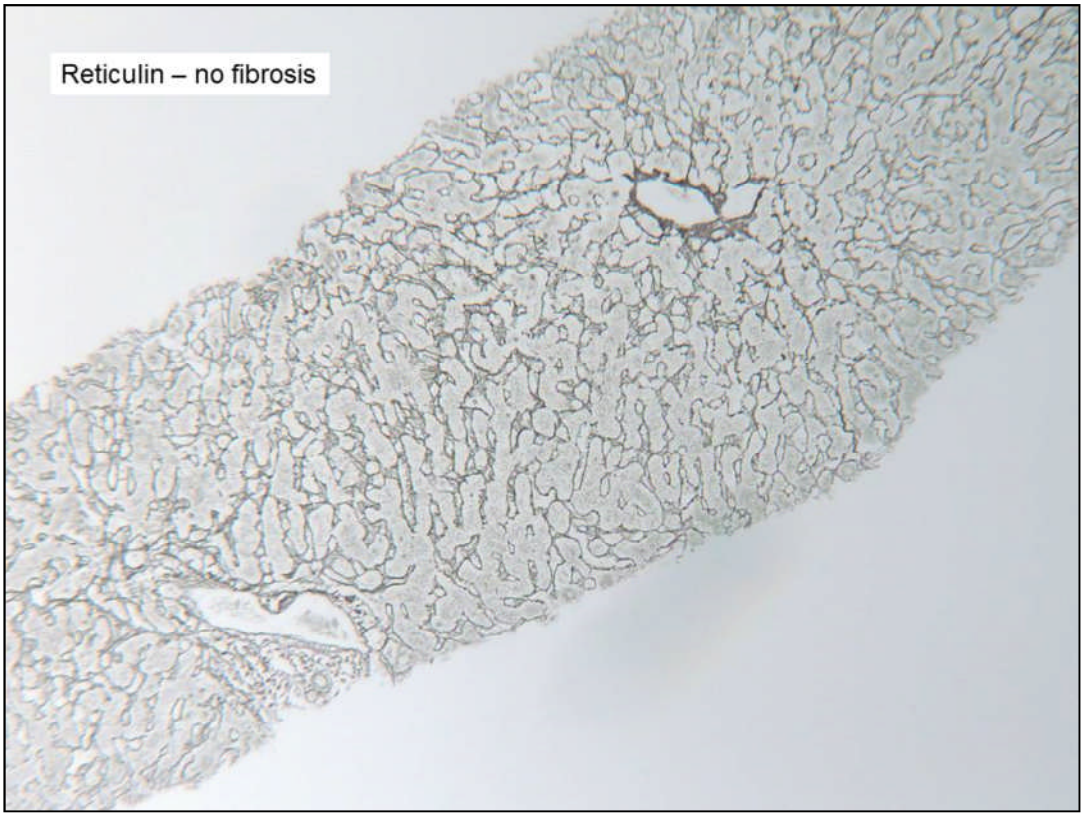
Van Gieson stains type 1 collagen in scar tissue. This distinguishes chronic disease with scarring from recent acute injury with necrosis and collapse. In this case, all the fibrous septa are densely staining with crisp edges – this is characteristic of long standing and non-progressing fibrosis.



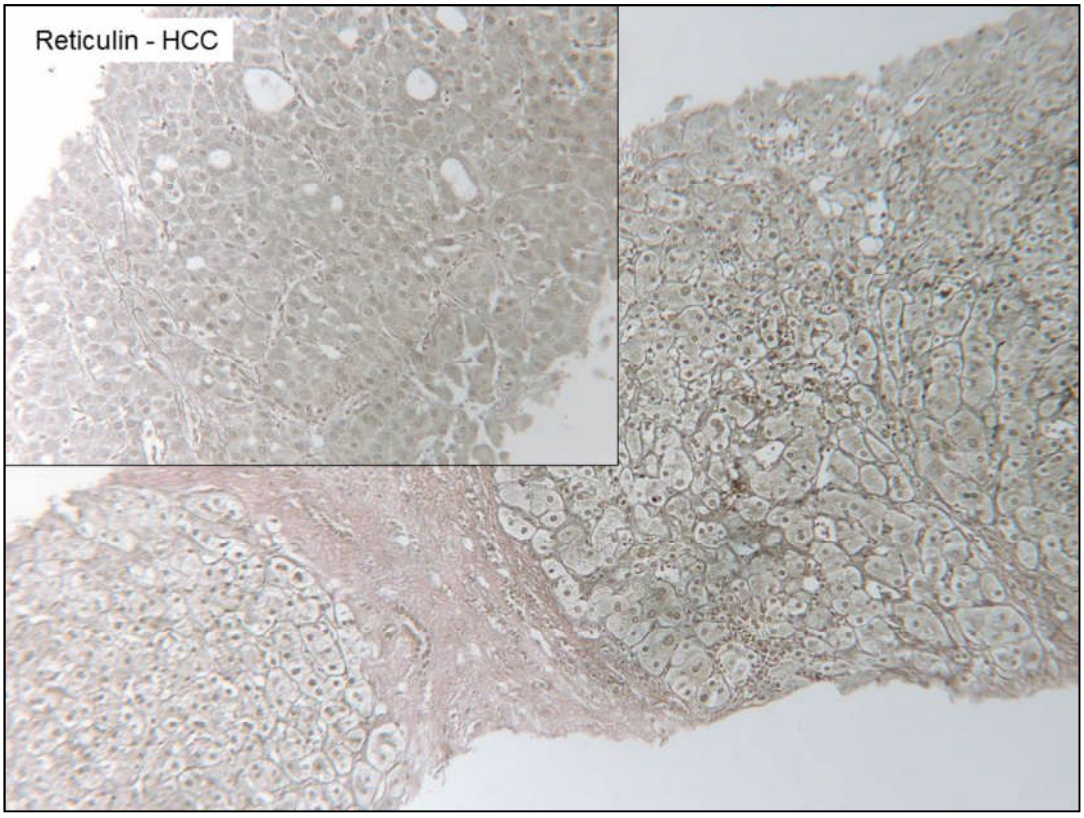
The third stains for architecture is the Shikata method of using the Orcein stain. This shows where elastic fibres are – e.g. normally in the wall of arteries and in the liver capsule. In this example of long standing chronic disease, all the fibrosis positive on van Gieson is also positive for elastic.



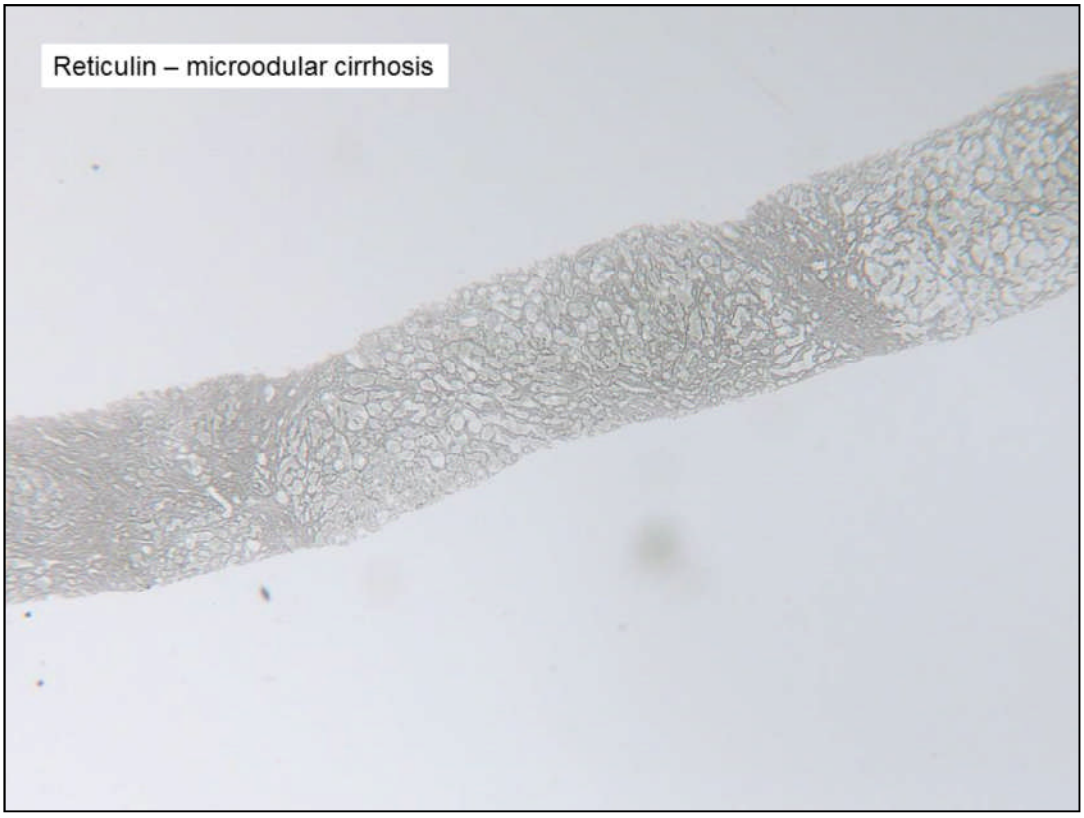
Shikata also stains hepatitis B surface antigen in hepatocyte cytoplasm. – and also copper associated protein, see later.



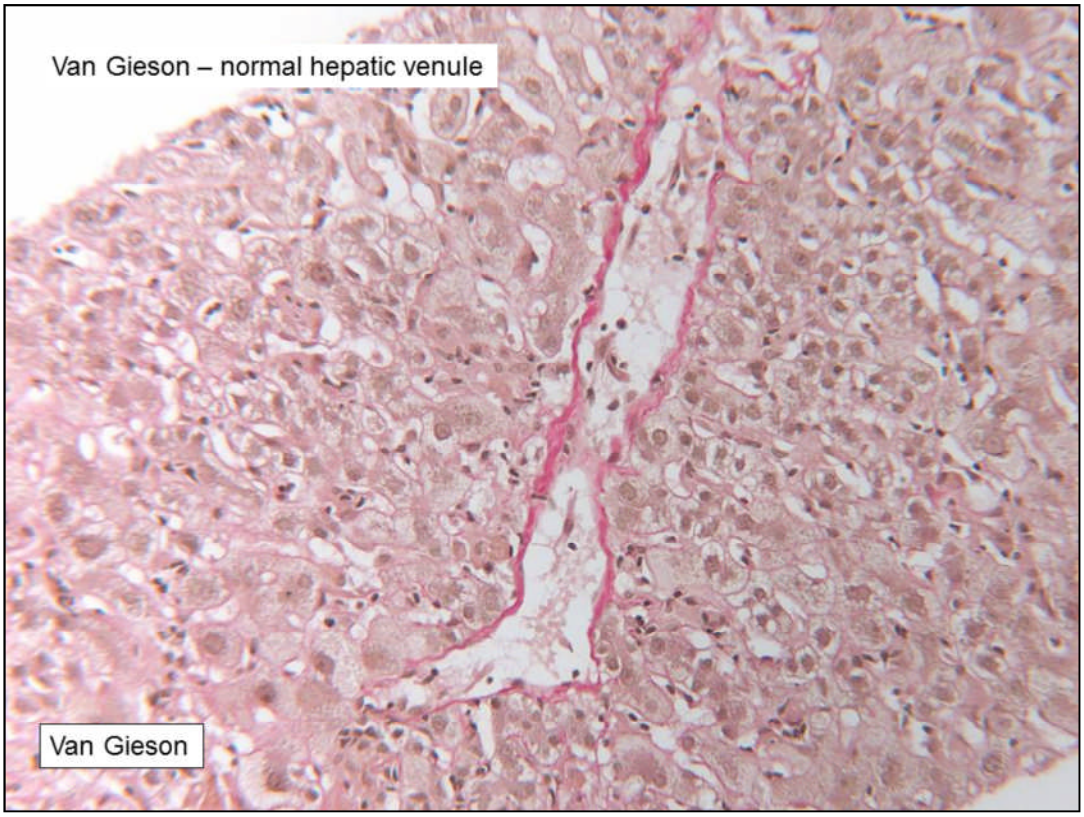
Reticulin normally shows just enough connective tissue to support portal tracts, with a rounded outline. Liver cell cords radiate between portal tracts and hepatic veins, with a few foci of thickened plates and interrupted plates – these are both present in this biopsy, and suggest there has been some acute liver injury recently.



Reticulin can be absent in hepatocellular carcinoma, which can be a useful sign in well differentiated hepatocellular neoplasms.



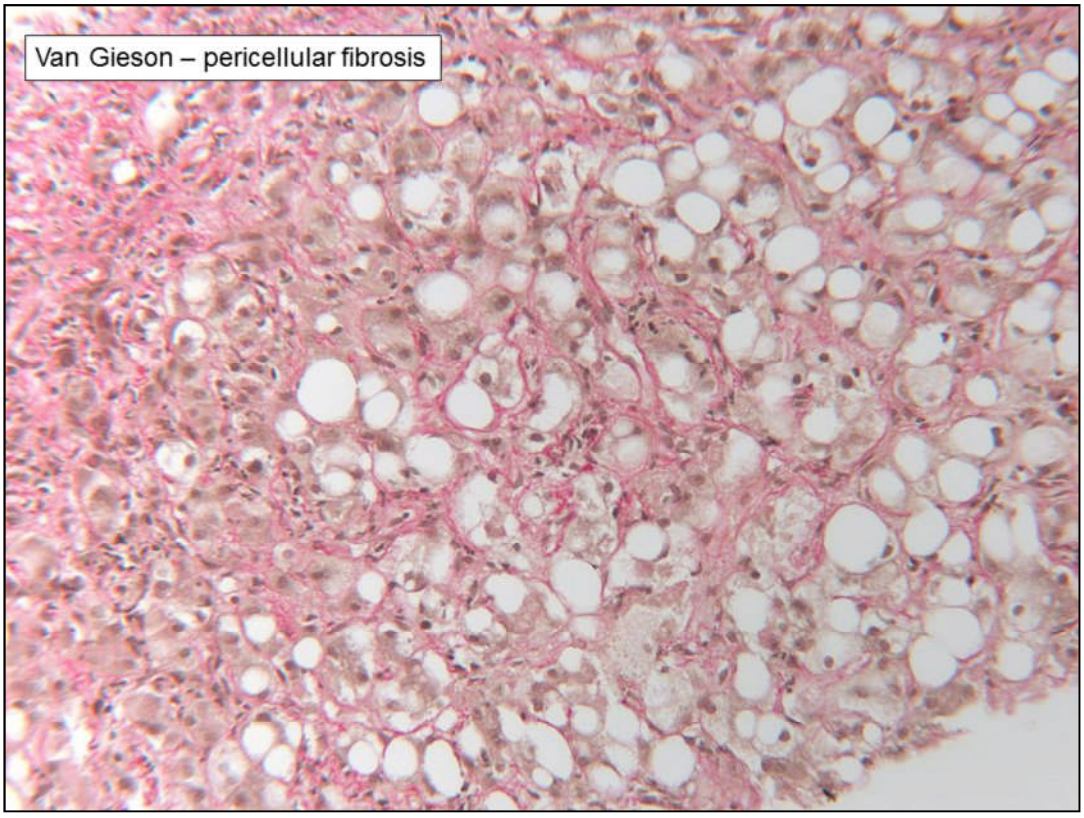
This biopsy shows bands of fibrosis and also increased fibrosis in sinusoids, with little nodular regeneration – this pattern is characteristic of micronodular cirrhosis in alcohol related liver disease.



Van Gieson demonstrates the collagen in hepatic vein walls. Normally there is no red staining collagen in the sinusoids.

Different departments use different collagen stains – some prefer Masson trichrome, which stains collagen blue. Some use Sirius red, which stains collagen red, more strongly than van Gieson. It is used for quantitating collagen by image analysis morphometry. It may suggest more perisinusoidal fibrosis than other stains.

Van Gieson also stains bile bright green – sometimes useful in confirming bilirubinostasis.



IN this example of steatohepatitis, the van Gieson shows collagen in the sinusoids and surrounding the fatty and ballooned hepatocytes (pericellular fibrosis) in a ‘chicken wire’ pattern.

If there is congestion of sinusoids around the hepatic veins, look at the van Gieson stain – in this case there is occlusion of the lumen by loose connective tissue, which may be seen in veno-occlusive disease or in venous outflow obstruction in Budd Chiari syndrome.

Usual panel

Architecture:
- Retic
- van Gieson
- Shikata

Hepatocytes:
- PAS, PASD,
- Shikata

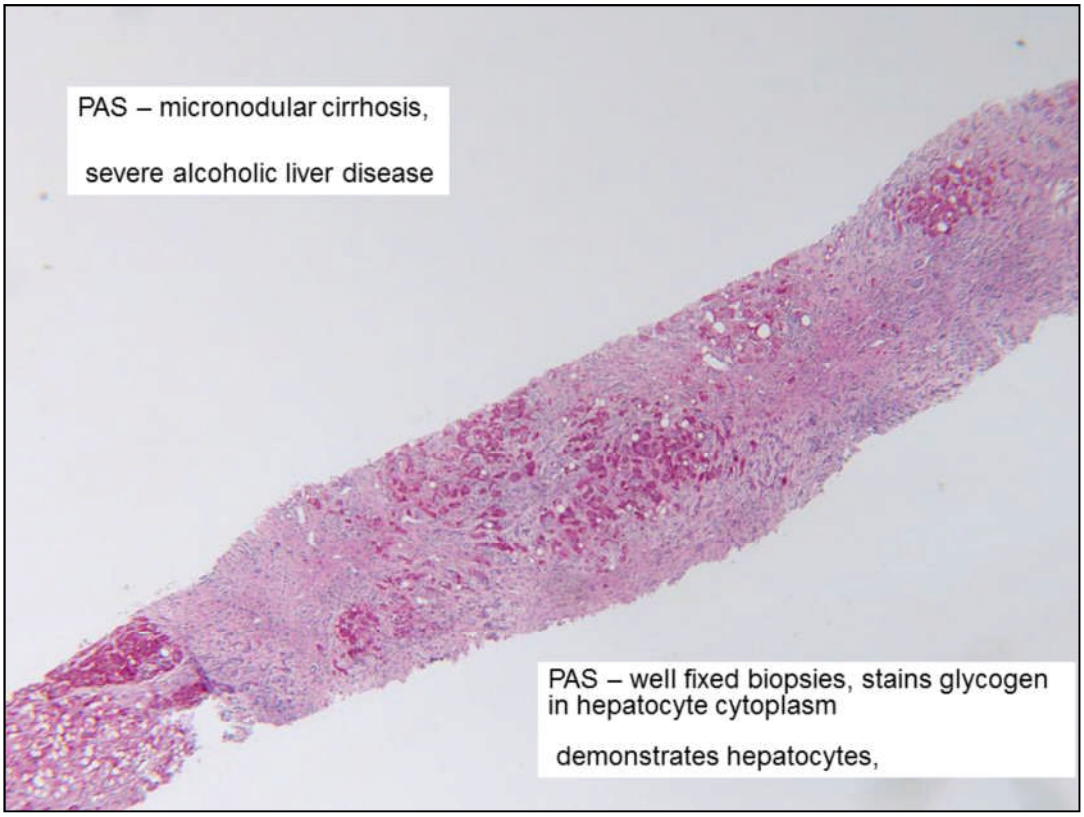
Pigment:
- Perl's

Usual panel

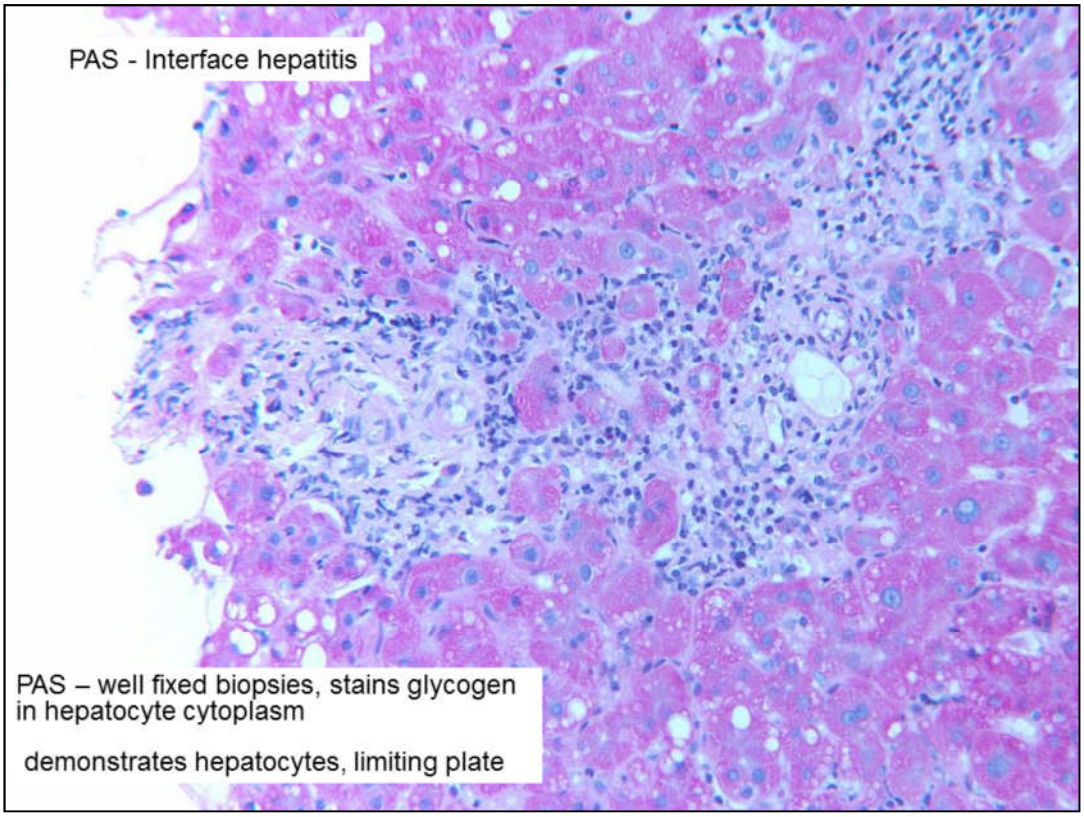
Architecture:
- Retic
- van Gieson
- Shikata

Hepatocytes:
- PAS – well fixed biopsies, stains glycogen in hepatocyte cytoplasm
  demonstrates hepatocytes, limiting plate
- PASD – stains glycoproteins, glycogen removed by diastase
  active Kupffer cells, A1ATD, bile ducts, basement membrane
- Shikata – copper-associated protein, HBsAg

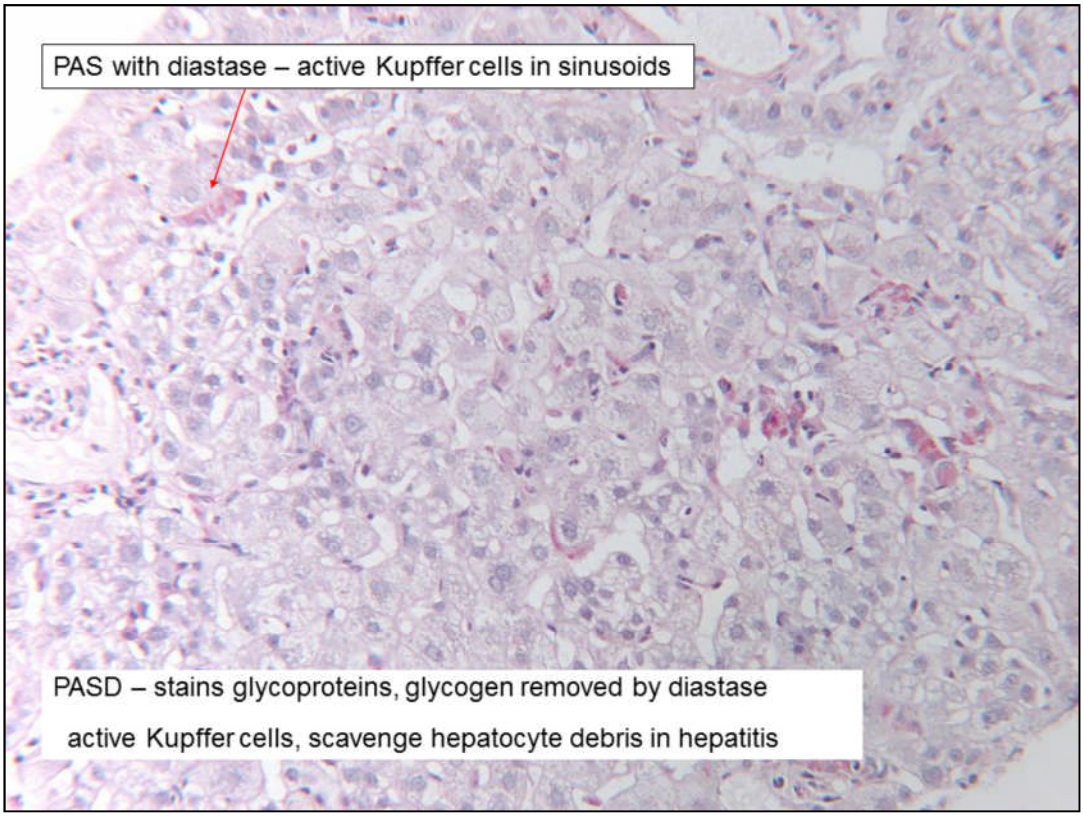
Pigment:
- Perl's



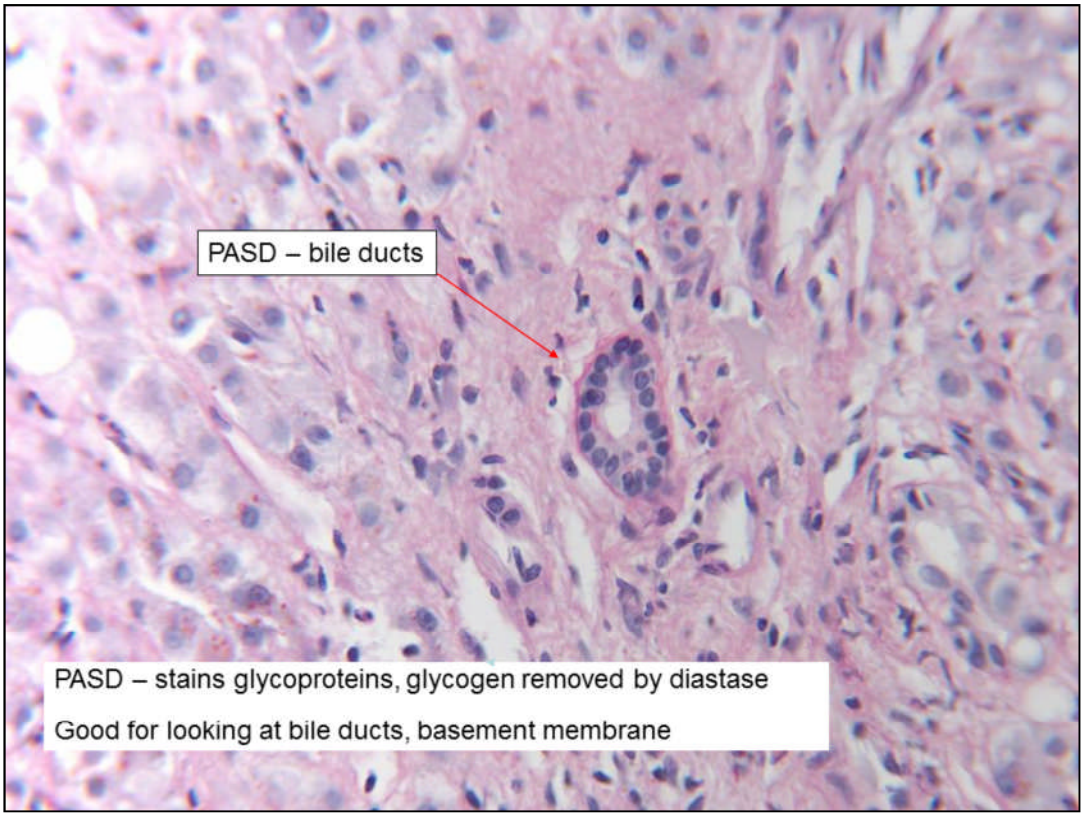
Glycogen is lost from hepatocytes when biopsies aren't well fixed. But in well fixed biopsies it can be helpful I demonstrating hepatocytes, e.g. highlighting where parenchymal granulomas are, or illustrating the texture as in this case – the same case as the reticulin stain a few slides earlier.



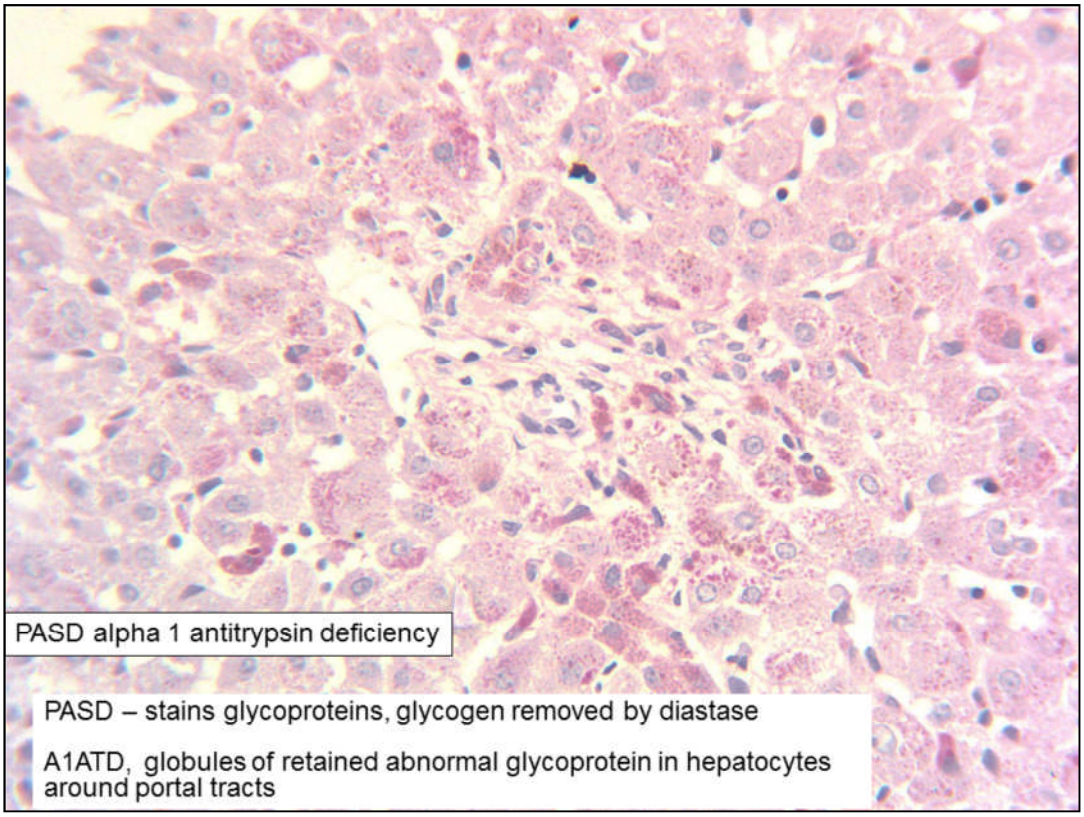
At high magnification, the interruption of the limiting plate at the margin of portal tracts which identifies interface hepatitis, is well seen on PAS.



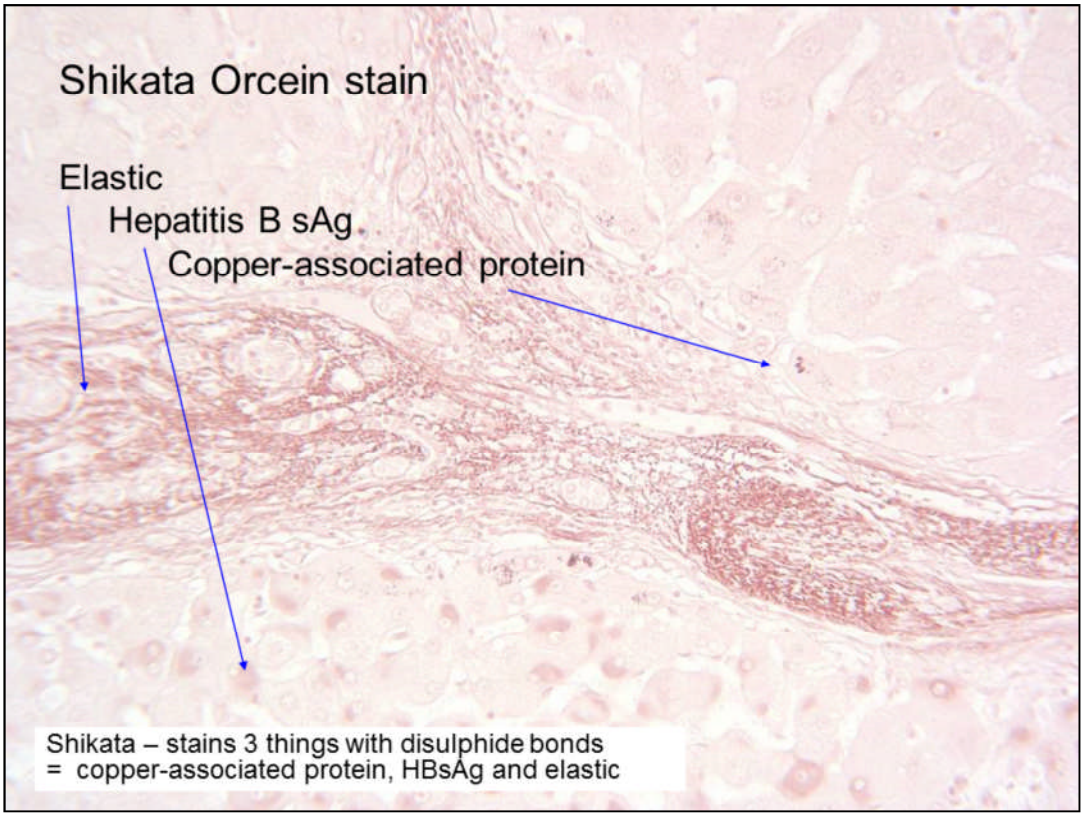
PAS with diastase is more useful than PAS alone. The diastase dissolves the glycogen, glycoproteins remain positive. Therefore scavenging macrophages are highlighted, and these are a useful sign of recent hepatocyte injury, which may persist for a few weeks after the acute hepatitis activity has subsided.



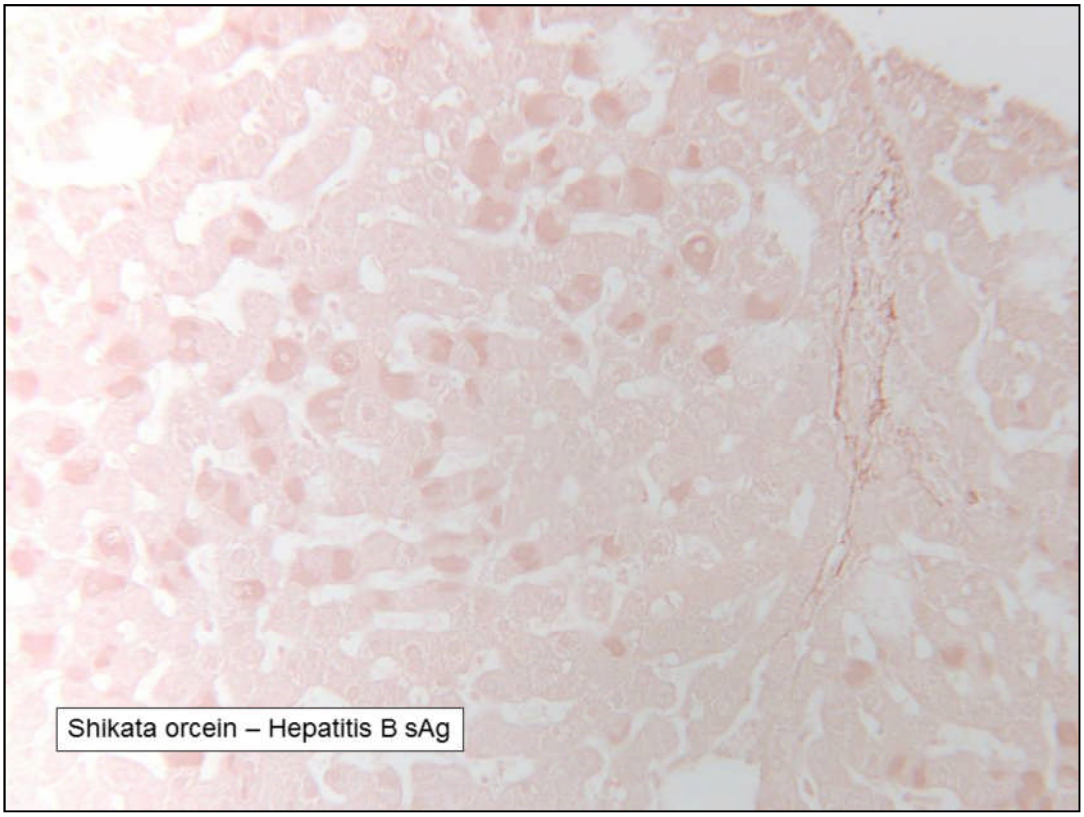
The basement membrane of bile ducts stains for PASD, and this is generally a good stain for looking at the detail of bile ducts. In PSC, the basement membrane can be thickenend.



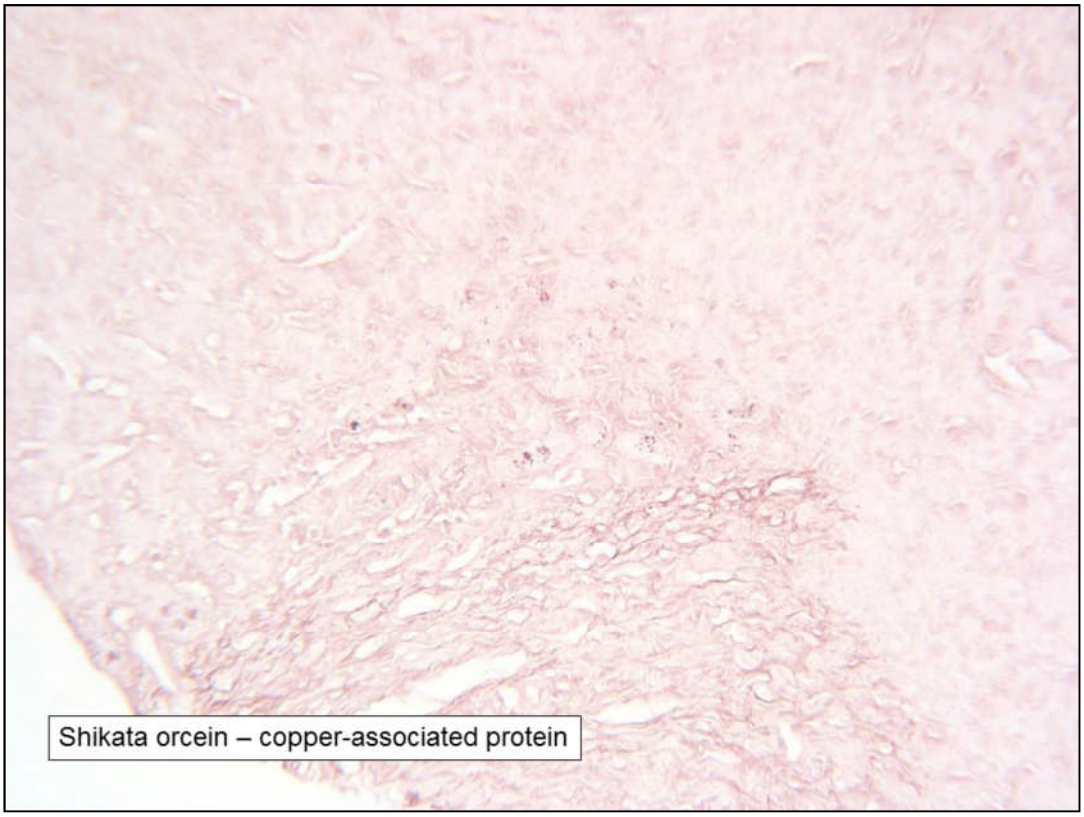
In alpha 1 antitrypsin deficiency, the PASD globules in periportal hepatocytes are characteristic – individual hepatocytes either have small numbers of big globules or lots of small globules. Copper associated protein can also be PASD positive but unlike A1AT is positive on Shikata.



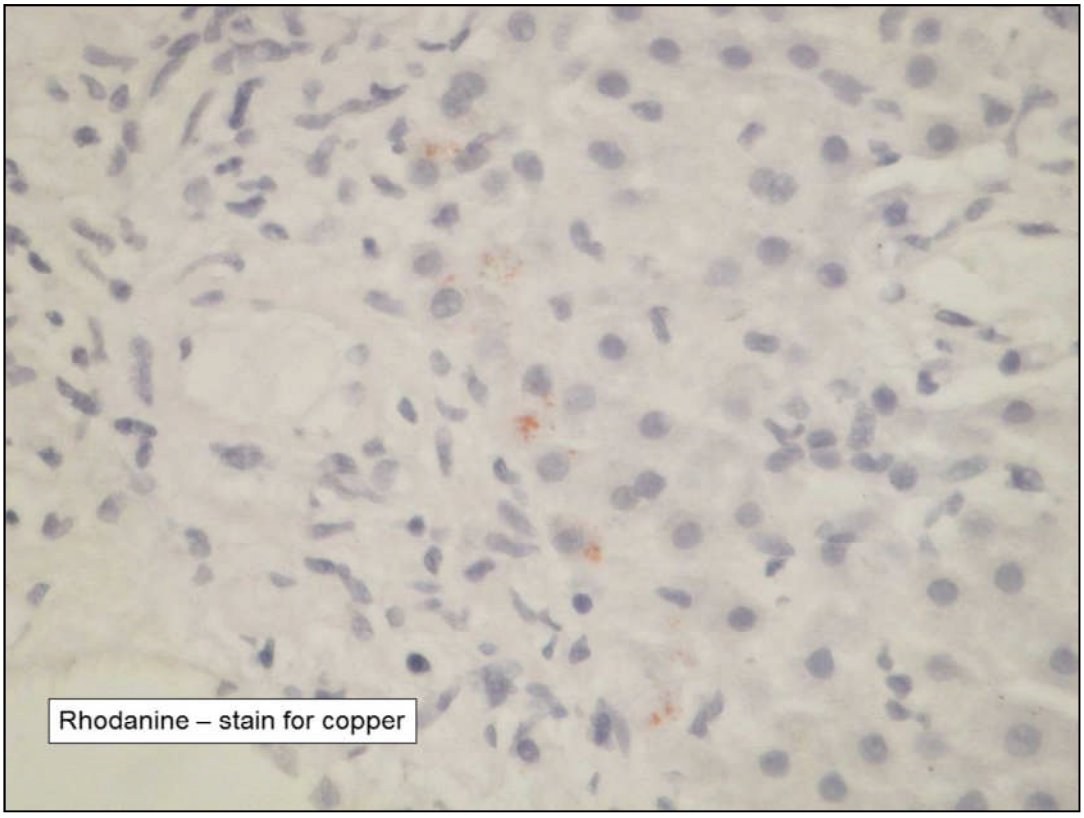
The Shikata stain has three uses in liver histology.



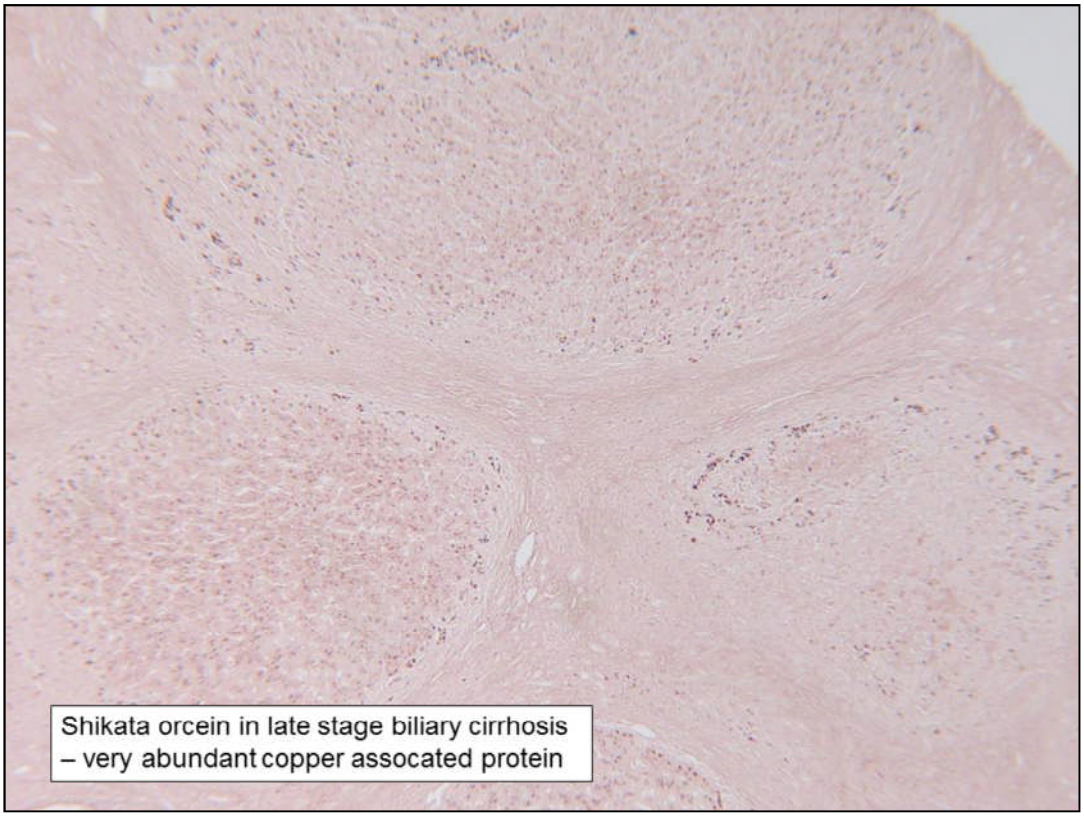
Hepatitis B is usually included in clinical details. If the Shikata is unclear, confirm whether HBsAg is present by immunohistochemistry – although looking up the results of blood tests is an alternative for discovering the patient's hepatitis B status.



In chronic cholestatic diseases, copper accumulates in periportal hepatocytes, complexed with copper associated protein which stains as black granules on Shikata. Both copper and bile salts are excreted into bile and accumulate in hepatocytes in chronic cholestatic disease. The patient may be itchy (bile salts in skin?) but not jaundiced unless there is obstruction of the bile duct. In diseases where individual intrahepatic ducts are damaged/destroyed, the copper associated protein accumulates around the affected portal tracts but not tracts with healthy ducts.



Shikata can be a technically unpredictable stain – if in doubt use Rhodanine to directly stain copper.



Small amounts of copper associated protein in liver without much cirrhosis – is very likely to be due to chronic cholestatic liver disease.

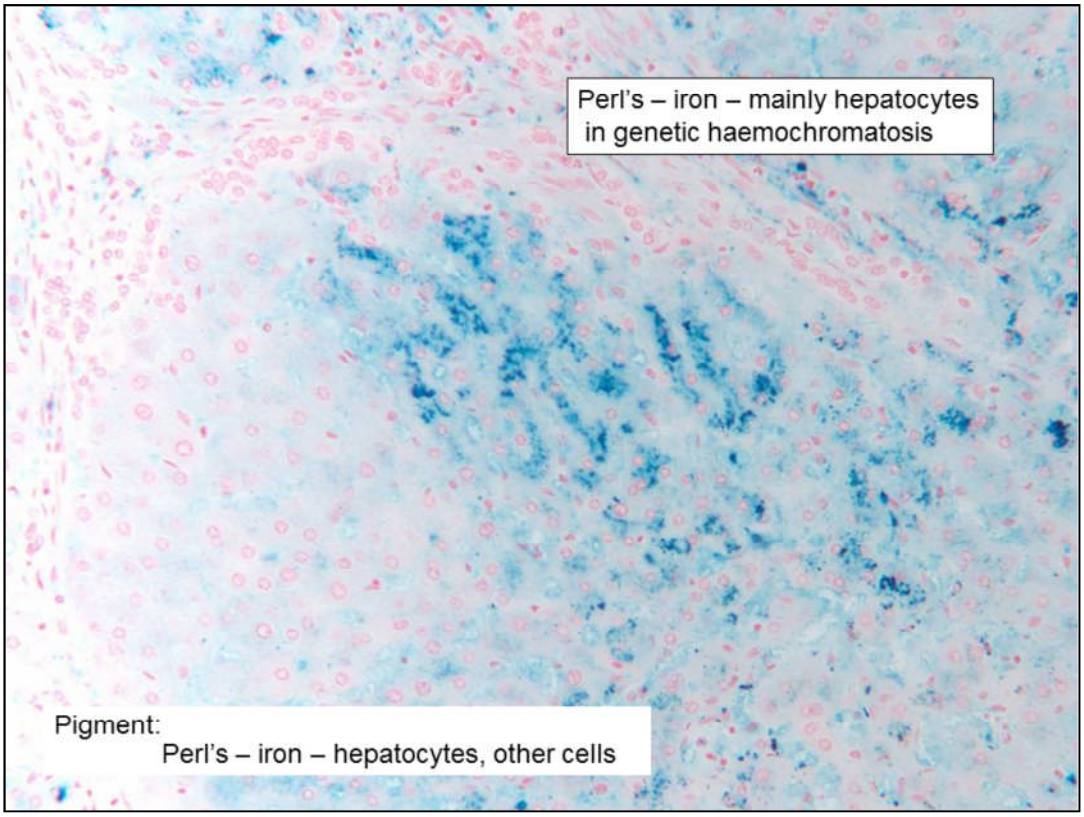
In cirrhosis – small amounts of copper associated protein can be present, just as a consequence of distortion of the biliary tree. In cirrhosis due to chronic cholestatic disease (PSC, PBC) – there can be large amounts of copper associated protein, and also clearly demarcated nodules with a halo around the perimeter of the nodules.

Usual panel

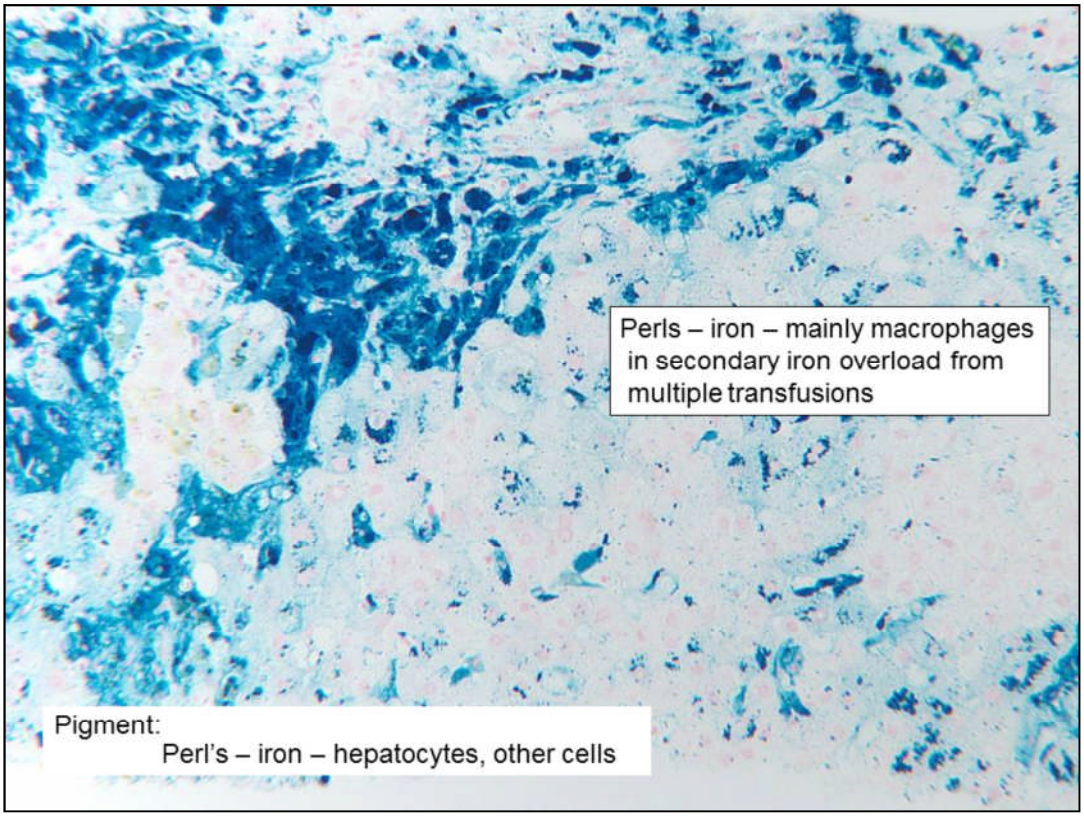
Architecture:
- Retic
- van Gieson
- Shikata

Hepatocytes:
- PAS, PASD,
- Shikata

Pigment:
- Perl's – iron – hepatocytes, other cells



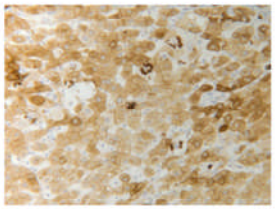
The Perl's stain shows iron and where it is. Iron in hepatocytes is usually graded 0-4 with grade 4 being iron in nearly all hepatocytes. In genetic haemochromatosis, iron absorbtion from the gut is not switched off when iron stores are full. Excess iron is stored in various body sites including hepatocytes.



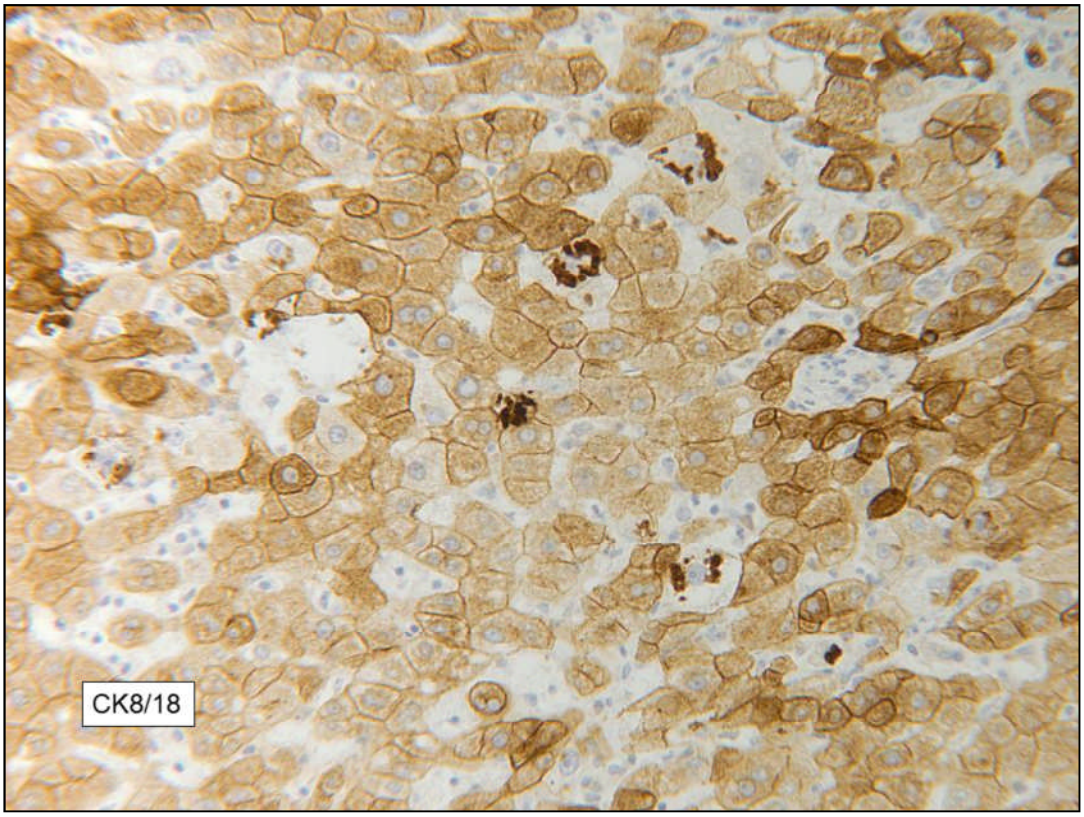
When iron is derived from the breakdown of erythrocytes, it is stored in macrophages more than hepatocytes. In acute hepatitis, the macrophages also are often positive for iron as well as PASD.

Immunohistochemistry?

- Traditional panel of special stains depends on affinity of dyes for different chemical moieties. Plant or chemical dyes
- Can use immunohistochemistry for specific antigenic proteins
- Mallory bodies in steatohepatitis
  - Ubiquitin
  - CK8/18
  - Cam 5.2

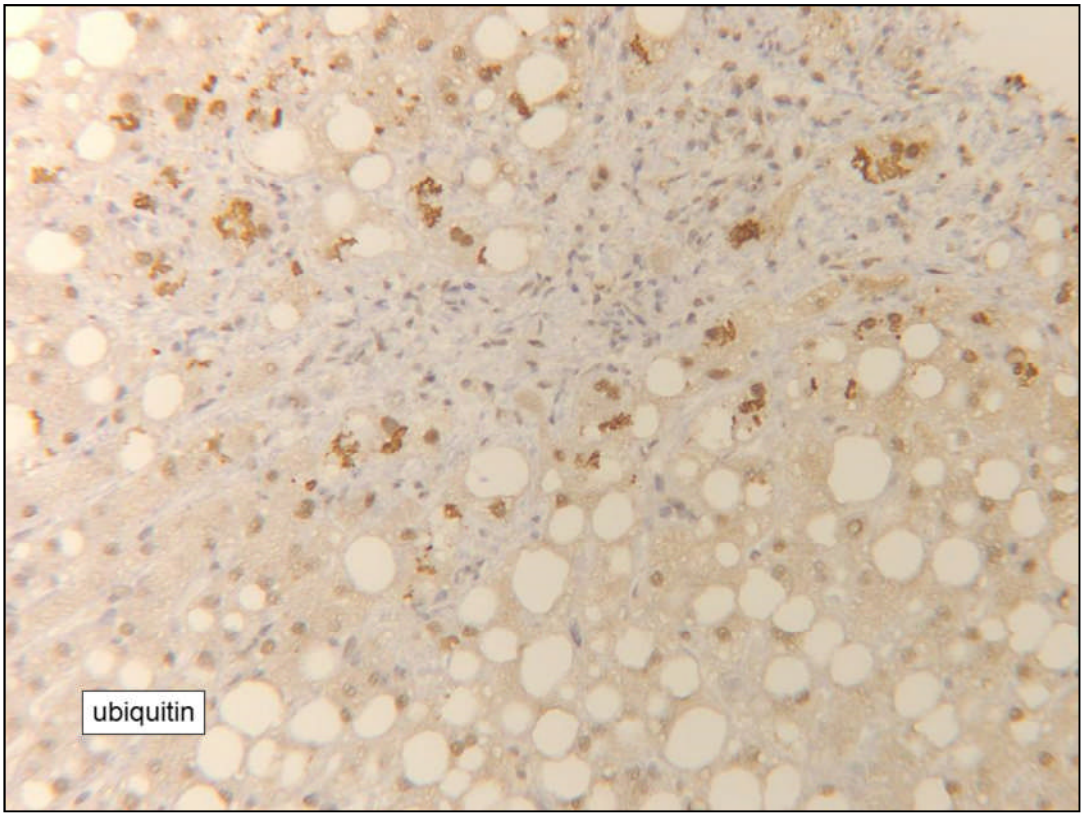


- Bile ducts and ductular reaction
  - CK7
- Others – alpha 1 antitrypsin,
  viruses – hepatitis B, CMV, HSV



In steatohepatitis, free radical damage from oxidative stress results in damage to the cytoskeleton with unfolding of the cytokeratins which are complexed with ubiquitin into structures called Mallory Denk bodies. Cytokeratin stains show loss of cytoplasmic staining in ballooned hepatocytes as well as the strong positive MDBs.

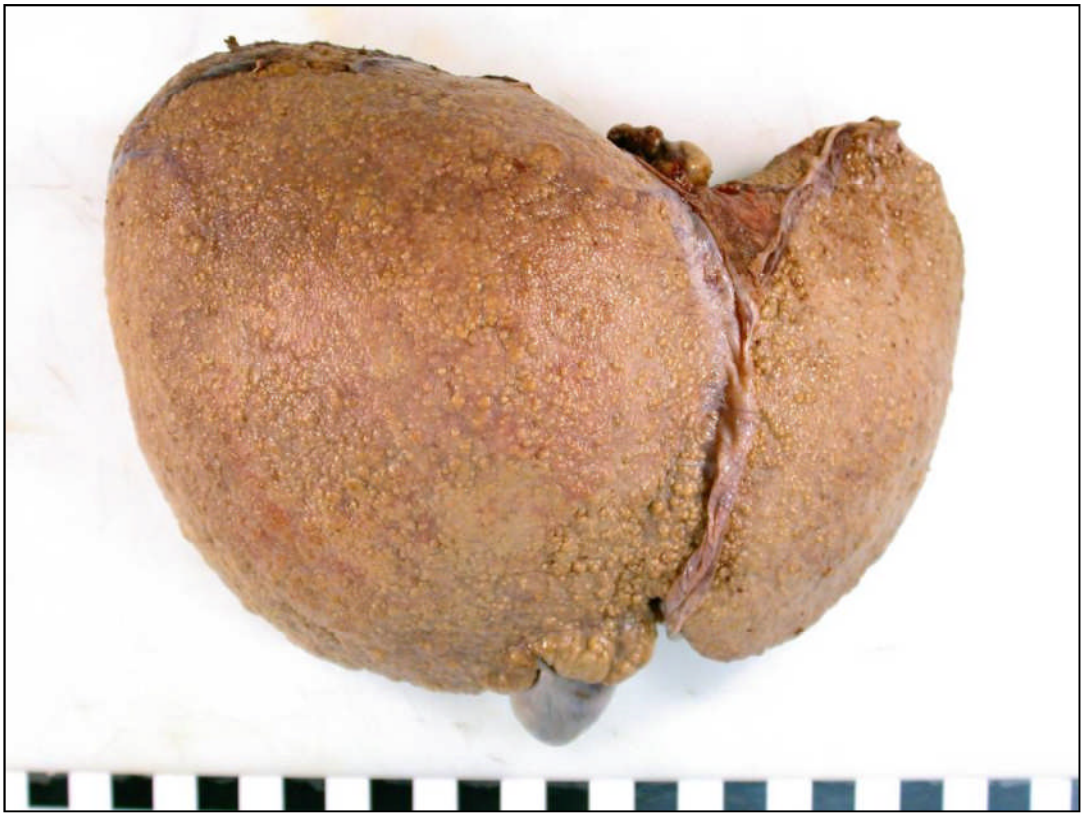
We have switched to Cam 5.2 for this purpose, following a change in laboratory IHC platform.



Ubitquitin also shows MDBs but not the loss of cytokeratin.

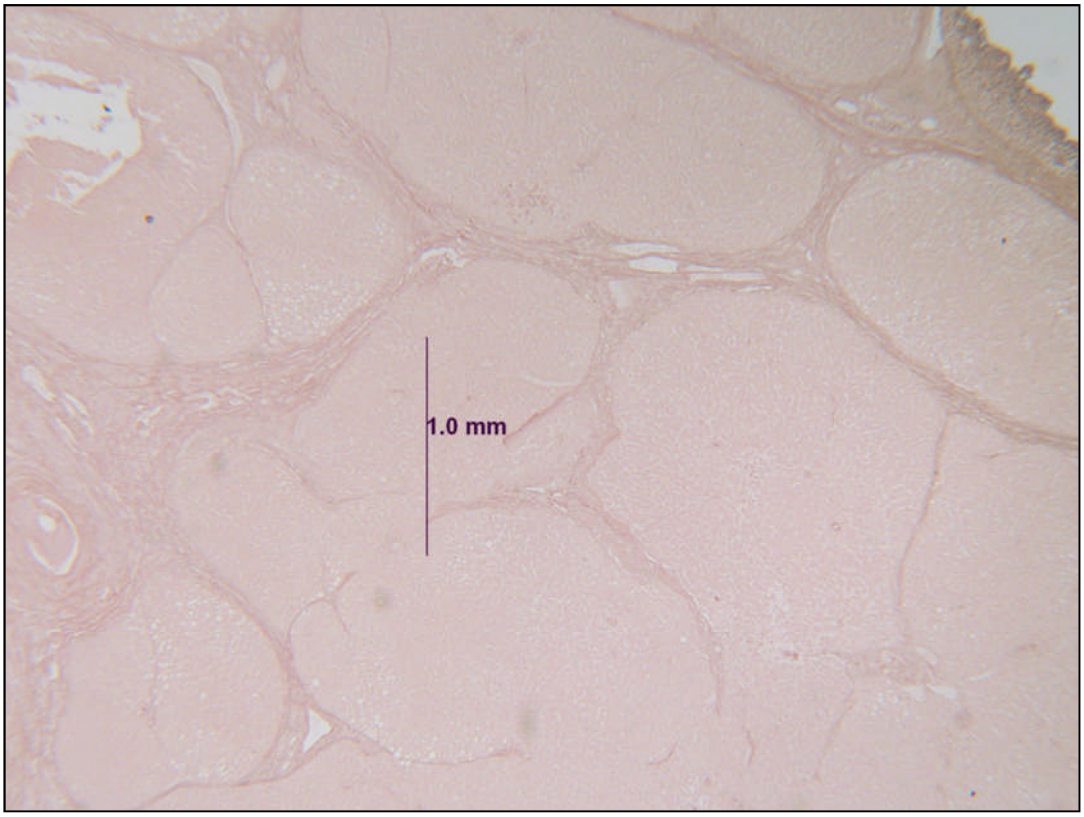
Microscopy

- Recognise normal liver on needle biopsy
- Value of special stains
- Identify presence of cirrhosis, hepatitis or metastatic tumour in needle biopsy

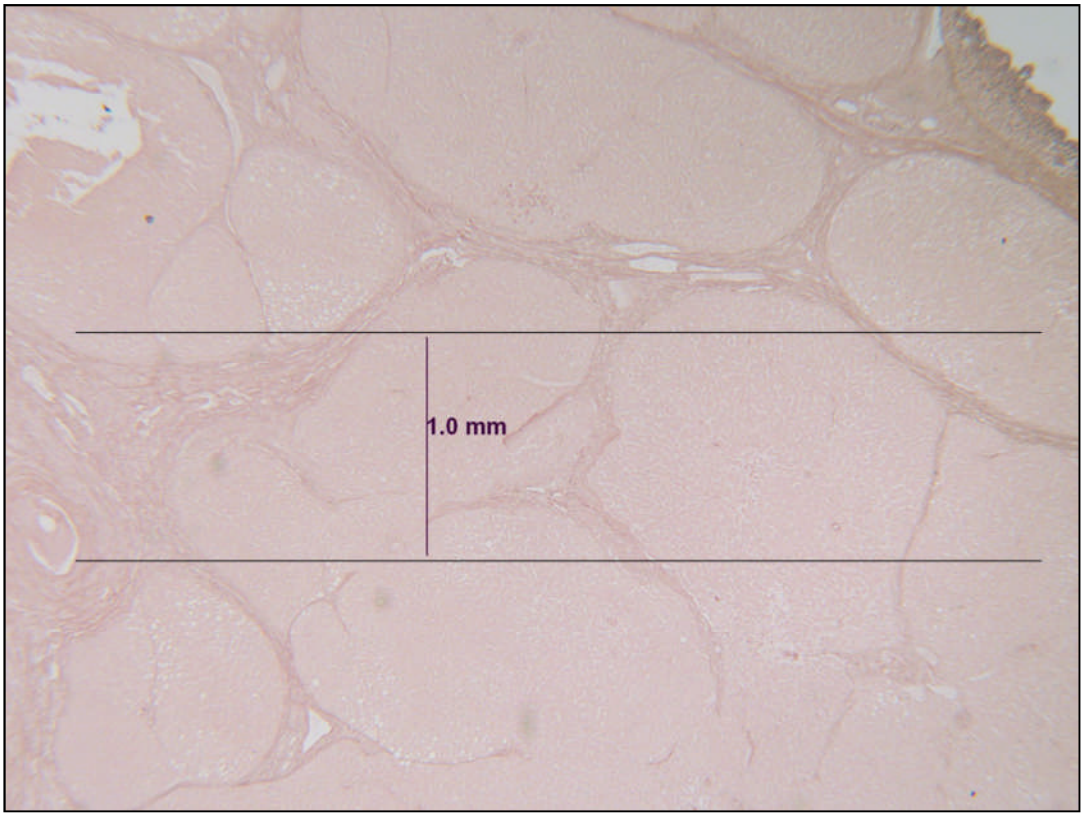


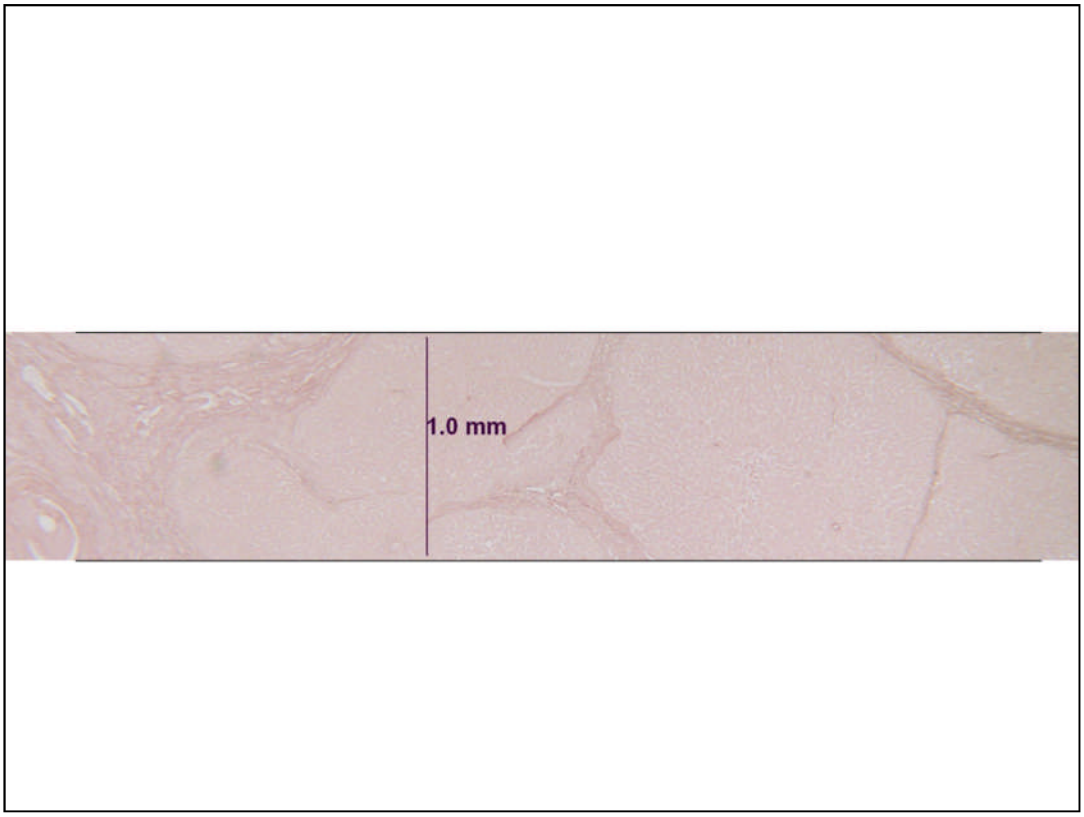
A micronodular cirrhosis liver – transplant for alcohol related liver disease

Sliced explant liver – macronodular cirrhosis (nodules mainly >3mm diameter) with hepatocellular carcinoma.



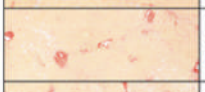
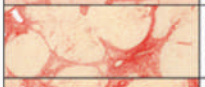
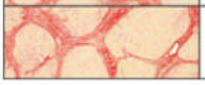
Cirrhosis is the diffuse change in architecture of the liver, with fibrosis and nodular regeneration. It is easier to identify in whole livers – the core obtained by needle biopsy is too narrow to include whole nodules in macronodular cirrhosis.


1.0 mm



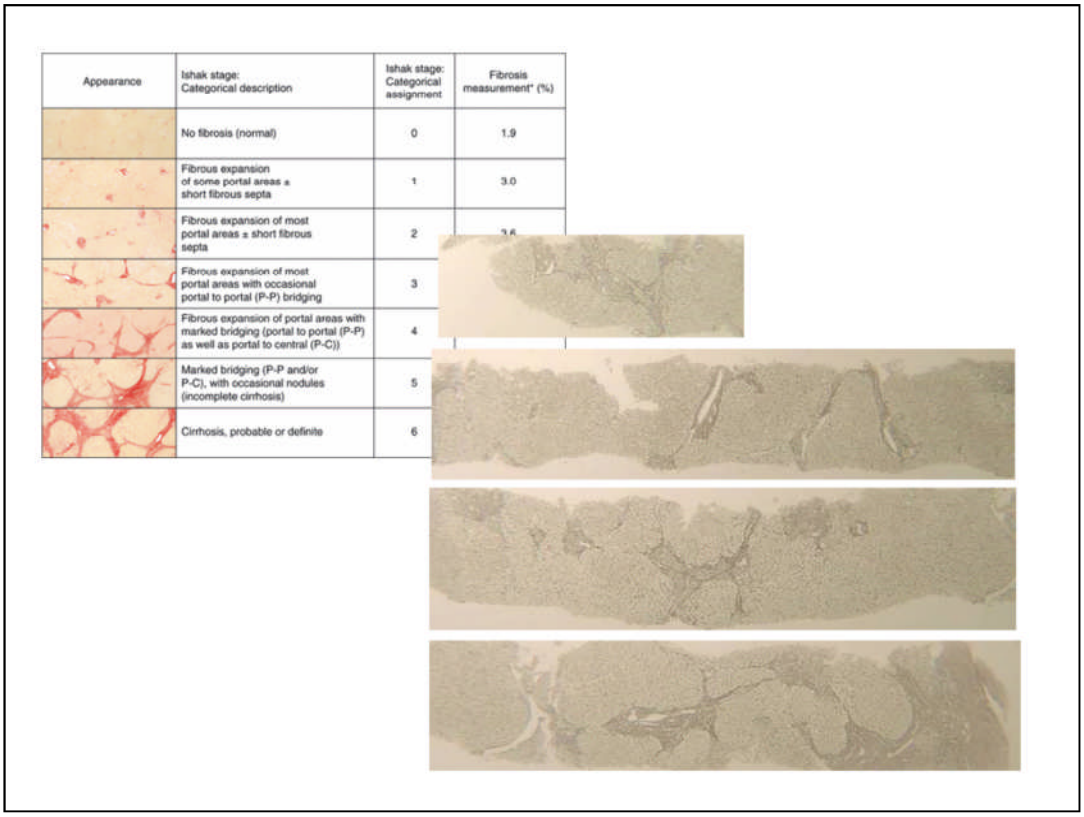
For this reason ‘late stage’ or ‘advanced’ chronic liver disease is preferred by some to a statement of whether or not cirrhosis is present. This is also because cirrhosis is no longer a final irreversible end stage of liver disease, but can to some extent be remodelled if the cause of disease is removed e.g. hepatitis C, haemochromatosis.

Chronic liver disease stage scores and quantitative liver fibrosis measurements.

Appearance	Ishak stage: Categorical description	Ishak stage: Categorical assignment	Fibrosis measurement* (%)
	No fibrosis (normal)	0	1.9
	Fibrous expansion of some portal areas ± short fibrous septa	1	3.0
	Fibrous expansion of most portal areas ± short fibrous septa	2	3.6
	Fibrous expansion of most portal areas with occasional portal to portal (P-P) bridging	3	6.5
	Fibrous expansion of portal areas with marked bridging (portal to portal (P-P) as well as portal to central (P-C))	4	13.7
	Marked bridging (P-P and/or P-C), with occasional nodules (incomplete cirrhosis)	5	24.3
	Cirrhosis, probable or definite	6	27.8

Standish RA *et al, Gut 2006;55;569-578*

This table shows different ways of communicating abnormality of architecture. Quantitation of fibrosis shows the increase in the proportion of connective tissue, but there is also a change in the structure of the liver, expressed here in words and illustrations



Appearance	Ishak stage: Categorical description	Ishak stage: Categorical assignment	Fibrosis measurement* (%)
	No fibrosis (normal)	0	1.9
	Fibrous expansion of some portal areas ± short fibrous septa	1	3.0
	Fibrous expansion of most portal areas ± short fibrous septa	2	
	Fibrous expansion of most portal areas with occasional portal to portal (P-P) bridging	3	
	Fibrous expansion of portal areas with marked bridging (portal to portal (P-P) as well as portal to central (P-C))	4	
	Marked bridging (P-P and/or P-C), with occasional nodules (incomplete cirrhosis)	5	
	Cirrhosis, probable or definite	6	

In real life, the pattern of fibrosis is often variable along the length of the needle biopsy core.

The presence of broad and narrow septa is a useful feature of late stage cirrhosis, which correlates with increased portal pressure and risk of liver failure.

Microscopy

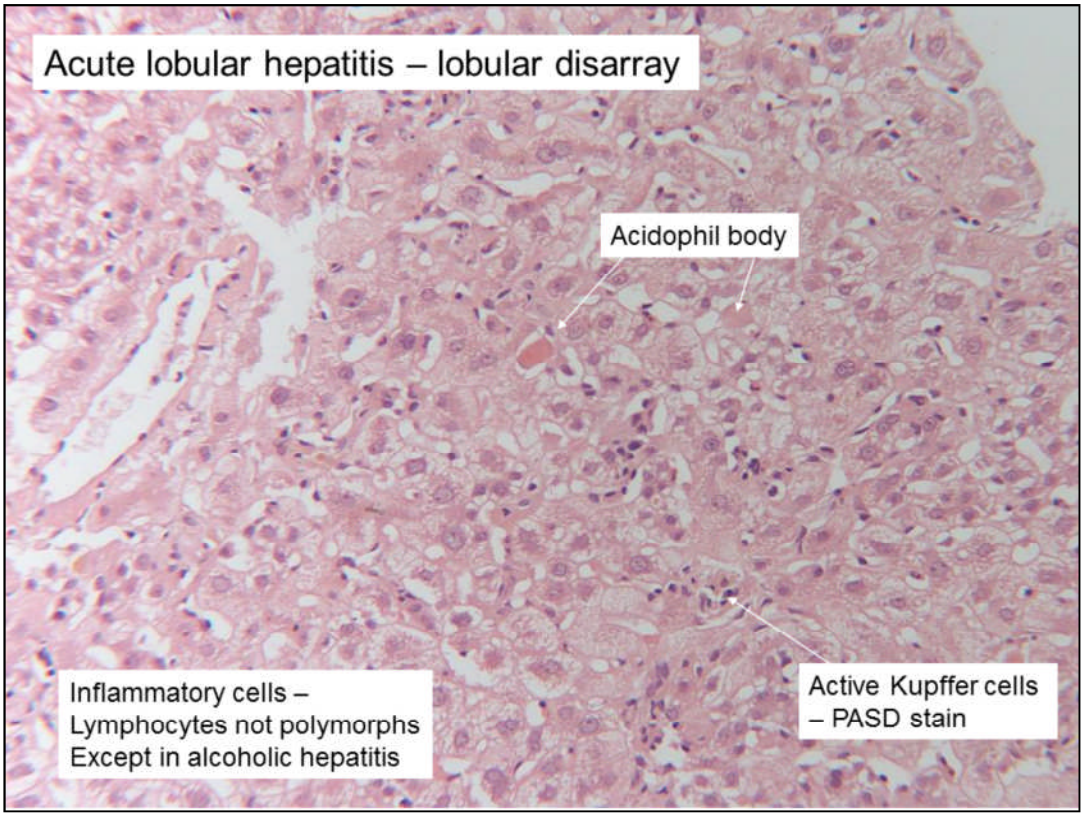
- Recognise normal liver on needle biopsy
- Value of special stains
- Identify presence of cirrhosis, hepatitis or metastatic tumour in needle biopsy

Hepatitis = inflammation in the liver

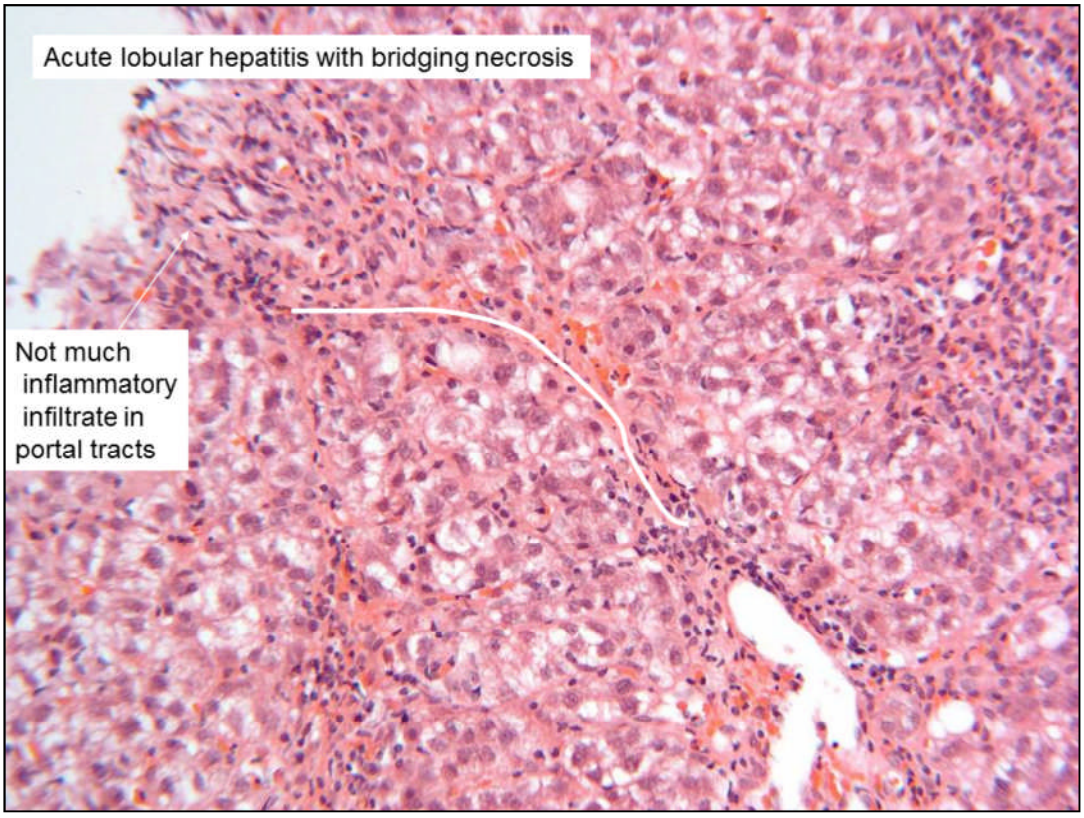
acute, chronic

caused by something –

- may see cause and effect in the biopsy
- evolves over time.

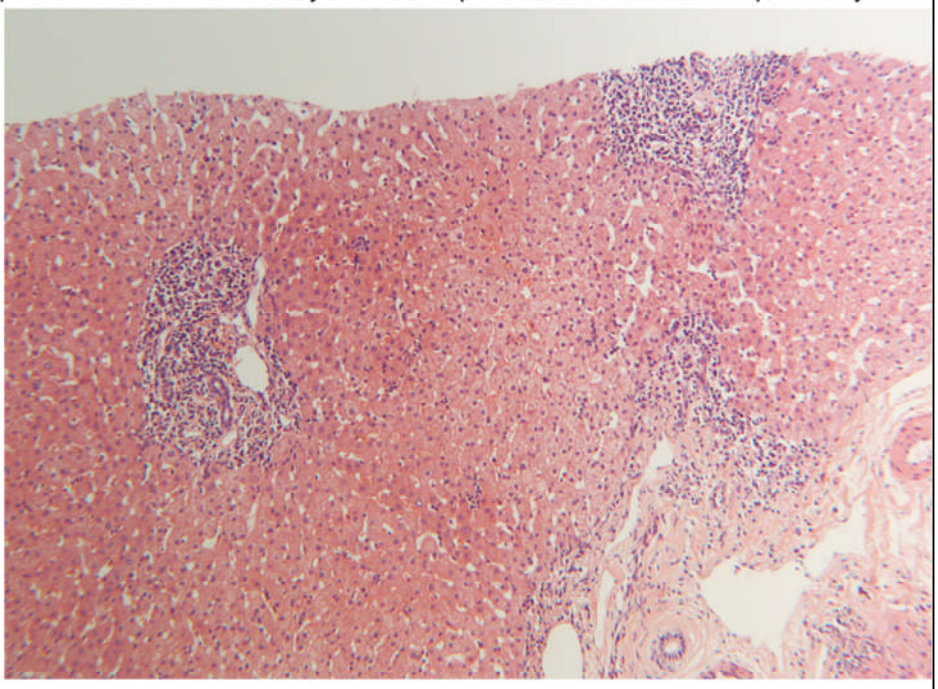


Lobular disarray – the feature of acute hepatitis resulting from several changes – acidophil bodies are apoptotic hepatocytes, which are quickly degraded leaving scavenger Kupffer cells positive on PASD stain. There are also increased lymphocytes in sinusoids, variation in hepatocyte size, and interruptions in liver cell plates. Overall the usual uniform pattern of liver parenchyma changes to a higgledy-piggledy pattern. There may also be bilirubinostasis (in cholestatic hepatitis).



When hepatitis is more severe, contiguous hepatocytes die, resulting in ‘bridging necrosis’ – linking portal tract with hepatic vein. This is initially negative on collagen stains.

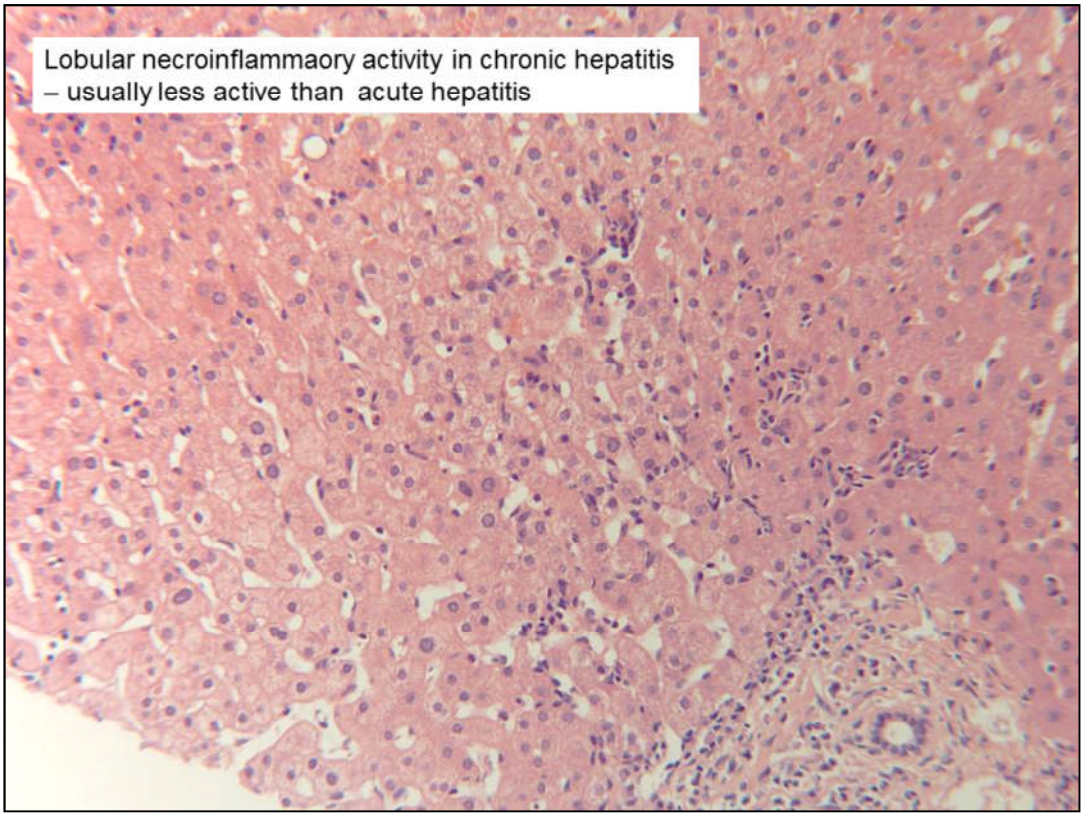
Chronic hepatitis – more inflammatory infiltrate in portal tracts than lobular parenchyma



To indicate the severity – grade the inflammation,
stage the fibrosis

In chronic hepatitis, the portal inflammation is more evident than the lobular inflammation, except in acute flares of disease.

There are grading/staging systems (e.g. Ishak. Metavir) used to indicate severity of disease – especially useful in research to demonstrate effects of treatment in chronic viral hepatitis.


Lobular necroinflammaory activity in chronic hepatitis
– usually less active than acute hepatitis

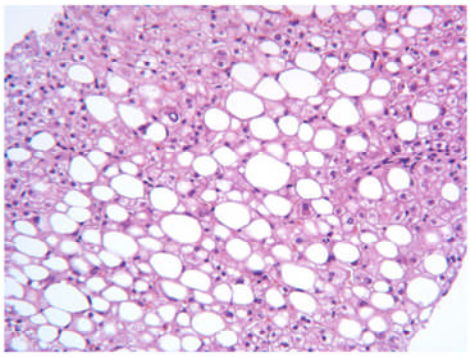
Knowledge base

- Steatosis,
- cirrhosis NOS,
- chronic hepatitis NOS,

Knowledge base

- Steatosis
- Fatty liver disease:
  - Steatosis

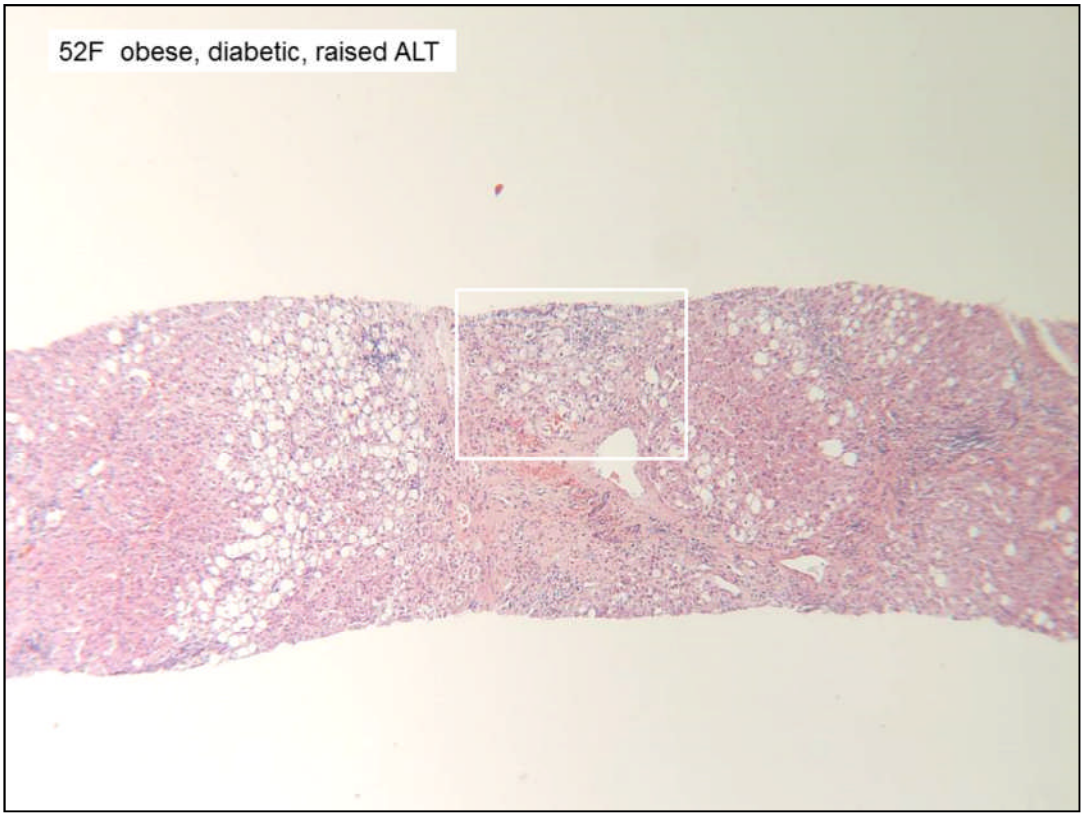
Liver can cope with the stress



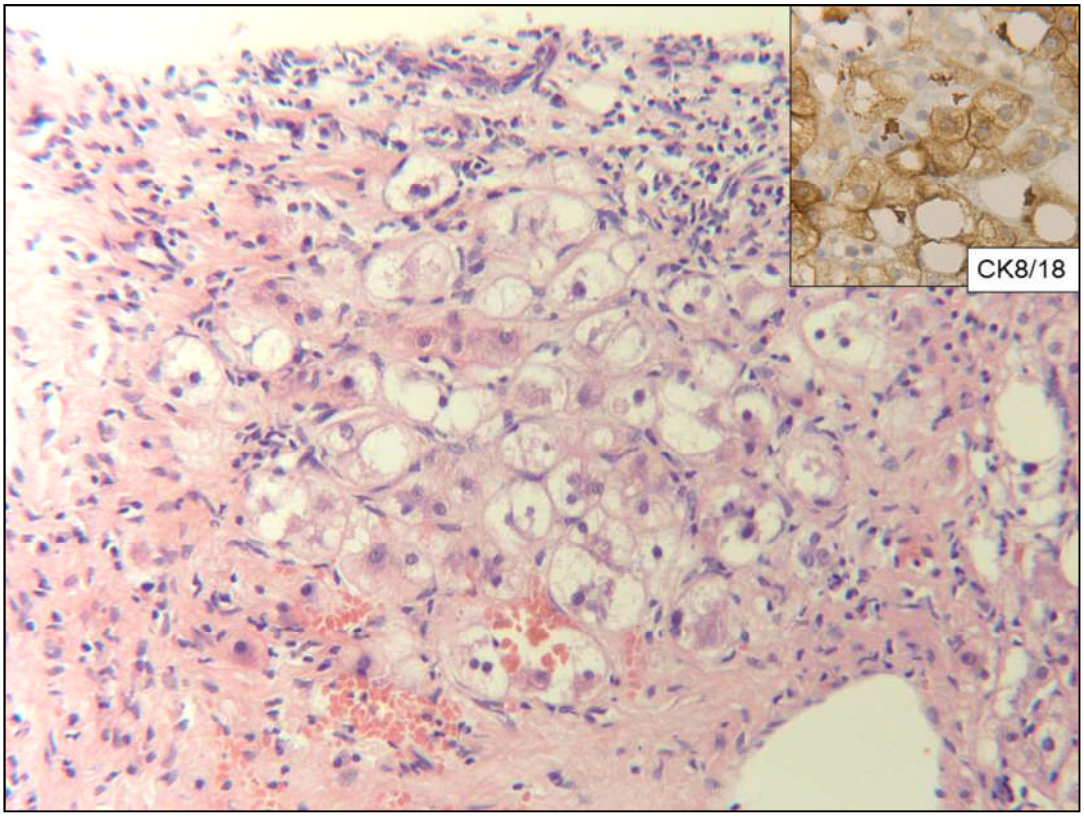
  - steatohepatitis

Liver can't cope with the stress
cells are injured, die,
in response – inflammation,
fibrosis

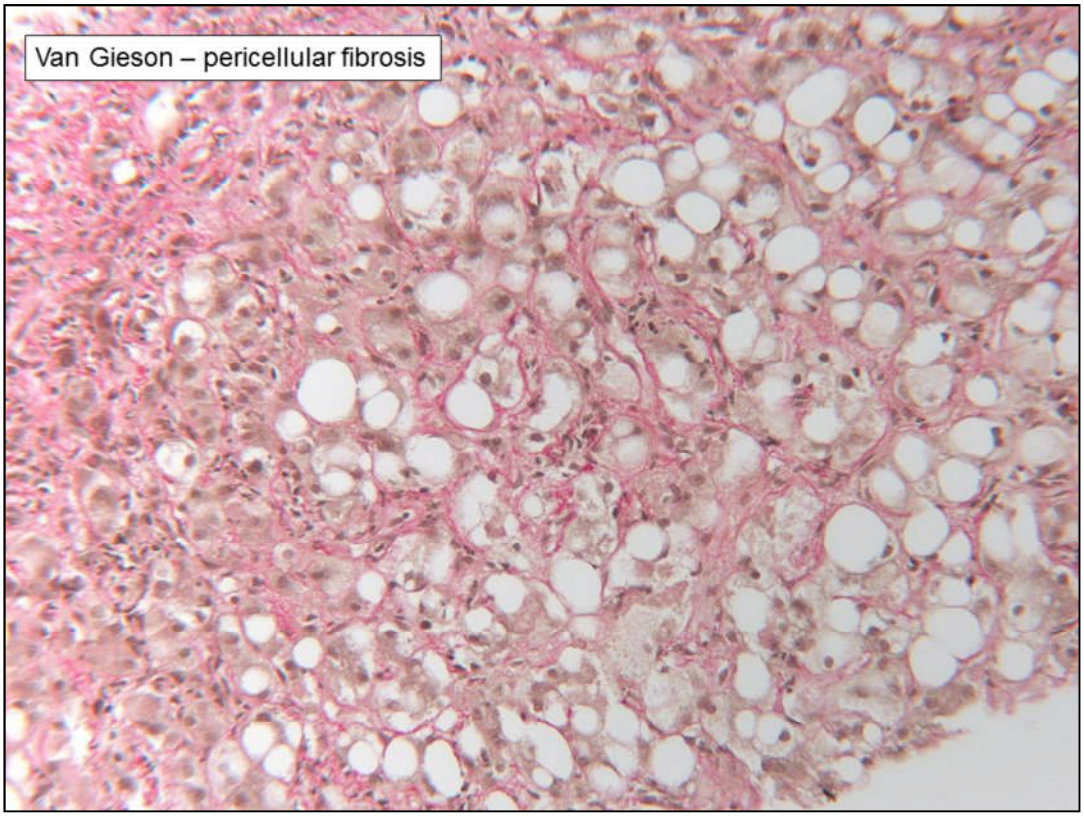
Steatosis and steatohepatitis both occur either due to alcohol related liver disease, or non-alcoholic fatty liver disease, usually associated with metabolic syndrome. Most of the time, histology cannot distinguish the case of fatty liver disease, but does have an important role in identifying steatohepatitis and its grade/stage.



An example of non-alcoholic fatty liver disease (NASH). The degree of steatosis, ballooning and fibrosis varies along the biopsy core. Higher magnification of the box in the next slide.



In this area – hepatocytes show ballooning degeneration – swollen, round, spidery cytoplasm, some steatosis. Immunostain demonstrates Mallory Denk bodies in the cytoplasm of ballooned hepatocytes. There is also inflammatory infiltrate and fibrosis.



The fibrosis is confirmed by van Gieson – chicken wire pericellular pattern as well as broad septa.

Steatosis v steatohepatitis

Look for signs of cell injury as well as fat

What there is -

- Ballooned hepatocytes
- Mallory bodies
- Inflammation (mild)
- Fibrosis (pericellular, zone 3)

And what there isn't –

- More than mild portal inflammation, plasma cells,
- cholestasis, etc.

Fatty change is common and may also be present in biopsies with other types of liver disease. So features '*and what there isn't*' are an indication that there is a second pathological process in addition to fatty liver disease.

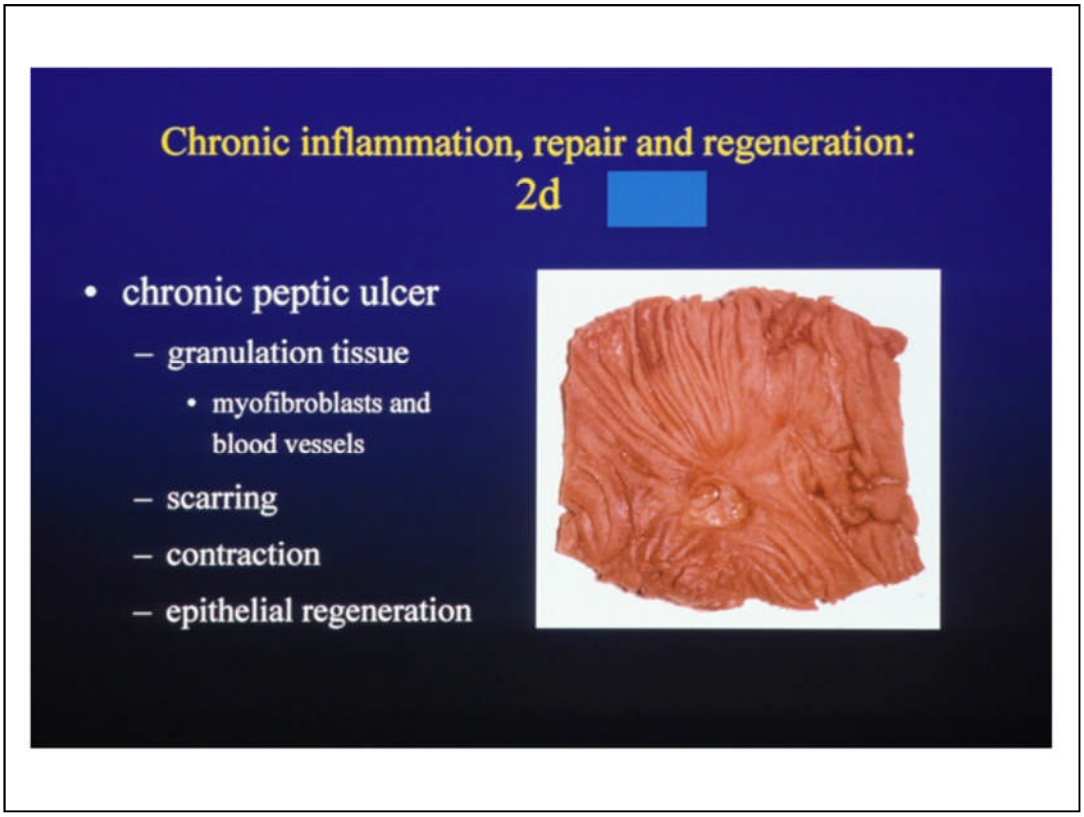
Clinico-Pathological Diagnosis of fatty liver disease

	Steatosis	Steatohepatitis
alcoholic		
Non-alcoholic		

Clinical information is essential to complete the clinic-pathological diagnosis.

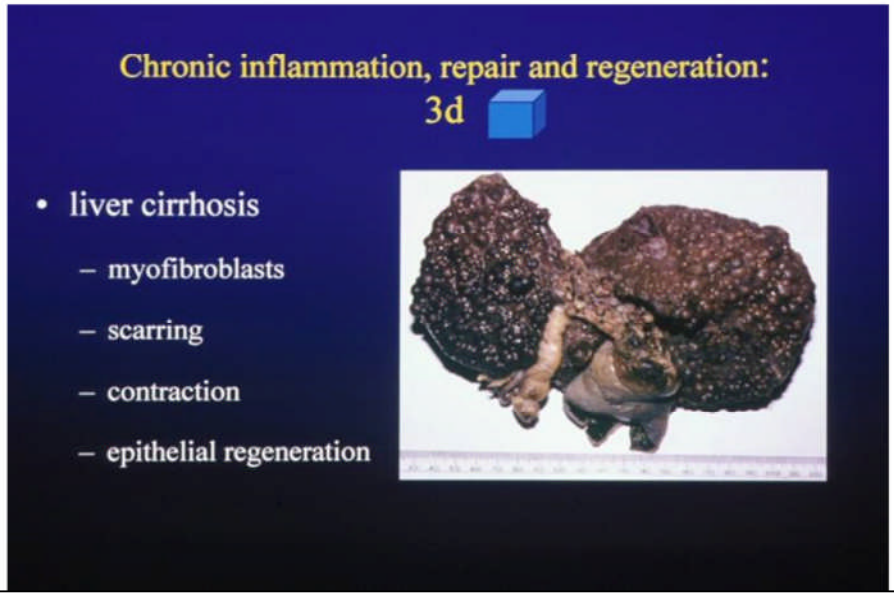
Knowledge base

- Steatosis,
- cirrhosis



Mechanisms of chronic inflammation, repair and regeneration – think about that in 2 dimensions – epithelial surface -

Cirrhosis is defined anatomically by the presence throughout the liver of
fibrous septa that subdivide the parenchyma into nodules
= *end stage of all chronic liver diseases*

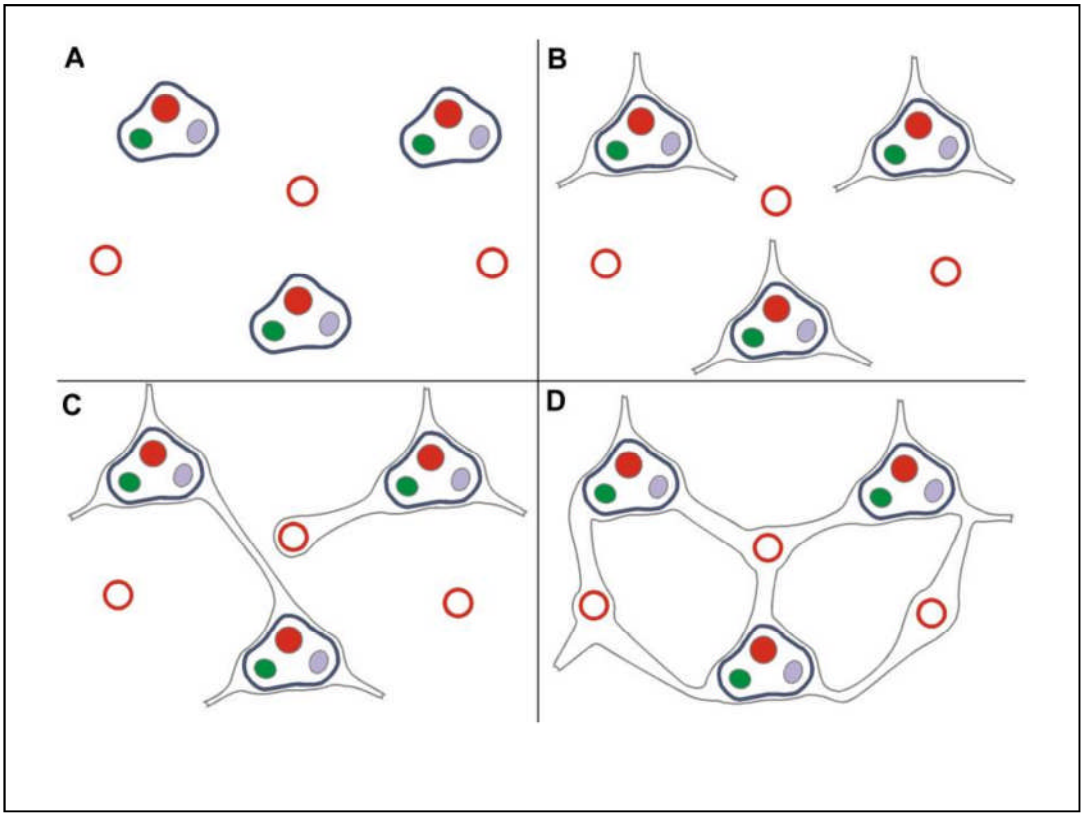


- and 3 dimensions – a solid organ where these processes result in the final common morphology of a scarred nodular liver.

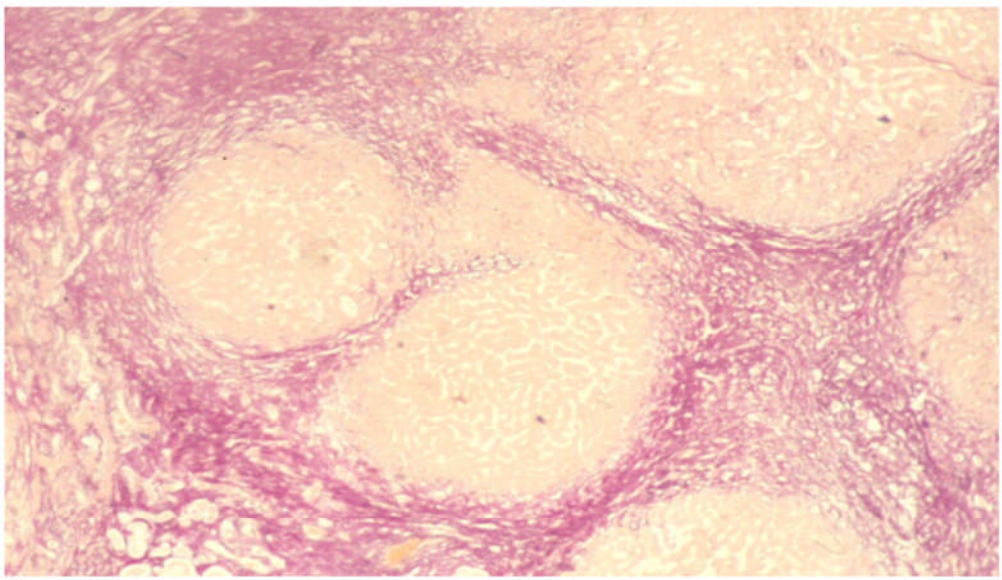
Cirrhosis = end point of chronic liver disease

- alcohol
- viruses (hepatitis B&C)
- autoimmune
- metabolic (iron and copper storage)
- bile duct obstruction

= continuing damage to the liver
during repair and regeneration



Cartoon illustration of architectural changes in cirrhosis. In 3d, the ‘nodules’ are not separate spheres but knobbly lumpy masses – imagine lots of Jerusalem artichokes packed together – or the spaces in a bathroom sponge.



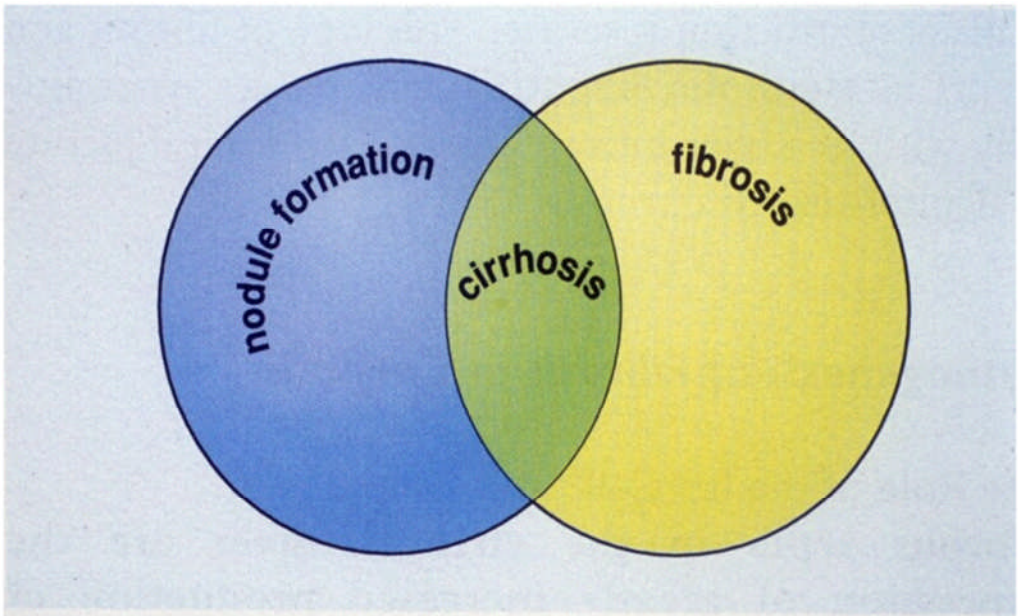
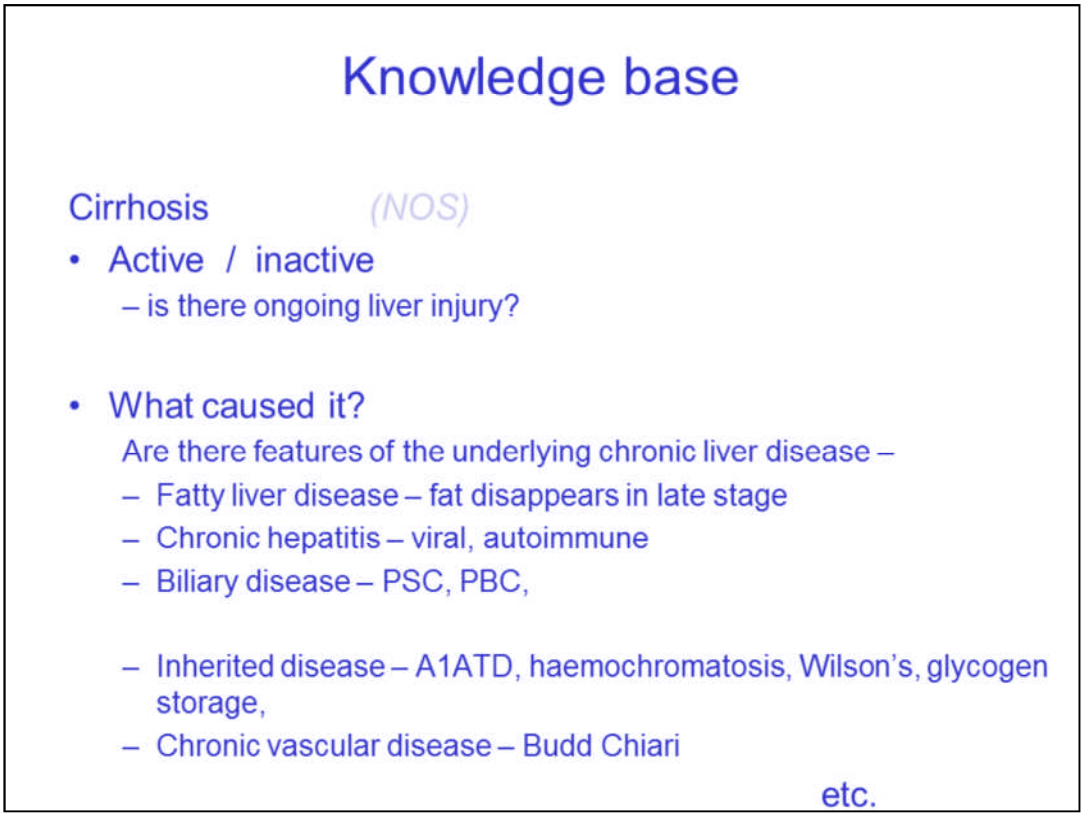
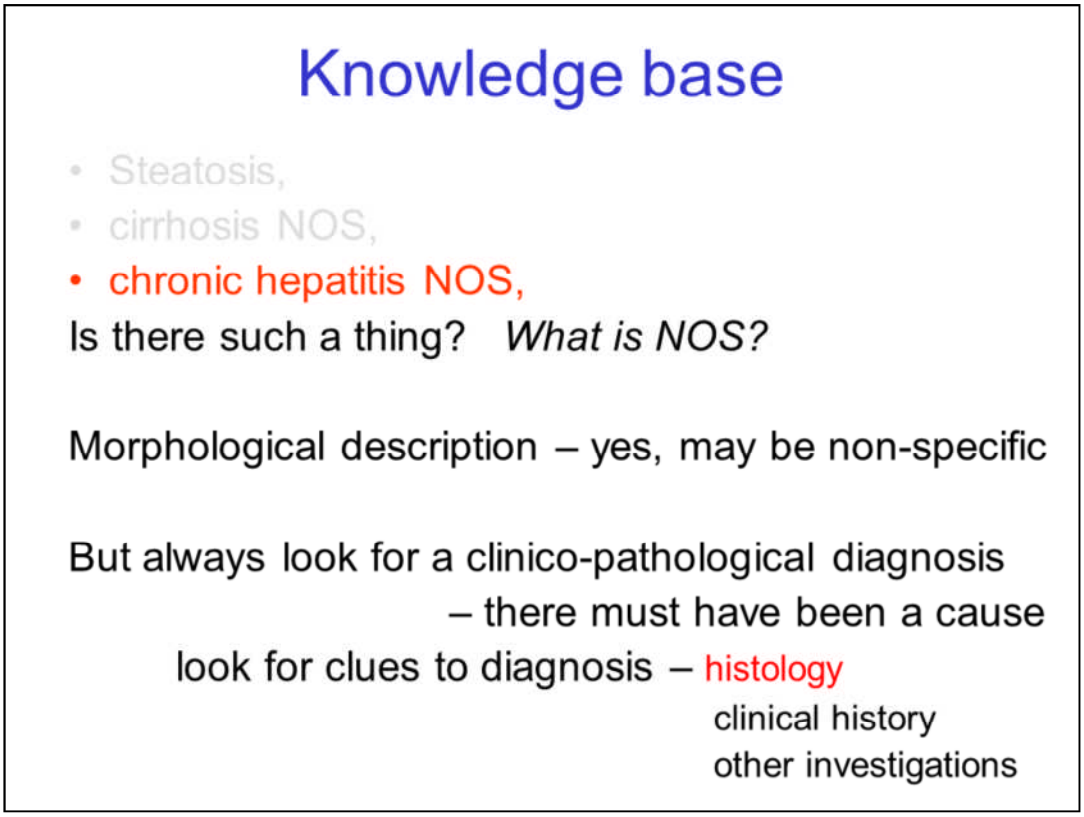


FIGURE 35.15 Two processes are involved in true cirrhosis.

Just fibrosis – without nodular regeneration – e.g. congenital hepatic fibrosis.
Just nodule formation without fibrosis – e.g. nodular regenerative hyperplasia.



What to report in biopsies of cirrhosis (or late stage liver disease)



'Chronic hepatitis NOS' – there must always have been a cause of chronic liver disease, and pathologists should look for features to indicate what this was. 'Chronic hepatitis' is a morphological pattern of portal fibrosis and portal tract inflammation. Activity is an indication of current liver damage, either in the form of interface hepatitis (damaging hepatocytes adjacent to portal tracts) or lobular hepatitis or both.

If the disease is currently inactive, then features of the cause will often not be present. – the biopsy just shows the altered architecture, but the clinical history and investigations may indicate what the cause was. Most often 'cryptogenic cirrhosis' is the end stage of steatohepatitis (NASH or ASH) – steatosis disappears in end stage disease.

Identify presence of hepatitis:

- Histopathologist ….itis = inflammation
  – a morphological feature of liver disease
- Clinician – hepatitis, acute or chronic is a clinical condition, recognised by raised liver enzymes in blood
- investigate to find the specific cause = diagnosis
- Acute hepatitis – virus, drug, autoimmune, 'seronegative' = no known cause
- Chronic hepatitis – virus, autoimmune, drug

Alcoholic hepatitis, non-alcoholic steatohepatitis

Also biliary disease, metabolic disease, vascular disease

What do we mean by 'hepatitis' - used differently by pathologists = a morphology and clinicians = raised liver enzymes.

Inflammation in liver biopsies

- A few mononuclear cells are normal in the liver

  Non-specific increase in any form of liver injury or in severe systemic disease

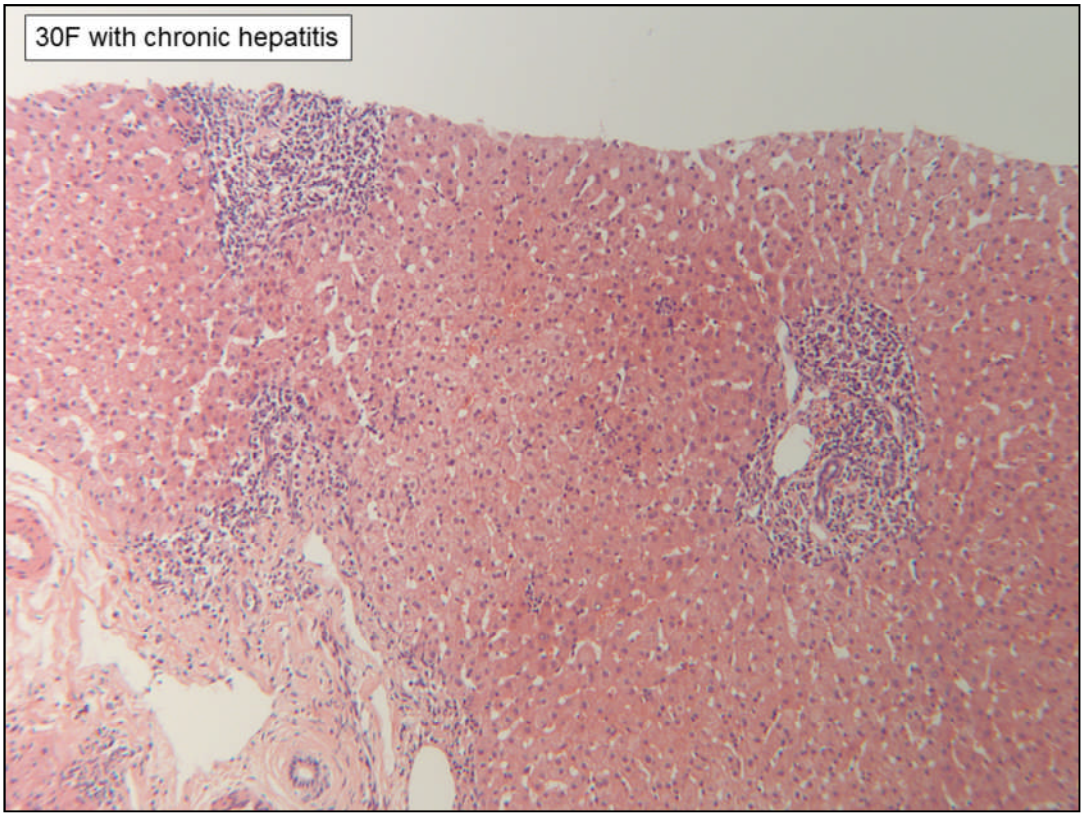
- 'hepatitis' when there is liver injury and inflammation – when main pathology is inflammatory

- Cause may be self-limiting – virus, drug (acute hepatitis)

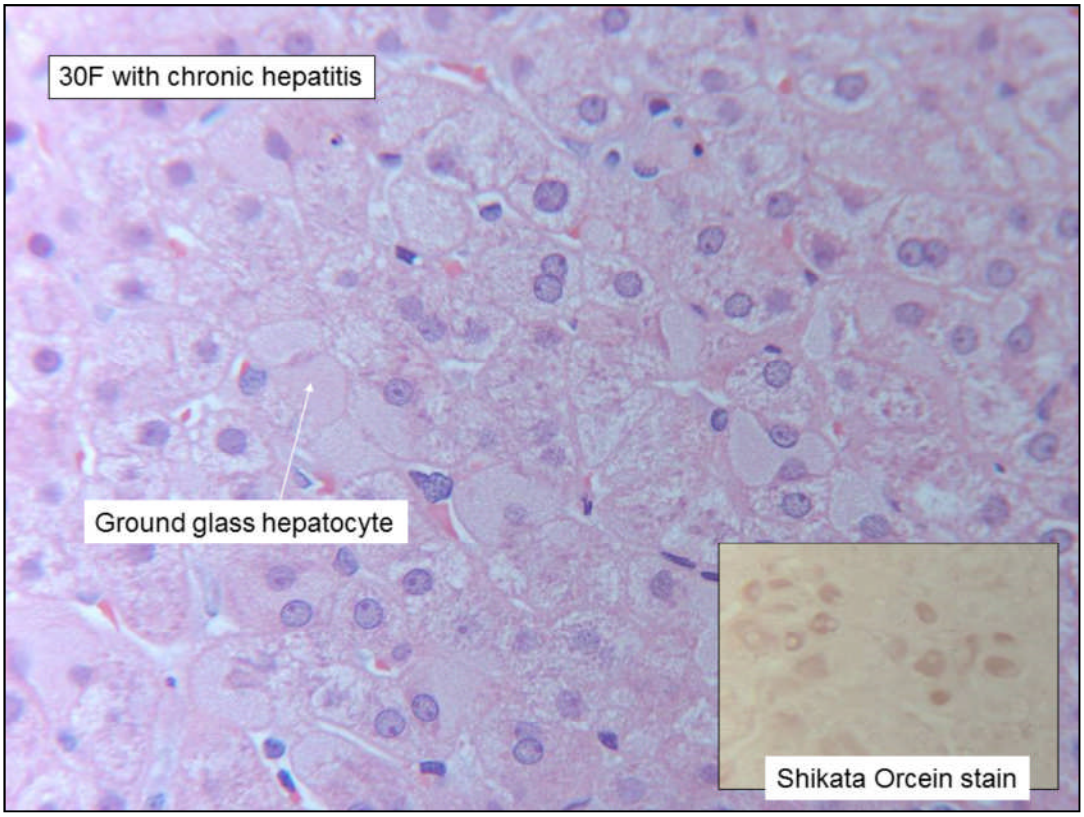
- Or persistent – virus, autoimmune hepatitis (chronic hepatitis)

- Fibrosis and some portal inflammation – non-specific effects of any liver injury

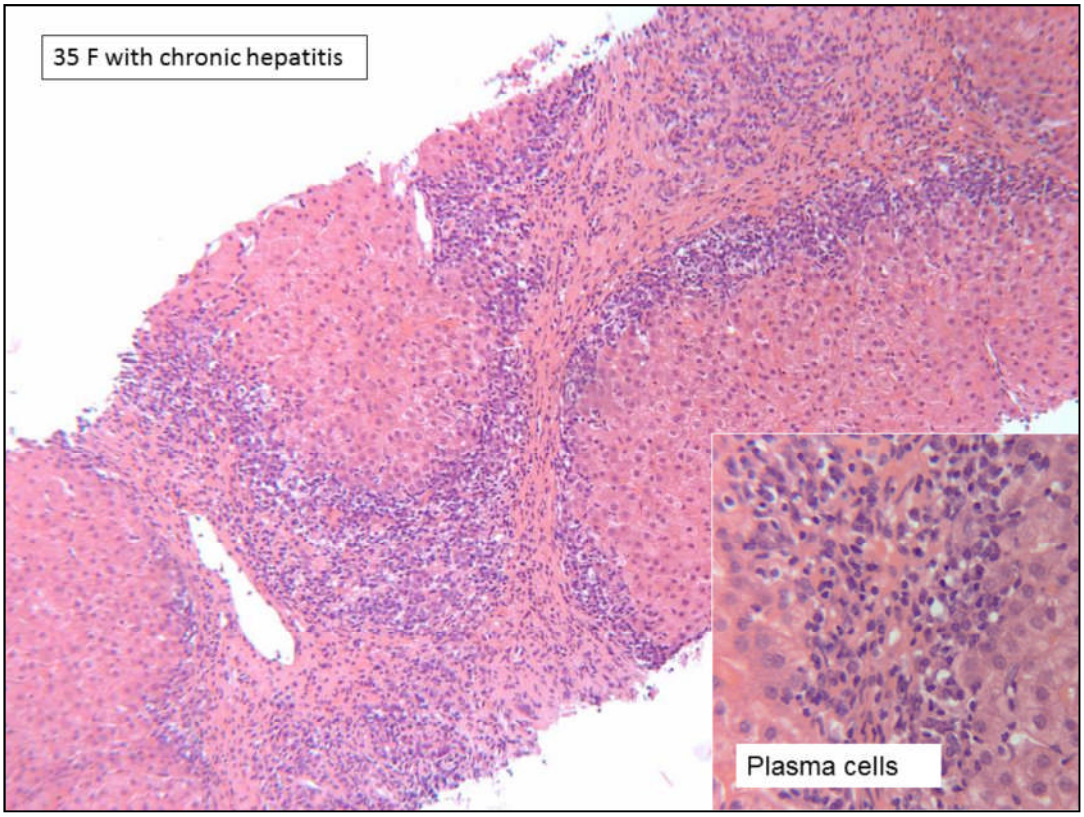
Distinguish non-specific reactive change = minor inflammatory changes in patients with systemic illness e.g. systemic viral infection – from hepatitis when the primary injury is in the liver e.g. from hepatotrophic virus.



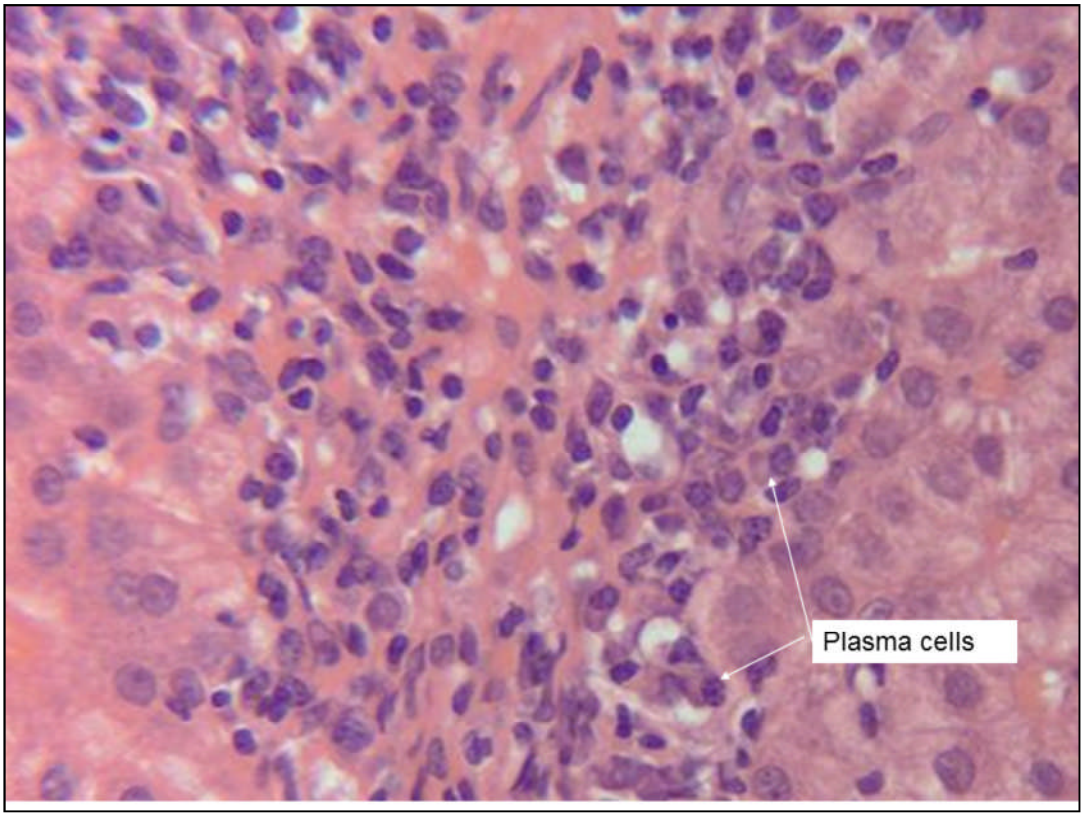
The pattern of chronic hepatitis



The cause is evident in the biopsy – ground glass hepatocytes of hepatitis B



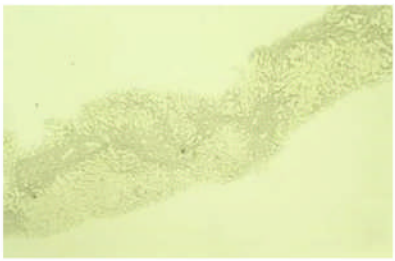
This case is severe chronic hepatitis with unusually marked interface hepatitis. There are numerous plasma cells in the inflammatory infiltrate.

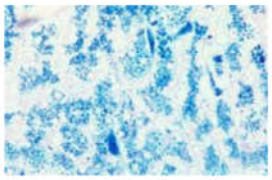


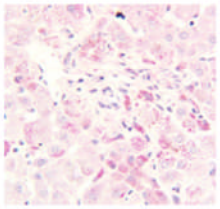
If you can easily find plasma cells in the inflammatory cell infiltrate – suggest autoimmune hepatitis.

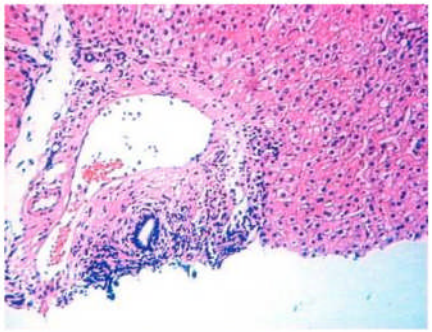
Chronic hepatitis

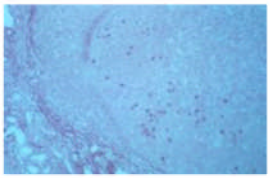
- Portal expansion and fibrosis
- Portal tract chronic inflammatory infiltrate
- Interface hepatitis
- Absence of features of biliary disease

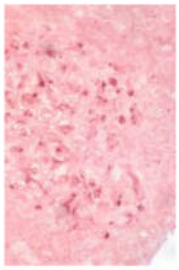




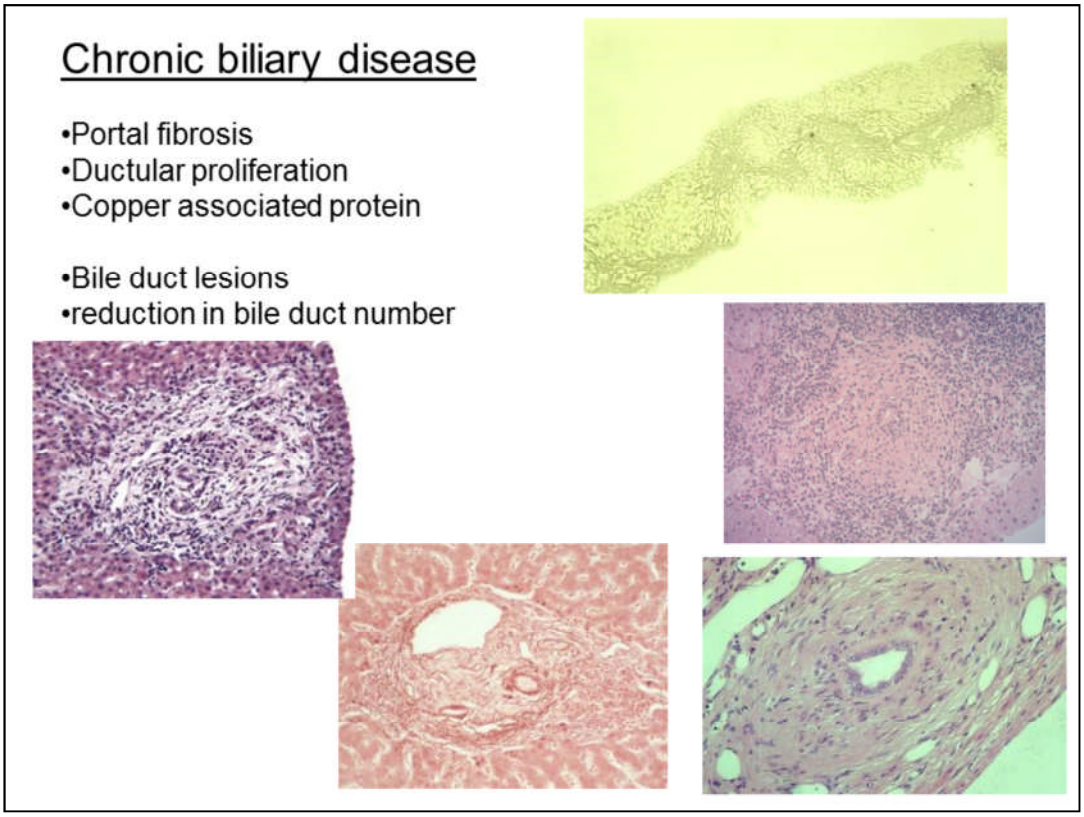








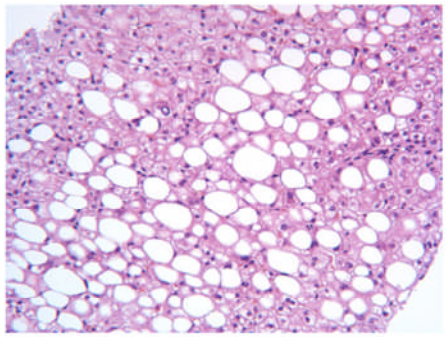
Special stains can indicate the cause of the chronic hepatitis – portal based inflammation and fibrosis is common to various diseases – including alpha 1 antitrypsin deficiency, haemochromatosis, chronic cholestatic diseases.

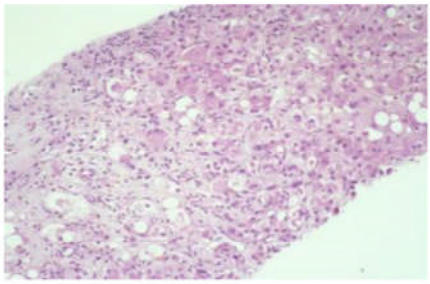


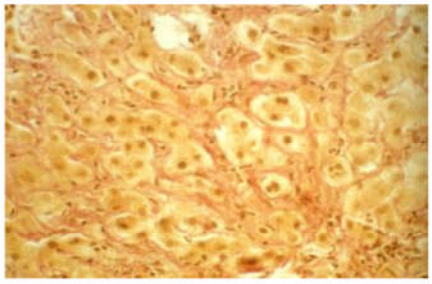
In chronic cholestatic diseases, there is portal fibrosis and portal inflammation, and there can also be interface hepatitis. Look for ductular reaction = prominent ductules around portal tracts, associated with bile duct injury and copper associated protein.

Fatty liver disease

- micro/macro vesicular fat
- Variable but mild portal inflammation
- ? Other features of steatohepatitis
  - ballooning, Mallory bodies,
  - lobular inflammation
  - pericellular fibrosis







Fatty liver disease – usually easy to recognize, but fat may be less in severe alcoholic steatohepatitis (dominated by ballooning, inflammation, pericellular fibrosis) and in late stage cirrhosis.

Macroscopic pathology

- Open biopsy of liver
- Resections for metastatic tumour
- Cholecystectomy

- Why do we bother??

Audit GB cancers since Jan 2008 (5 years)

- Total GB 4,245 (767-1075 per year)
- Total GB cancers 15
- Previously known/suspected 9
- Incidental 6 (per year 0,1,1,3,1)
- % incidental GB cancer 6 / 4245 (0.142%)
- Suspected at cut up 3 – 4
- First detected at histology 2 - 3

How often is there unexpected cancer in routine cholecystectomy specimens? – in Leeds, 0.2% = <1 in 500 or 1-2 cases per year.

All gall bladders are sent to histology for examination, mainly to exclude malignancy. Chronic cholecystitis and/or gall stones are usually present but their presence does not affect subsequent clinical management.

Macroscopic gall bladder – distinguish liver bed from serosal surface, open longitudinally along serosal surface, in case there is unexpected cancer – in which case it will be important to be able to asses the resection margin of the liver bed.

Gall stones come in many shapes and sizes. Cholesterol, pigment, calcified, mixed – have some association with aetiology, but is rarely of clinical importance.

Cholecystectomy:

- Macro examination is very important,
- Open along serosal (shiny) surface
- Gallstones – number, size
  - Appearance – cholesterol (80%) pigment, mixed
- Histology from any mucosal abnormality, and thickest part of the wall
  - Cholesterolosis – 25% 'strawberry gall bladder'
- Gall stones are associated with cancer, and are the usual reason for cholecystectomy
  - >80% cancer have gallstones
  - <0.2% cholecystectomy for gallstones has cancer

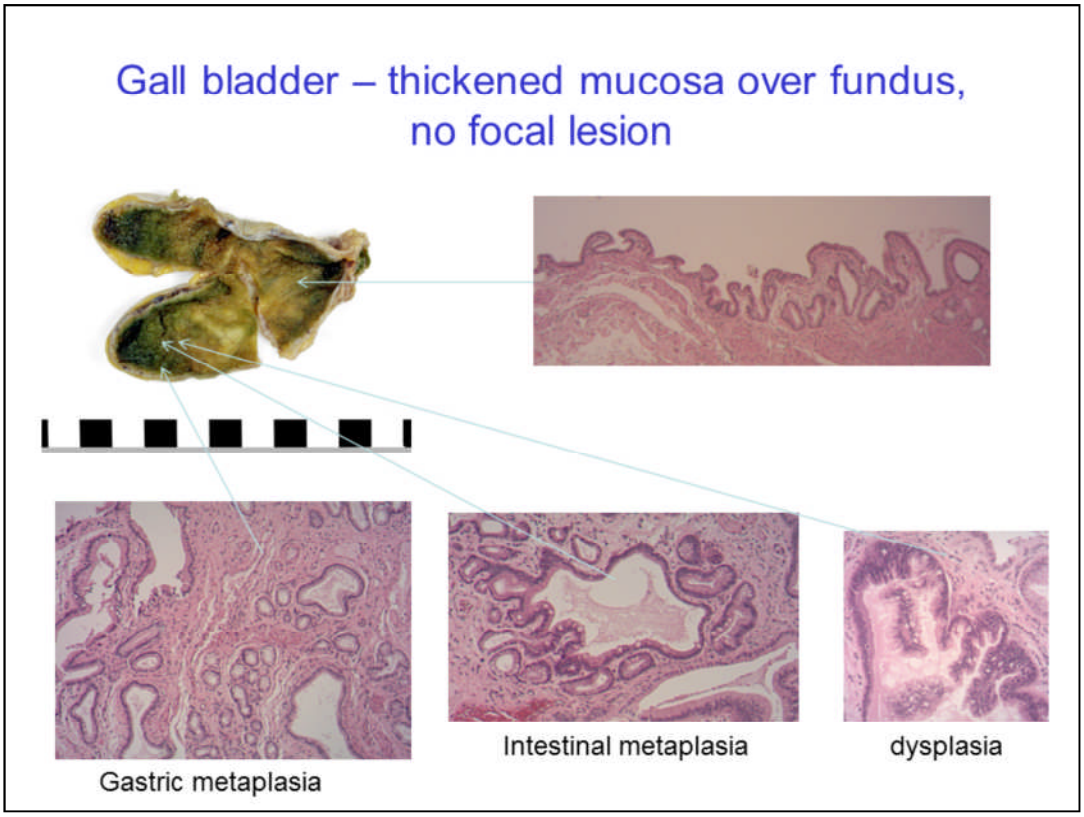
Microscopy

- Report cholecystectomies

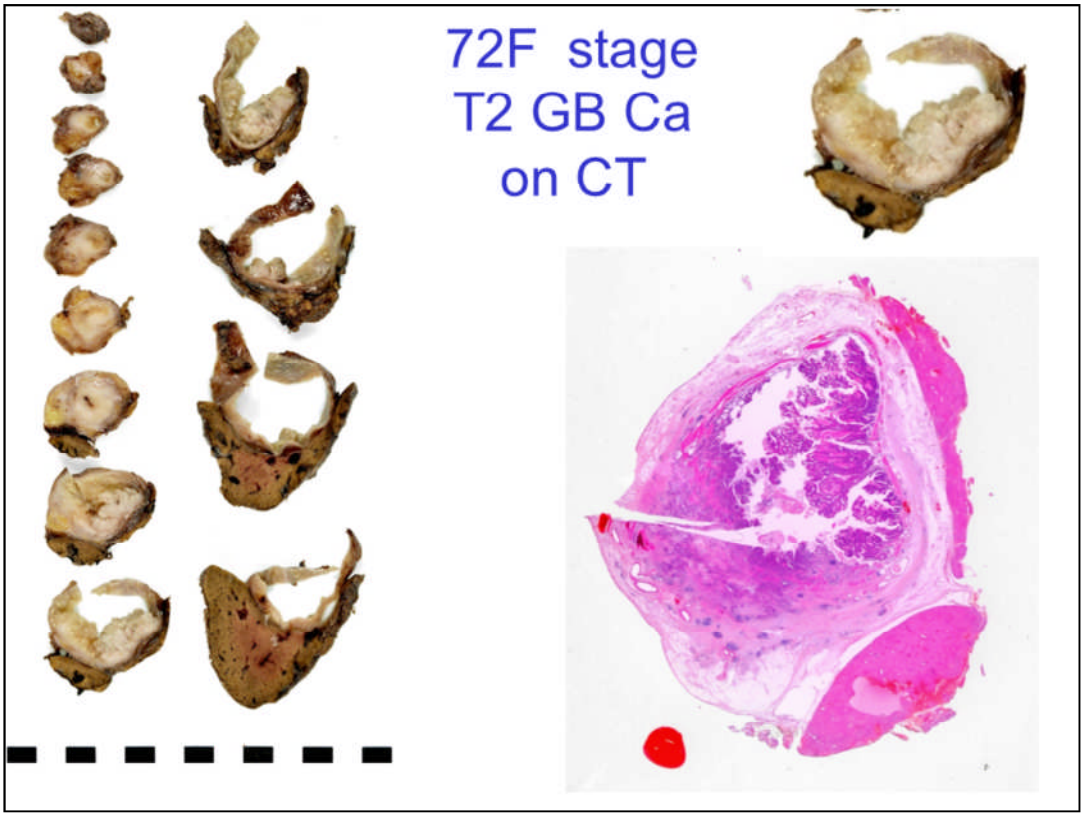
Microscopy

- Report cholecystectomies
- Inflammation – acute, chronic, both
  - Xanthogranulomatous – makes a mass lesion, mimics cancer
- Fibrosis
- Rokitansy-Ashoff sinuses = epithelium-lined diverticula into and through muscle wall
- Metaplasia
- Dysplasia
- Cancer
- Vasculitis, granulomas, cholesterolosis

Originally described as normal mucosa – but the fundal half is grandular/nodular and thickened.



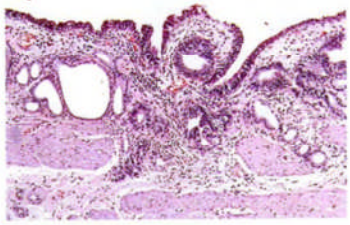
Chronically inflamed gall bladders often have a range of epithelial changes – you can recognise gastric and intestinal metaplasias. Dysplasia is important – there is typically extensive dysplasia in patients with early gall bladder adenocarcinoma. Therefore if you find dysplasia in the initial routine blocks of gall bladder, go back and embed multiple extra blocks to make sure there isn't also an early adenocarcinoma which may be macroscopically invisible. No cancer found in this case.

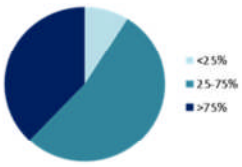


Some gall bladder cancer is associated with exophytic polypoid tumour – polyps >1cm identified on ultrasound are an indication for cholecystectomy. Sample these extensively to ensure early invasive cancer isn't missed. This one is stage pT2 adenocarcinoma = invasion through the gall bladder muscle wall into surrounding tissue but not reaching the serosal surface or invading liver. Remember – the gall bladder doesn't have a muscularis mucosae. pT1a adenocarcinoma is into lamina propria and pT1b into but not through the muscle wall.

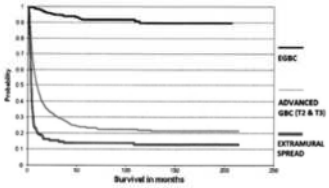
Early gallbladder carcinoma has a favourable outcome but Rokitansky-Aschoff sinus involvement is an adverse prognostic factor

- In Chile – 20% of GB cancer is early, of which 60% were not identified macroscopically





Extent of mucosal involvement by Intramucosal carcinoma



Stage Tis, T1a, T1b = Early GB cancer (not beyond muscle wall), Better prognosis.

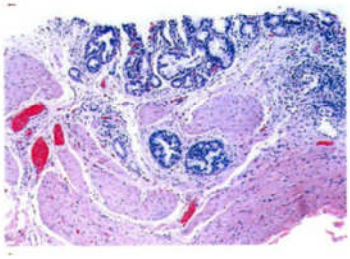
Roa JC *et al.* Virchows Arch 2013;463;651-661

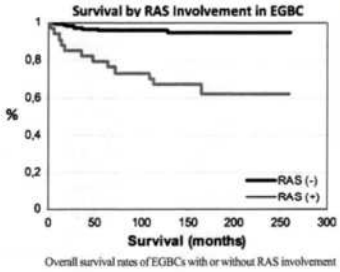
Gall bladder cancer has a marked geographical variation. High incidence countries are able to study large cohorts of patients, and identify the better prognosis of early stage disease. 'Intramucosal carcinoma' or high grade dysplasia is often very extensive in patients with early cancer.

Early gallbladder carcinoma has a favourable outcome but Rokitansky-Aschoff sinus involvement is an adverse prognostic factor

- Stage Tis, T1a, T1b = Early GB cancer,

- High grade dysplasia in RAS
- 'difficult if not impossible to distinguish from invasive carcinoma'



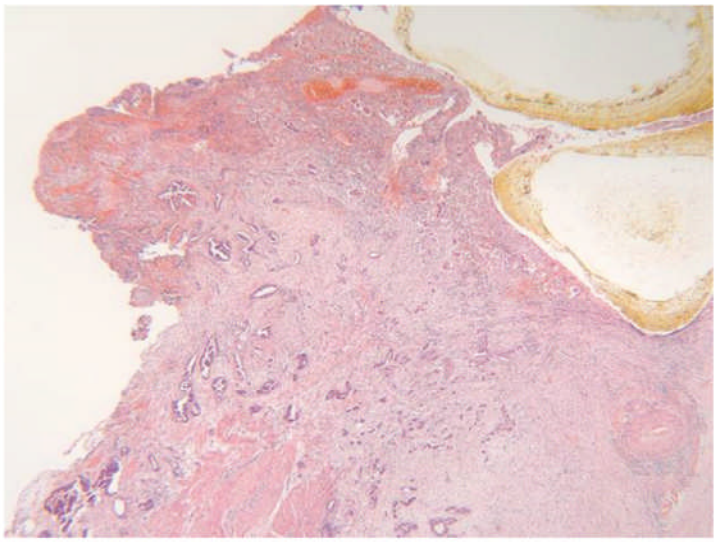


Overall survival rates of EGBCs with or without RAS involvement

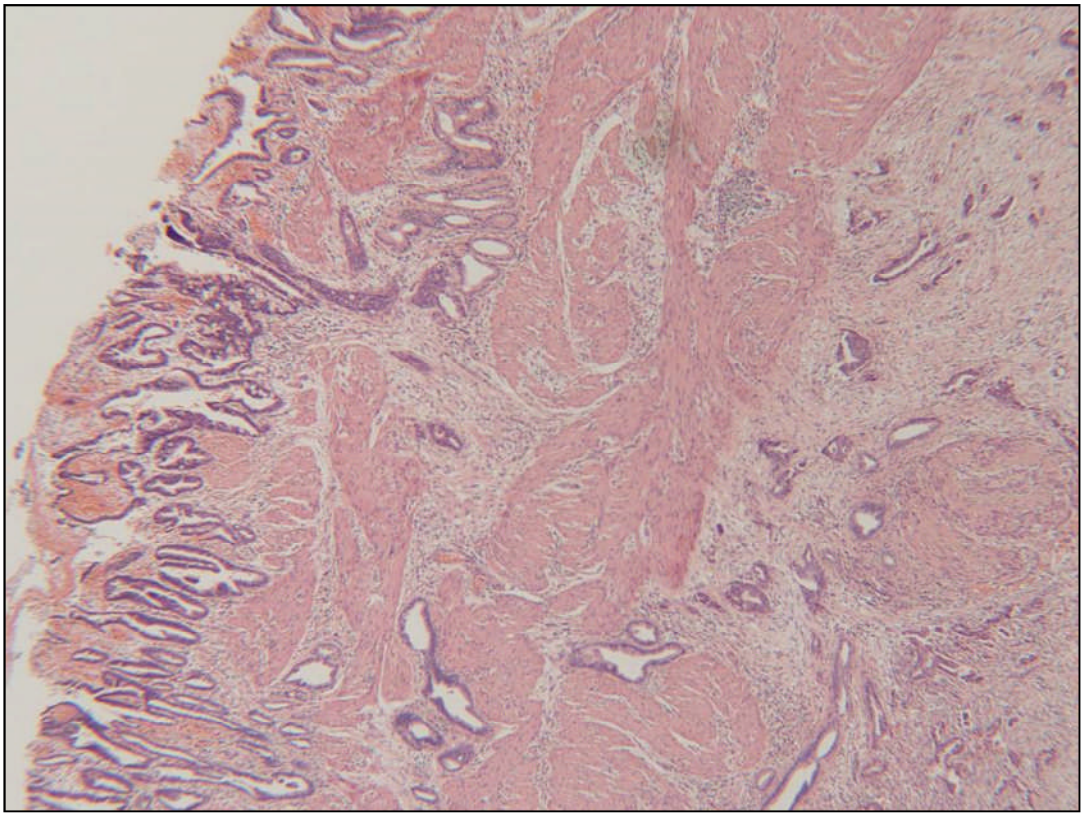
Roa JC *et al.* Virchows Arch 2013;463;651-661

Dysplasia in Rokitansky –Ashoff sinuses is difficult to distinguish from invasive adenocarcinoma.

72F RIF pain, duodeno-cholecystic fistula on imaging
gall bladder opened and distorted, 5x2.5x2cm, wall up to 7mm thick.
No stones, no focal lesions.



Fistulous connections to the GI tract can develop in chronic cholecystitis (the origin of gall stone ileus) – but have a higher risk of adenocarcinoma, sample thoroughly.



High power – mucosal and invasive adenocarcinoma which can be very well differentiated.

Stage A curriculum
hepatobiliary pathology and gall bladder

- Metastatic tumour
  - Macroscopic - Resections for metastatic tumour
  - metastatic tumour in needle biopsy
  - Knowledge base – metastatic carcinoma
- Medical liver biopsies
  - Macroscopic – liver biopsy
  - Microscopy - Recognise normal liver on needle biopsy
  - Value of special stains
  - Identify presence of cirrhosis, hepatitis in needle biopsy
  - Knowledge base - Steatosis, cirrhosis NOS, chronic hepatitis NOS,
- Gall bladders
  - Macroscopic pathology - cholecystectomy
  - Microscopy – report cholecystectomies
  - Knowledge base – chronic cholecystitis; cholesterolosis

The stage A curriculum topics re-arranged – the order followed in this lecture.