An Approach to Liver Biopsies.

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These notes on liver histopathology describe a logical approach to liver specimens and aim to highlight the role of special stains in biopsy interpretation.

They include some tips which are difficult to find in books, but are by no means comprehensive!

First decide which broad category this biopsy fits into.

There are two categories of liver biopsy – done to investigate medical liver disease, or targeted biopsies for tumour diagnosis. Both are usually taken in radiology departments under ultrasound guidance.

Look at H&E section for

inflammatory, fibroticfatty, cholestatic, near normal appearance or tumour?

architecture preserved or altered?

Look at reticulin slides for more detailed appraisal of architecture

- portal tracts normal, expanded, linking up?

Shape of portal tracts

Rounded (duct obstruction)

Stellate (chronic hepatitis)

- liver cell plates normal or thickened indicating regeneration?

Perivenular areas – reticulin collapse? (recent hepatitis)

And bridging to portal areas?

Any peri-cellular fibrosis? better seen on van Gieson (alcohol or non-alcoholic steatohepatitis (NASH)? or venous outflow obstruction)

-part of all of biopsy is tumour?

By now you should have some idea of whether this is a normal or near-normal biopsy, liver disease with normal architecture, a chronic liver disease with scarring, or a tumour biopsy.

It is good practice, and more fun, to look at the liver biopsy before reading the clinical details. But do find out the clinical details before writing the report, in order to give as useful a clinico-pathological diagnosis as possible.

NORMAL LIVER

There is some histological variability among normal livers. These are some of the changes that you might see:

Portal tracts – there should nearly always be an artery, vein and bile duct branch (>90% of tracts). Smaller portal tracts are rounded in shape while larger ones are triangular. The amount of fibrous tissue increases slightly with age, and there may also be age related changes in the hepatic artery branches. There is a little loose fibrous tissue around bile ducts, but this is not normally in concentric layers. There may be a few lymphocytes within the portal area (physiological lymphoid tissue), but not normally plasma cells, eosinophils or polymorphs.

Efferent veins (=terminal hepatic venules; old name = central veins). The larger ones normally have some collagen in the wall, best seen on the van Gieson (VG) stain. This layer is often incomplete, and there should not be any VG positive collagen fibres extending out into the sinusoids from the efferent veins. Efferent veins should be approximately evenly spaced between portal areas, but this appears very variable and the normal relationships can often not be visualised in small needle biopsies.

Liver parenchyma Liver cells are approximately distributed in radiating cell plates converging on efferent veins. In children under about 6 these plates are 2 cells thick, while in adults they are 1 cell thick although because of their anastomosing pattern you can see wider plates in some areas. The reticulin stain shows the architecture best, and jumbling up of the cell plates or areas where liver cells form a pavement rather than a cord are indications of previous regeneration.

Liver cells themselves vary in size. Their glycogen content varies according to the time of day and nutritional state of the patient, and the amount of ceroid pigment in the liver cells is variable and increases with age. This forms coarse granules around the canalicular pole of zone 3 (perivenular) liver cells.

The degree to which liver cell nuclei vary in size increases with age – large nuclei correspond to natural polyploidy in liver cells, and about 10% hepatocytes are binucleate . Clear spaces within nuclei due to glycogen accumulation are frequently seen in children but are less common in adults when they may be associated with diabetes or Wilson disease.

There is often a little stainable iron in periportal liver cells especially in adult men. In neonates periportal liver cells normally contain iron and copper

Non-specific reactive changes

There are often minor changes in the liver of patients who are ill for other reasons. This is to be distinguished from a primary liver disorder, and the term ‘non-specific reactive changes’ is preferable to ‘non-specific reactive hepatitis’ which may be misinterpreted as a form of hepatitis by the person reading the report.

The sorts of things you may see are:

Increased portal tract inflammatory cells – this usually does not involve all the portal tracts in the biopsy

Mild fatty change

Occasional degenerating liver cells or small groups of inflammatory cells in the parenchyma

One or two PAS diastase (PASD) positive Kupffer cells.

Changes like this are difficult to distinguish from genuine liver diseasee, and clinic-pathological correlation is very important.

Acute and chronic liver disease

The clinical indications for liver biopsy in the investigation of medical liver disease are now clearly defined –

a. to make the diagnosis or decide relative contribution of diagnoses, where this is not clear from the comprehensive non-invasive liver screen (includes clinical history, and algorithm based blood tests for liver enzymes, liver function tests, viral and autoimmune serology, and ultrasound scan).

b. to determine the severity (grade and stage) of a known disease

c. to monitor effects of treatment.

The indications for the biopsy and suspected clinical diagnoses should be included on the request form.

Acute hepatitis

Physicians biopsy patients with chronic liver disease much more often than those with an acute disease. It is quite unusual to see an acute hepatitis in a biopsy.

This may be due to viruses, drugs, or recent onset autoimmune hepatitis. The degree of portal inflammation is outweighed by the parenchymal changes.

The lobular features of acute hepatitis are: acidophil bodies (apoptotic hepatocytes, even small numbers are significant), PAS positive Kupffer cells, lobular disarray, (variation in hepatocyte size, regeneration, inflammatory cells). If these features are present, the portal inflammation may be part of an acute hepatitis rather than a sign of underlying chronic liver disease. There may be cholestasis.

The severity of an acute hepatitis is judged by the presence and extent of hepatocyte destruction :

Spotty necrosis – individual hepatocyte necrosis/apoptosis = acidophil bodies

Confluent necrosis – affecting an area of contiguous hepatocytes, which may be:

Zonal necrosis – loss of hepatocytes in a zone, usually perivenular, zone 3

Bridging hepatic necrosis – that extends to link hepatic veins to portal tracts
Panacinar necrosis – when all of the liver cells in the acinus have been destroyed.
There is usually ductular reaction at the margins of the portal tracts.

These terms describe the severity of the hepatitis and not its cause.

Features which suggest a drug induced hepatitis (drug-induced liver injury, DILI) are:

Cholestasis, may also be cholangiolitis
Relatively little portal inflammation for the degree of damage
Eosinophils in the inflammation
A clear-cut edge to the areas of lobular zonal necrosis
A fairly uniform degree of involvement throughout the liver (useful in transplant or post mortem livers)

Features which support a viral cause are:

Prominent portal inflammation
Spotty necrosis throughout the acindus although this is usually most severe around efferent veins
Variations in severity from place to place.

Features which support autoimmune hepatitis are: (but both may be absent).

Prominent plasma cells, in portal tracts and/or in parenchyma
Portal inflammation especially if interface hepatitis

The aetiological diagnosis therefore depends on the clinicians – evidence of

Careful drug history
Viral serology (including hepatitis E and EBV)
Autoantibodies and immunoglobulin levels

However the distinction of viral/drug/autoimmune acute hepatitis cannot be made with any certainty on the basis of histology, and for many patients the aetiology is never established (seronegative hepatitis).

Liver biopsy showing cholestasis

Liver biopsy may be used in the investigation of jaundiced patients once bile duct obstruction has been excluded by ultrasound, and if the cause is not clear from the clinical history/liver screen investigations.

Bilirubin pigment is often visible in liver biopsies – the liver cells may show a greenish speckling which is difficult to distinguish from ceroid pigment. If you look hard, canalicular bile plugs are nearly always visible in cholestatic liver biopsies, and are a much more reliable sign of cholestasis –

some use the term ‘bilirubinostasis’ to distinguish this from cholate stasis of bile salts in periportal hepatocytes in chronic biliary disease. PAS diastase positive macrophages (‘scavenger macrophages’) will also be present if bilirubinostasis has been present for more than a few days. Don’t report bilirubinostasis if there are neither bile plugs nor PASD +ve Kupffer cells present.

If the biopsy shows cholestasis, this may be due to large duct obstruction, hepatitis, or drugs, or to inherited disorders of bile metabolism. (Duct obstruction is occasionally present without detectable duct dilatation on ultrasound). Therefore look for features of any of these conditions.

Large bile duct obstruction

Once present for a few days, in addition to cholestasis there will be enlargement of the portal tracts. Characteristically they show oedema, ductular reaction associated with neutrophil accumulation next to ductules. The enlarged portal tracts take on a rounded outline, and look pale. (This contrasts with the stellate enlargement of portal tracts in chronic hepatitis). The presence of neutrophils does not indicate an infective cholangitis – this is only suggested by accumulation of neutrophils within the lumen of bile ducts themselves. The degree to which portal areas show these features is very variable, even in patients with radiologically proven large bile duct obstruction. Also the time course and sequence of the development of histological features is variable (See also primary sclerosing cholangitis, p9). Typically, oedema is an early manifestation, later replaced by increasing fibrosis.

Sepsis:

Patients with severe sepsis/septicaemia shock may show cholestasis with some ductular reaction and characteristically have bile concretions within distended marginal ductules / canals of Hering. This is a distinctive histological sign which should prompt a clinical enquiry about current or previous sepsis.

Cholestasis in hepatitis

Hepatitis due to drugs or viral infection may be predominantly cholestatic. Conversely cholestasis for other reasons which has been present for some time will be associated with liver cell death and Kupffer cell activity. These two situations are difficult to tell apart – any acidophil bodies, or PASD positive Kupffer cells distributed throughout the liver acinus favours a cholestatic hepatitis, in contrast to Kupffer cell activity restricted to the perivenular areas of bilirubinostasis.

If the biopsy shows bilirubin stasis, with no other histological changes, this may be

- Drug induced cholestasis
- Early large bile duct obstruction
- Cholestasis due to infection or septicaemia
- Cholestasis due to inherited metabolic disorder – may be recurrent, familial, pregnancy, pill

Fatty change only

Steatosis (fatty change) has a list of causes including obesity, diabetes, steroid treatment, other drugs, other inborn metabolic disorders (causes of non-alcoholic fatty liver disease, NAFLD) or can be alcohol related. The clinical history of alcohol use is essential for the distinction to be made. Fatty change that is seen as round empty spaces of variable size within hepatocyte cytoplasm is macrovesicular steatosis. Microvesicular steatosis is barely perceptible without a fat stain on a frozen section, and is a rare feature of specific conditions (e.g. acute fatty liver of pregnancy)

Fatty change affecting <5% hepatocytes and without fibrosis or any other abnormality is considered to be within normal limits. Fatty change >5% without features of steatohepatitis (see below) or fibrosis is reversible if the cause is withdrawn, and not a sign of chronic liver disease.

Chronic liver disease

This is the usual indication for medical liver biopsy, which is performed to establish

a. the most likely aetiology of the chronic liver disease, when this is not clear from non-invasive liver investigations.

a. the severity of chronic liver disease, in terms of fibrosis/progression towards cirrhosis (stage) and current disease activity (grade).

Nearly all chronic liver disease results in fibrosis and hepatocyte regeneration potentially leading to cirrhosis, although the rate of progression is very variable. Recently, non-invasive tests for liver disease stage, such as fibroscan, are increasingly used as an alternative to liver biopsy – in particular to identify patients with low risk of fibrosis, where biopsy can be avoided.

Histological indicators of the aetiology of chronic liver disease/cirrhosis are appreciated when the disease is 'active' i.e. there is ongoing liver cell damage.

There are 4 main patterns of progression of chronic liver disease:

Two have portal based inflammation and fibrosis:

a. Chronic hepatitis – (see page 7) portal tract inflammation with increasing portal fibrosis without features of biliary tract or fatty liver disease. Re-classified in 1994 to emphasise the aetiology, this may be

viral (hepatitis B, C, D, others)
autoimmune
drug related (methyl dopa, isoniazid etc)
metabolic (e.g. alpha 1 antitrypsin deficiency, Wilson).

'Interface hepatitis' is the term for inflammation that targets the limiting plate of periportal hepatocytes, leading to expansion of the portal tract by fibrosis, and is the identifying feature for chronic hepatitis, although minor degrees may be seen in other liver diseases.

NB clinical details of viral serology, autoantibodies, drug history and character of liver enzyme tests are essential for the classification of chronic hepatitis.

For chronic viral hepatitis B, liver biopsy is necessary to decide treatment for patients with raised ALT and high viral load on PCR testing, or with evidence of late stage disease on fibroscan. For hepatitis C, treatment is no longer dependent on biopsy findings, and biopsy is now only done if non-invasive tests suggest an additional liver disease aetiology.

b. Chronic cholestatic liver disease (See page 8)

the characteristic features are:

- portal tract expansion with ductular reaction (previously 'proliferation')
- later fibrosis, with linking of portal tracts – hepatic veins not incorporated into bridges until late
- cholate stasis recognised by copper associated protein (CAP) in swollen periportal hepatocytes,

The degree of chronic inflammation is very variable, and the differential diagnosis from the 'chronic hepatitis' pattern may be difficult – Shikata +ve copper associated protein in non-cirrhotic liver is the most important distinguishing feature. CK7+ve periportal hepatocytes (intermediate hepatobiliary cells) are also seen in chronic biliary disease - not usually the same cells that contain CAP. CK7 is also useful to demonstrate portal tracts that lack a bile duct.

Two have fibrosis that is sinusoidal, and later incorporates portal tracts:

c. Fatty liver disease. (See page 10).

d. Chronic vascular diseases (see page 11).

Patterns of chronic liver disease and contribution of special stains;

Chronic hepatitis

The PAS stain without diastase is useful in seeing whether there is or has been interface hepatitis, since then liver cells are left stranded behind the advancing edge of portal fibrosis and inflammation.

Interface hepatitis is characteristic for chronic hepatitis pattern of disease – if there is only portal fibrosis with lymphocytes within the fibrous scar tissue, this is non-specific evidence of chronic liver disease without indicating the cause.

When interface hepatitis is present, additional features may be helpful in the differential diagnosis:

Autoimmune hepatitis: a rather uniform degree of interface hepatitis around most or all the portal tracts, which is moderate or severe in degree, and contains clearly visible plasma cells, perhaps also with rosetting of hepatocytes – this would be the characteristic histology of an auto-immune hepatitis, (but all features may not be present).

Viral hepatitis – Hepatitis B – Shikata stain for hepatitis B surface antigen, and ask for immunoperoxidase for Hepatitis B core and surface antigen if diagnosis is unclear – helpful in some circumstances but not often needed now serum HBV PCR is routinely done.

Hepatitis C – interface hepatitis usually mild degree. Other histological features include steatosis (especially genotype 3), portal tract lymphoid aggregates, bile duct inflammation (but not ductopenia or CAP).

Inflammation in chronic cholestatic disease – patchy interface hepatitis with CAP and reduced bile ducts – likely to be chronic cholestatic disease.

Alpha 1 antitrypsin deficiency – globules in periportal hepatocytes on the PASD stain. If there is a suggestion of these, (negative Shikata stain CAP can be PASD+ve) – then confirm with immunohistochemistry for A1antitrypsin (larger globules have a paler centre with this stain), and ask clinicians to check serum levels. Diffuse positivity is sometimes seen in regenerating hepatocytes.

Wilson disease – look at a Shikata stain for abnormally large amounts of CAP, or in non-periportal location and do a stain for copper (rhodanine) The clinician should be alerted to the possibility of this disease. It is usually screened for in patients with liver disease – diagnosis depends on a combination of low caeruloplasmin, high copper excretion in urine, increased copper in liver, etc.).

Drugs – the presence of the eosiniophils is suggestive of drugs, mainly in acute disease, and by no means always present. Long term nitrofurantoin and minocycline are causes of chronic hepatitis (these may also have autoimmune features), but many drugs are rare causes, check clinical drug history against information on the LiverTox website.

Acute hepatitis with bridging hepatic necrosis – this can look very like a chronic hepatitis with cirrhosis – find out from clinicians if the history is short, if the ALT is very high (>1000) and look for PASD +ve Kupffer cells as sign of recent injury. Passive septa, due to bridging necrosis and collapse – usually contain looser reticulin and less collagen (van Gieson stain) than cirrhotic septa and frequently contain a few red blood cells. The Shikata stain shows elastic fibres within the septa of cirrhosis in cases of some years' duration. The absence of Shikata positivity in the septa raises the possibility that they are due to passive collapse of acute hepatitis.

Chronic cholestatic diseases

Primary biliary cholangitis (PBC)

The biopsy shows a chronic biliary disease, as described above. Bile ducts seem reduced in number, or bile ducts present are surrounded by inflammatory cells, show degeneration of ductal epithelium, or ideally have an associated granuloma. Normally each portal tract, identifiable by the presence of a hepatic artery branch, should contain a bile duct approximately the same size as the hepatic artery, and within 3 diameters of it. .

Nowadays, patients are diagnosed with PBC on the basis of raised alkaline phosphatase and mitochondrial antibodies, +/- raised IgM; biopsy is no longer required for diagnosis. It is only performed if there are atypical clinical features, typically clinical evidence of autoimmune hepatitis as well as PBC.

Liver biopsy in patients with PBC may show either:

a. the presence of bile duct granulomas which is virtually diagnostic of this condition

b. histology which is consistent with the diagnosis in the correct clinical setting – this is much the more common situation. Features common in primary biliary cirrhosis

- a dense lymphocytic aggregate within the portal area, at the site of a previous bile duct – a 'tomb stone' of the destroyed duct

- a mild to moderate degree of interface hepatitis which is often variable from one porta area to another

- ductular reaction is very variable at the margin of the portal tracts; do not confuse these small proliferating ductules with the bile ducts centrally located within the portal tract

- don't expect to see bilirubinostasis – visible bile is only present at a late stage of PBC

- there may be some inflammation or nodular regeneration in the hepatic parenchyma

- the Shikata stain shows the presence of CAP granules in periportal hepatocytes from the early stage of the disease.

Copper associated protein reflects the presence of cholate stasis (the accumulation of bile salts as opposed to pigment) and affected liver cells are usually swollen and pale staining. They may contain MDBs (not an indication of steatohepatitis if in a periportal location).

If CAP is seen before the development of cirrhosis it is very suggestive of a biliary disease such as PBC (also PSC, biliary stricture). Conversely, small amounts of CAP in periportal hepatocytes may be seen non-secifically in established cirrhosis of any cause.

<u>Primary sclerosing cholangitis (PSC)</u>

This is a diagnosis best made on radiology. Liver biopsies may show:

- a classical bile duct lesion of an obliterated duct, its position marked by a dense fibrous scar – very rare in needle biopsies

- a reduction in the number of bile ducts in the portal tracts
- existing bile ducts showing concentric periductal onion skin fibrosis and some attenuation of the epithelial lining
- Histological features of large bile duct obstruction only.
- There may be a degree of interface hepatitis
- Visible bilirubinostasis is often present relatively early in this condition – a point of distinction from PBC.

Shikata +ve periportal CAP in a non-cirrhotic biopsy is usually present in patients with PSC even if the biopsy otherwise shows mild disease. If not seen but clinical suspicion is strong, repeat the Shikata. Also request CK7 – highlights ductopenia, ductular reaction, and may show intermediate hepatobiliary cells (hepatocyte morphology but CK7+ve) in a periportal location – a feature of chronic cholestatic disease.

Clinical context – raised alk phos, may be pANCA antibody, 70% patients have ulcerative colitis. MRCP has often been done already – followed by liver biopsy if findings are indefinite. 'Small duct' PSC is characterised by typical clinical and histological features without imaging changes of PSC.

'**Overlap syndromes'** – patients have clinical features of autoimmune hepatitis and biliary disease (PBC or PSC) – i.e. raised ALT and alk phos, raised IgG and IgM, autoantibodies of ANA/SMA and AMA or pANCA or imaging evidence of PSC. They may fail to respond to medical treatment of their autoimmune hepatitis. In these circumstances, liver biopsy is done to look for features of both diseases, and to indicate which it the dominant disease process. Clinico-pathological correlation is essential in diagnosis and treatment of 'overlap syndromes'

Fatty liver disease

This is the commonest finding in liver biopsies. There may be

- Fatty change only
- Steatohepatitis = fatty change together with hepatocyte ballooning, MDBs, inflammation (usually very mild) and often with fibrosis which is initially with pericellular (around hepatocytes) sinusoidal, and perivenular distribution.
- Cirrhosis – in late stage disease, the steatosis diminishes and disappears, although ballooning/MDBs may survive longer.

There may be some help from special stains:

The Perls stain may show mild to moderate hepatocyte siderosis, especially in alcoholic aetiology.

Van Gieson stain – is very useful in showing pericelular collagen disposition in zone 3, resulting in a 'chicken wire' pattern. Anything more than a minimal staining is significant. The collagen fibres often have a characteristic wavy configuration and completely surround individual hepatocytes.

MDBs – stain well with CK8/18 or Cam 5.2, these also highlight the –ve cytoplasm of ballooned hepatocytes in contrast to the +ve cytoplasmic staining of neighbouring hepatocytes.

Differential diagnosis:

Non-alcoholic steatohepatitis (NASH) v. alcoholic steatohepatitis. The clinical history of alcohol use is essential for the aetiological diagnosis of patients with fatty liver disease. There are some histological pointers though –

Acute alcoholic hepatitis – a clinical presentation where the biopsy shows bilirubinostasis, numerous Mallory bodies, typically associated with polymorph satellitosis, and often prominent pericellular fibrosis. Assess for underlying cirrhosis and evidence of sepsis. This is a serious acute medical condition and steroids may be needed.

Ballooning/ MDBs infrequent compared to degree of steatosis, glycogenated nuclei, relatively slender fibrosis – would be typical of non-alcoholic steatohepatitis.

Vascular disorders

These are uncommon in biopsy, and have subtle features – usually no inflammation or steatosis. Look out for nodular regeneration of parenchyma, with hepatocyte plate atrophy (thin trabeculae of small hepatocytes, often with linear sinusoidal fibrosis and perhaps siderosis). Portal tracts may appear sclerotic- due to fibrosis with loss of portal vein – may be associated with dilated periportal sinusoids (shunt channels) and are suggestive of obliterative portal venopathy (previous thrombosis of main portal vein) and non-cirrhotic portal hypertension.

Vascular disorders include venous outflow obstruction (acute or chronic), veno-occlusive disease and acute hypotension – usually left heart failure in a patient with congestive cardiac failure. Occluded vessels are difficult to see by usual liver stains – if there is any sinusoidal dilatation in the biopsy ask for a trichrome stain and look hard at the efferent veins.

Chronic venous congestion – there is sinusoidal dilatation in zone 3, accompanied by shrinkage or loss of hepatocytes in this region. There may be some fibrosis. Cardiac cirrhosis is rarely seen now that constrictive pericarditis is surgically treated.

Acute venous congestion – in Budd Chiari syndrome – classically there is loss of liver cells in zone 3, with the liver cell plates being replaced by red cells in the space of Disse. Efferent veins may be dilated or thrombosed. Long term this results in fibrosis and cirrhosis – in general the presence of cords of very small liver cells within or adjacent to fibrous septa and a pattern of cirrhosis without inflammatory cells suggests there may be a vascular cause.

Veno-occlusive disease is rarely seen in this country. The occluded small hepatic vein branches are most easily seen on a trichrome stain once this has been prompted by the observation of dilated sinusoids.

The liver in shock – patients with previous congestive heart failure who then develop hypotension get a zonal necrosis of zone 2&3 hepatocytes. This looks very like the acute zonal necrosis seen in paracetamol overdose.

Nodular regenerative hyperplasia

A vague nodularity of the liver tissue seen on reticulin and not accompanied by fibrous septa is seen in nodular regenerative hyperplasia. This may result in portal hypertension. Minor degrees of this lesion are quite common - it is believed to be due to irregular partial occlusion of blood vessels within the liver and is usually of no clinical significance.

Grading and staging in chronic liver disease

The liver biopsy can indicate

a. the stage of liver disease – how far the architecture has changed on a continuum between normal and end stage cirrhosis

b. the grade of liver disease – how active is the liver injury at the time of biopsy, with the implication that more severe activity implies more rapid progression.

These concepts are analogous to the stage and grade of malignant tumours.

This information can be indicated by text in the report, or by using a semi-quantitative numerical scoring system. Commonly used systems are Ishak for viral hepatitis and Kleiner for NASH. Summaries of both are on the UKLPG histopathology trainees/specialty attachment liver section.

Adequate biopsy size is important for staging – there is a risk of under-estimating the stage or grade of disease in small biopsies – to be reasonably reliable a biopsy with more than 10 portal tracts is needed. This is achieved by a biopsy >20mm long obtained with a 16G biopsy needle.

Liver biopsy in cirrhosis

In any biopsy of chronic liver disease, comment on

1. whether there is late stage disease (cirrhosis - definite or possible/developing)
2. Whether there is ongoing active disease, liver injury
3. whether there are clues as to the aetiology

1. Is there cirrhosis?

It can be very difficult to decide whether or not a needle biopsy shows cirrhosis. With micronodular cirrhosis this is often quite clear. With macronodular cirrhosis, the suspicion can be raised, but

macronodular cirrhosis can rarely be confirmed or completely excluded in needle biopsy specimens, In biopsies with a lot of fibrosis and vague micronodularity, the term 'developing ' or 'early' cirrhosis is useful.

NB consider whether the cirrhosis could be an acute hepatitis with bridging hepatic necrosis – the two can look very similar.

Clues to presence of cirrhosis

a. one or more fibrous bands crossing the biopsy, and not containing longitudinally orientated ducts or vessels, are suspicious of cirrhosis

b. if hepatocytes on opposite sides of such bands show different appearances either smaller hepatocytes on one side, or fatty change, this is suspicious of cirrhosis

c. disturbance of the normal vascular relationships so that you can't see efferent veins in appropriate places

d. the Shikata stain – if you know the liver disease is not biliary in nature, the presence of small foci of granules of CAP in periseptal hepatocytes is an indication of cirrhosis. These granules indicate cholate stasis which does not occur in liver disease other than biliary except when cirrhosis has developed. (The rare exceptions are the presence of CAP in Wilson disease and chornic vascular disease). The Shikata stain is also useful for showing elastic fibres in fibrous septa in established cirrhosis, only when the cirrhosis has been present for some years.

2. Is the cirrhosis active?

Aggregates of lymphoid cells which are variable and occur only within areas of fibrosis are often present in cirrhosis and do not indicate continuing disease activity.

Active cirrhosis implies continuing interface hepatitis and/or inflammation within parenchymal nodules.

3. What is the cause of the cirrhosis?

There may be features diagnostic or suggestive of:

- Alpha 1 antitrypsin deficiency
- Haemochromatosis
- Wilson disease (copper throughout nodules, not just periseptal)
- Fatty liver disease - pericellular collagen, ballooned hepatocytes, MDBs that are not just periseptal (which may occur in biliary disease in association with CAP)
- Particularly dense diffuse fibrosis with sinusoidal fibrosis and without nodular regeneration is suggestive of alcoholic liver disease, whether or not there is fatty change.
- Biliary cirrhosis – reduced bile ducts – unaccompanied hepatic arteries, but more difficult to appreciate in cirrhosis. Also marked ductular reaction, abundant CAP around all fibrous septa.

Biopsies with inactive liver disease are a common end stage and are histologically 'cryptogenic'. The Shikata +ve elastic typically matches the van Gieson +ve collagen, because there has been no recent change in the fibrosis/scar tissue in the liver. These patients often have features of metabolic syndrome, and this is thought to represent end stage NASH in many cases, but 'burnt out' autoimmune hepatitis or alcoholic disease in long term abstainers may look the same.

Regression of cirrhosis – effective treatment of viral hepatitis or iron depletion in cirrhosis from haemochromatosis, demonstrate that not all fibrosis in the liver is progressive and irreversible. Features of the 'hepatic repair complex' include perforated, incomplete fibrous septa, isolated thick collagen fibres and hepatocytes within portal tracts or prolapsed into hepatic veins. 'incomplete septal cirrhosis' may be an example of this. Patients may have non-cirrhotic portal hypertension.

Iron in the liver

All liver biopsies are routinely stained with Perls' stain. If iron is present it may be in either hepatocytes or in sinusoidal cells. If there is also cirrhosis, the distribution of the iron is relevant. The degree of iron staining in hepatocytes is conventionally graded 0-4, with grades 3 and 4 being suggestive of haemochromatosis. Grade 1 or 2 may be seen in alcoholic liver disease, and grade 1 in some normal livers. The age/sex of the patient is important. Any hepatocyte iron in a pre-menopausal female may be significant.

Iron staining present only in hepatocytes in a patient with cirrhosis suggests that the iron has accumulated after the development of cirrhosis, i.e. that the cirrhosis is not due to haemochromatosis. Iron present in macrophages within portal areas or fibrous septa and bile duct epithelium as well as in hepatocytes shows that the cirrhosis developed after iron deposition, and therefore may be due to haemochromatosis.

Patients with iron overload due to multiple blood transfusions usually have more iron in macrophages than in liver cells. Small amounts of iron present only in Kupffer cells is suspicious of recent hepatocyte necrosis, and is accompanied by PASD positivity in the Kupffer cells.

Venesection treatment for haemochromatosis – patients who have developed cirrhosis may still be at risk of hepatocellular carcinoma, even when the iron stores are fully depleted and the cirrhosis is regressing.

Tumours

Targeted liver biopsies are often taken of liver masses during imaging investigation. There are two questions – is this a tumour, and is the tumour primary or secondary? Include a comment on any background liver tissue in the report.

It is important that targetted biopsies are distinguished from medical liver biopsies so that they can be handled differently in the lab. We cut two shallow H&Es with spare sections saved between for immunohistochemistry. If no tumour in levels 1-2, check deeper levels before reporting no tumour present.

Focal nodular hyperplasia (FNH) – the biopsy here looks a bit like cirrhosis, often like PBC – and the history that the biopsy comes from a focal lesion is essential. The big septa near the middle contain abnormal large vessels, but no bile ducts. Arteries are often associated with ductular reaction and inflammatory infiltrate. A biopsy of unaffected liver is helpful in ruling out a generalised chronic liver disease. Glutamine synthetase gives a characteristic map-like positivity useful in diagnosis.

Hepatocellular neoplasia

Benign primary lesions composed of near-normal hepatocytes – include hepatocellular adenoma and FNH. In biopsies, it may be difficult to distinguish lesion from background liver – look for the presence of portal tracts in background liver while diffusely positive sinusoids for CD34 ('capillarisation of sinusoids') may help identify the lesion. The differential diagnosis is with very well differentiated hepatocellular carcinoma (HCC).

In adenoma, there is regular liver tissue with absence of any portal tracts, although arteries and veins may be present. Check reticulin -deficiency is a feature of HCC.

Hepatocellular adenomas are now sub-typed along genetic lines, and types are often predictable by morphology. Confirmatory immunohistochemistry is useful, especially in cases lacking these morphologies:

Inflammatory adenoma – i-HCA – mutation in the IL6 pathway. This results in adenoma with sinusoidal dilatation, inflammatory infiltrate and ductular reaction around arteries (but no bile ducts). This type may also have beta-catenin mutations, and risk of hepatocellular carcinoma.

Steatotic adenomas – h-HCA – mutation of hepatocyte nuclear factor with down-stream Liver fatty acid binding protein defiency. Usually diffuse steatosis. Stains negative for LFABP (background liver is positive). May be multifocal, Malignancy very rare.

Beta-catenin – mutated adenoma b-HCA – rare, more often in males. May be cytological atypia and rosettes. Positive for glutamine synthetases (diffuse or spotty, not map-like). May have rare nuclear

positivity for beta catenin (which normally shows membranous positivity – even one positive nucleus is significant).

Adenoma which lacks any of these distinctive features requires immunohistochemistry or molecular testing for diagnosis.

Benign bile duct lesions - usually in sub-capsular frozen section rather than needle biopsy - think of von Meyenberg complex and peribiliary gland hamartoma (formerly bile duct adenoma) - both have distinctive characteristics.

Malignant tumours

Hepatocellular carcinoma

These vary in appearance from something that looks almost like normal liver to a highly anaplastic tumour which could be anything. Usually a hepatocellular carcinoma will have:

- cells which resemble hepatocytes with plentiful eosinophilic cytoplasm although the nuclear-cytoplasmic ratio is higher. Nuclei usually have prominent nucleoli. There is usually little or no fibrosis unless the tumour is infiltrating a cirrhotic liver.

- sinusoidal spaces between trabeculae of liver cells lined by endothelium resembling the structure of normal liver.

- reticulin stain may show absence of reticulin in areas – this is a useful sign of HCC but is often patchy.

In a biopsy of a liver cell lesion, tumour cells forming rosettes with central bile plugs are the most reliable indication of hepatocellular carcinoma

Immunohistochemistry –

Alpha fetoprotein – even single cells positive are diagnostically useful – patients usually have high or rising serum levels

HepPar1 – marker of hepatocyte mitochondria, positive at least focally in most HCC unless poorly differentiated. Patchy absence of staining, compared to +ve in background liver tissue – can be a useful pointer to well differentiated HCC.

Polyclonal CEA and CD10 are markers of biliary canaliculi – evidence of hepatocyte differentiation in carcinomas – diffuse positivity is non-specific.

CK7 positive in around 15% HCC. CK19 positive in <10% HCC, and if present is associated with a poorer prognosis, and poor response to sorafenib treatment.

CD34 diffuse sinusoidal endothelial positivity in HCC, not in normal liver. However may be diffuse positivity in adenoma and FNH.

Glutamine synthetase - normally +ve in perivenular hepatocytes only. Also +ve in some adenomas, map like in FNH

Glypican 3 - an oncofetal antigennot present in normal hepatocytes,

Cholangiocaricnoma

Adenocarcinoma arising from bile ducts – distinguish intrahepatic, peripheral mass forming cholangiocarcinoma from perihilar cholangiocarcinoma which has a periductal infiltrating pattern of growth.

Diagnosis is mainly based on imaging – distinction between primary cholangiocarcinoma and metastatic adenocarcinoma particularly from the upper GI tract may not be possible on biopsy material.

Targetted Liver biopsy for suspected metastatic malignancy.

Diagnostic liver biopsy is avoided in patients who may be suitable for surgical resection of their tumour, because of the risk of chest wall recurrence or dissemination caused by biopsy.

For non-surgical patients, targeted biopsy under image guidance is often the best way to diagnose the tumour and plan treatment. Most are metastatic adenocarcinomas.

A panel of immunohistochemistry is appropriate, the choice of markers must be influenced by any previous cancer history, since the amount of tissue available may be limited. If no previous history, the panel for adenocarcinoma is CK7, CK20, TTF1, ER/PR (women), PSA (men), PLAP (germ cell).

JIW

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