Histological assessment of the liver

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Abstract

Histopathological assessments play an important role in the diagnosis and management of patients with liver disease. For some conditions, liver biopsy is still routinely used to establish the cause of liver disease. In other circumstances, evaluation of morphological changes provides additional information that is useful for clinical management, for example when assessing disease severity in chronic viral hepatitis and non-alcoholic fatty liver disease. However, with the increased use of non-invasive methods for assessing the severity of liver injury, particularly fibrosis, the role of liver biopsy in this respect is changing. In cases where a dual pathology is suspected, histological assessment can help to identify the main cause of liver injury. In addition, liver biopsy sometimes reveals abnormalities that have not been detected by previous investigations. Histopathological assessment of liver biopsies involves a systematic evaluation of changes involving individual components of the normal liver. The final interpretation of the abnormalities detected depends on clinico-pathological correlation. Sampling variation is a problem, particularly with small needle biopsies, and should be considered as a possible explanation when there is a disparity between clinical and pathological findings.

Keywords Liver biopsy; liver histology; liver pathology; MRCP

Key points

- Liver biopsy remains a key component in the diagnosis and management of medical liver disease
- Final interpretation is based on careful systematic assessment of the biopsy and close clinico-pathological correlation
- Sampling variation is a problem, particularly with small needle biopsies, and should be considered as a possible explanation when there is disparity between clinical and pathological findings
- Conventional histological assessments can be supplemented by scoring individual features, such as inflammatory grade and fibrosis stage; histological scoring is most appropriate in the context of clinical trials
- Non-invasive methods are increasingly used to assess severity of liver disease and have led to a changing role for liver biopsy
- Computer-assisted image analysis methods can provide accurate measurements of histological features such as fibrosis and steatosis — these methods are likely to be increasingly used as an adjunct to conventional histology, particularly as digital pathology evolves

Introduction

This article focuses on the role of liver biopsy in the assessment of medical liver diseases, where diffuse hepatic involvement is generally presumed to be present.[1,2] For some conditions (e.g. liver allograft rejection), histopathology is still regarded as the diagnostic gold standard. For other diseases, where a diagnosis is already suspected on the basis of other investigations (e.g. autoimmune hepatitis), liver biopsy is still routinely used, both to identify features supporting the suspected clinical diagnosis and to exclude the presence of features that might suggest an additional or alternative diagnosis.

Even in cases where the cause of liver disease has already been identified, the evaluation of morphological changes provides additional information that is useful for clinical management — examples include grading of disease severity (e.g. inflammatory activity in chronic viral or autoimmune hepatitis, steatosis severity in fatty liver disease) and staging of fibrosis in chronic viral, autoimmune and fatty liver diseases. In addition, liver biopsy may reveal abnormalities (e.g. iron overload, α_1-antitrypsin globules) not detected by previous investigations.

Types of specimens obtained

Specimens are most commonly taken for histological assessment by percutaneous needle biopsy. Needle biopsies may also be obtained via the transjugular route if there is massive ascites or a problem with blood coagulation, or under ultrasound guidance (or, less commonly, at laparoscopy) to sample focal lesions in the liver. Cytological specimens obtained by fine needle aspiration and wedge biopsies taken at laparotomy are also useful in the evaluation of focal liver lesions.

When interpreting liver histology, an adequate sample is vital as samples that are too narrow or too short increase problems with sampling variability and lead to understaging of liver disease. Recommendations for an adequate biopsy include the use of a 16-gauge (or wider) needle, a minimum of 10–12 portal tracts and a length of 20–25 mm. Unfortunately many specimens currently obtained fail to reach these definitions of adequacy. Surgical wedge biopsies taken from the subcapsular area can be misleading as there is more normal fibrous tissue in this region.

Interpretation of liver biopsy

The main features below should be included in the evaluation of all liver biopsies in which a diffuse liver injury is suspected.

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Liver architecture

An intact core at least 1 cm long is generally required to determine whether the normal vascular relationships between portal tracts and hepatic venules have been retained. For accurate assessment of fibrosis, a larger core (at least 20–25 mm long) is desirable. There may be problems with fragmentation of biopsies from cirrhotic livers. However, in these circumstances, the presence of fibrous septa with incomplete nodule formation usually enables a reasonably confident diagnosis to be made. In some patients with macronodular cirrhosis, an entire biopsy core taken from within a single macronodule may not show obvious fibrosis, but the presence of subtle architectural abnormalities can provide a clue to the underlying problem.

Connective tissue stains (Table 1) are required to distinguish long-standing fibrosis, in which mature elastic fibres are seen, from recent collapse following liver cell necrosis, in which there is condensation of the reticulin framework with immature collagen fibre deposition (Figures 1 and 2). This can be a problem in some patients with severe acute hepatitis, where surviving hepatocyte nodules surrounded by zones of necrosis or collapse can give a false impression of liver cirrhosis. The nodular shrunken liver that occurs in such cases can also be radiologically mistaken for cirrhosis.

Nodular regeneration without fibrosis (nodular regenerative hyperplasia) usually indicates a problem with the vascular supply to the liver, particularly portal venous insufficiency. Nodular regenerative hyperplasia is seen as part of the histopathological spectrum of 'idiopathic non-cirrhotic portal hypertension', in which the primary lesion is occlusion of small intrahepatic portal vein branches (portal vein stenosis). Distinction between non-cirrhotic and cirrhotic causes of portal hypertension is important, as patients with the former generally have a more indolent course with well-preserved synthetic function and less frequently progress to liver failure.

Portal tracts

Portal inflammation is common in many liver diseases but is particularly characteristic of chronic viral or autoimmune hepatitis. The nature of the inflammatory cells may provide a clue to the liver disease (e.g. numerous plasma cells in autoimmune hepatitis, granulomas in primary biliary cirrhosis (Figure 3), eosinophils in drug reactions).

The extension of inflammation from portal areas into the adjacent liver parenchyma with damage to periportal hepatocytes ('interface hepatitis' (Figure 4)) is thought to be important in the pathogenesis of periportal fibrosis, which occurs in many chronic liver diseases.

Bile ducts are the main targets for injury in some cholestatic liver diseases. Sclerosing cholangitis (whether primary or secondary) is characterized by concentric periductal 'onion skin' fibrosis and nodular scarring of the ducts, whereas primary biliary cholangitis is characterized by inflammatory bile duct destruction, frequently with a granulomatous component. The term 'vanishing bile duct syndrome' is used to describe cases with substantial bile duct loss (usually defined as ducts missing from >50% of portal tracts) (Figure 5). Counting bile ducts accurately in small biopsy specimens is complicated by sampling variation and the similarity between bile ducts and bile ductules, the number of which is increased in ductular reactions (see below). Nevertheless, a careful assessment of bile ducts should always be made if there is evidence of cholestatic liver disease.

Assessment of portal vessels (hepatic arterioles and portal venules) has a limited use in routine liver biopsy diagnosis. Arterial lesions are seldom seen in systemic vasculitides (e.g. polyarteritis nodosa). Obliteration of portal venules occurs in portal vein stenosis (see above).

In the liver allograft, the portal tracts are typically the main focus for acute cellular (T-cell-mediated) rejection, in which a mixed infiltrate of lymphocytes, plasma cells, eosinophils and

Special stains used in routine histological assessment of liver biopsies

Stain	Material demonstrated	Distribution in normal liver	Changes in liver disease
Reticulin	Type III collagen fibres	Portal tracts, hepatic sinusoids	Collapse of reticulin framework in areas of recent liver cell necrosis Thickening of cell plates in areas of nodular regeneration
Haematoxylin van Gieson[a]	Type I collagen fibres	Portal tracts, walls of hepatic vessels	Increased in hepatic fibrosis
Orcein	Hepatitis B surface antigen		Present in some patients with chronic hepatitis B virus infection
	Copper-associated protein[b]		Present in chronic cholestasis
	Elastic fibres	Portal tracts Walls of hepatic vessels	Found in long-standing fibrosis/cirrhosis
Periodic acid–Schiff	Glycogen	Hepatocytes	
Periodic acid–Schiff diastase	Mucin	Bile ducts	
	α_1-Antitrypsin globules		Present in α_1-antitrypsin deficiency
Perls' reaction	Haemosiderin		Increased in haemosiderosis/haemochromatosis

[a] Alternatives include the trichrome and Picrosirius red stains, which also stain collagen fibres.
[b] Rhodanine staining, which detects copper, can also be used.

Table 1

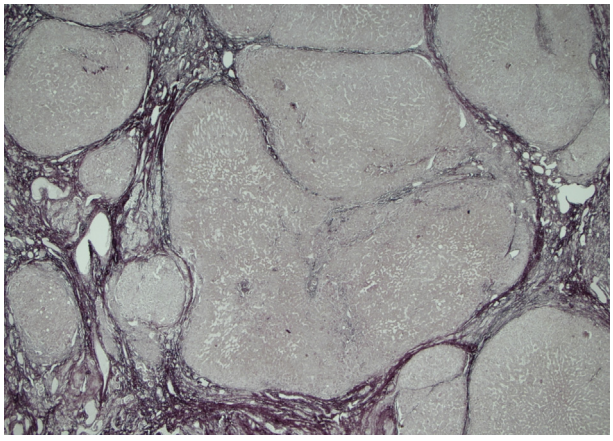

Figure 1 Orcein staining in a cirrhotic liver. Nodules of regenerating hepatocytes are completely surrounded by darkly staining elastic fibres, indicative of long-standing fibrosis.

blast-like cells is seen, associated with inflammation of bile ducts and portal vein branches (portal vein endotheliitis).

A bile ductular reaction is commonly seen in many forms of liver injury, but is particularly prominent in chronic cholestatic liver diseases (Figure 5), where it may lead to the development of progressive periportal fibrosis.

Liver parenchyma

Important lesions in the liver parenchyma include inflammatory infiltration, hepatocellular degenerative changes (e.g. fatty change, bile stasis, ballooning, Mallory–Denk bodies) and liver cell death by necrosis or apoptosis. In fatty liver disease (alcoholic, non-alcoholic), liver biopsy is important for the distinction between simple steatosis – a generally reversible and non-progressive condition – and steatohepatitis, which has greater potential to progress to fibrosis and cirrhosis.

Many conditions involving the liver parenchyma tend to have a zonal distribution, particularly involving regions around the terminal hepatic venules (acinar zone 3). Conditions that typically produce zone 3 liver cell damage include fatty liver disease, toxic liver injury (e.g. paracetamol poisoning), ischaemia and some immune-mediated diseases (e.g. bridging necrosis in acute or chronic viral hepatitis).

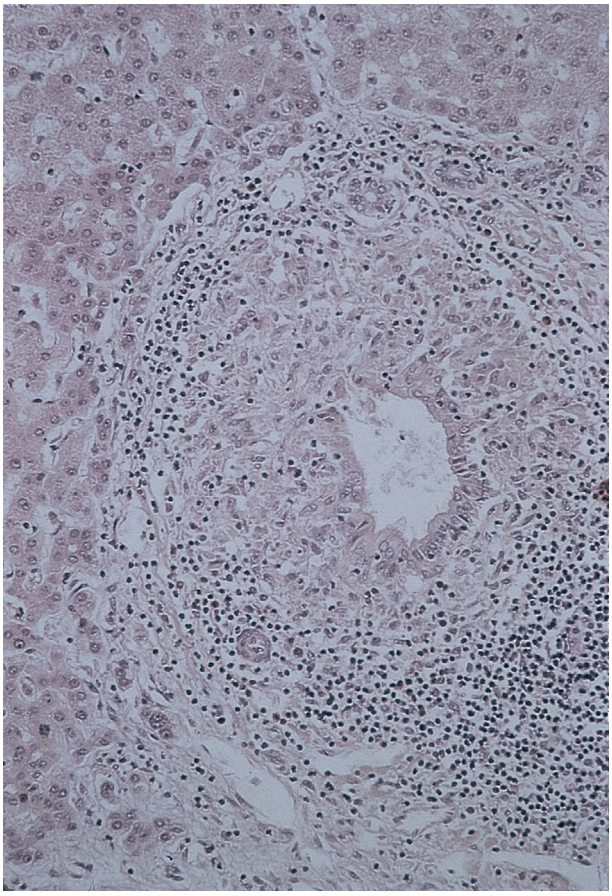

Figure 3 Primary biliary cirrhosis. Granulomatous bile duct destruction is characteristic of primary biliary cirrhosis. However, this lesion is patchy in distribution and is absent from many liver biopsies in patients with this condition.

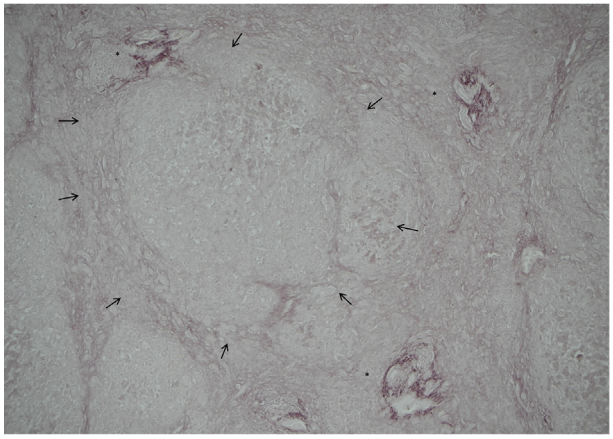

Figure 2 Orcein staining in a case of acute hepatitis. There is a nodule of regenerating hepatocytes in the centre of the field (outlined by arrows). This appearance can simulate cirrhosis on routine H&E staining but, in contrast to Figure 1, orcein staining shows elastic fibres largely confined to portal tracts (asterisks). The changes seen here are therefore relatively acute and potentially reversible.

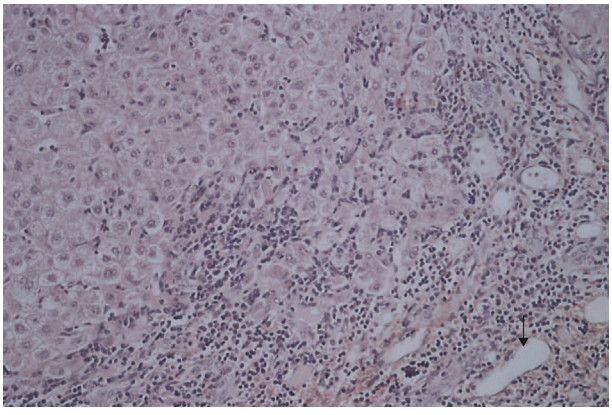

Figure 4 Portal and periportal hepatitis in autoimmune hepatitis. The portal tract (arrow) contains a dense infiltrate of mononuclear inflammatory cells. There is conspicuous spillover of lymphoid cells into the surrounding liver parenchyma ('interface hepatitis').

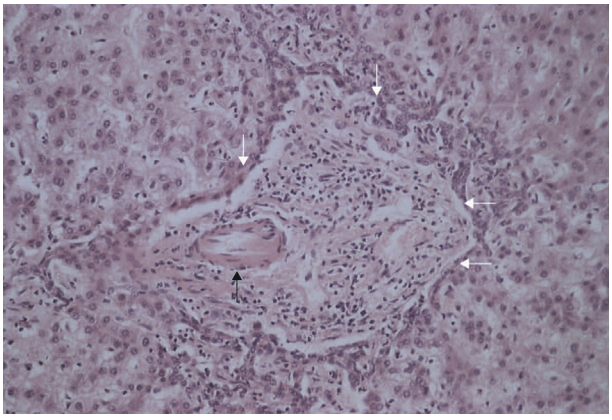

Figure 5 Loss of bile ducts in chronic biliary disease. This specimen, from a woman with primary biliary cirrhosis, contains a portal tract in which there is an arterial branch (black arrow) without an accompanying bile duct. The number of bile ductular structures is increased at the periphery of the portal area (bile ductular reaction, white arrows). In the absence of diagnostic granulomatous bile duct lesions, a definite histological diagnosis of primary biliary cirrhosis cannot be made from the changes illustrated here. Other chronic biliary diseases (e.g. primary sclerosing cholangitis) can have similar histological appearances.

Non-parenchymal cells

Non-parenchymal cells in hepatic sinusoids (Kupffer cells, hepatic stellate cells, sinusoidal endothelial cells) have an important role in the pathogenesis of many liver diseases, but are relatively less important in routine liver biopsy diagnosis. Abnormal Kupffer cells are found in some storage diseases (e.g. Gaucher's disease) and in certain intracellular infections (e.g. leishmaniasis). Enlarged Kupffer cells laden with ceroid 'wear-and-tear' pigment (or sometimes haemosiderin) are commonly present as a non-specific manifestation of recent hepatocyte injury.

Hepatic veins

Occlusion of terminal hepatic venules is characteristically seen in veno-occlusive disease of the liver. In most cases, this is thought to represent toxic liver injury, and it is particularly associated with certain forms of chemotherapy. The primary site of injury in hepatic veno-occlusive disease is now thought to be the endothelium lining the hepatic sinusoids ('sinusoidal obstruction syndrome'), with occlusion of hepatic venules probably occurring as a secondary event.

Features of sinusoidal obstruction syndrome are frequently seen in patients treated with oxaliplatin-based chemotherapy for metastatic colorectal carcinoma. Veno-occlusive lesions can also occur as a secondary phenomenon in other causes of venous outflow obstruction (e.g. Budd–Chiari syndrome, constrictive pericarditis) and, to a lesser degree, in many forms of chronic liver disease (e.g. alcoholic cirrhosis).

Special stains

Special stains used in routine liver biopsy assessment and their diagnostic applications are shown in Table 1.

Immunohistochemical and other ancillary studies

Immunohistochemical studies are used as an adjunct to conventional histological diagnosis. Substances that can be detected immunohistochemically in tissue sections include viral antigens (e.g. hepatitis B surface antigen, hepatitis B core antigen, hepatitis delta virus, cytomegalovirus, Epstein–Barr virus), Mallory–Denk bodies (which contain material immunoreactive for ubiquitin, p62 and keratin 8), α_1-antitrypsin and bile duct keratins such as keratin 7 (to detect residual biliary epithelial cells, highlight foci of ductular reaction in vanishing bile duct diseases and identify cells of intermediate hepatobiliary phenotype).

Tissue processed for histological examination (i.e. formalin-fixed and paraffin-embedded) can also be used for non-histological investigations such as biochemical measurements (e.g. liver copper or iron). However, fresh tissue is preferable for copper measurement, and for molecular analysis using techniques such as polymerase chain reaction analysis and chromogenic *in situ* hybridization (e.g. to facilitate diagnosis of viral diseases).

Clinico-pathological correlation

The individual features described above should be assimilated to identify the main pattern of liver injury (e.g. acute or chronic hepatitis, fatty liver disease, chronic biliary disease, etc.), for which there are usually a number of possible causes; clinico-pathological correlation is then required to establish a diagnosis. In some cases where more than one pattern of liver injury is present, dual pathology may be suspected – in such cases, liver biopsy may help to identify the predominant cause of liver injury.

Clinical meetings between pathologists and hepatologists can provide a useful forum for discussing more complex cases. To regard a liver biopsy report as a 'result' (analogous to measuring serum transaminase concentration) is clearly inappropriate and should be strongly discouraged.

Problems with interpretation of liver biopsy

Sampling variation

The average liver biopsy samples only about 1/100,000th of the whole liver. Fortunately, the distribution of pathological changes is reasonably uniform in many conditions, such as steatosis in fatty liver disease (alcoholic, non-alcoholic) and inflammation in chronic hepatitis (viral, autoimmune). However, in chronic biliary disease, fibrosis and cirrhotic transformation is often unevenly distributed. Variation in the severity of fibrosis occurs to a lesser degree in chronic viral hepatitis and fatty liver disease. Liver damage is also non-uniform in vascular diseases and severe acute hepatitis with multiacinar necrosis. An adequate sample is therefore vital in all liver diseases.

Insufficient clinical details

As noted above, liver biopsy assessment relies on careful clinico-pathological correlation. Many liver diseases have overlapping histological features, and small needle biopsies may not contain the pathognomonic features that allow a definite diagnosis. In the absence of detailed clinical information, the pathologist may be able to make only general statements regarding the overall pattern of liver injury. An example of the problems that can be encountered in the assessment of chronic biliary disease is illustrated in Figure 5. Likewise, alcoholic and non-alcoholic fatty liver disease have similar histological features, and the

distinction between the two generally requires additional clinical information.

Request forms for liver biopsies should always be accompanied by a clinical history, including the results of any relevant biochemical, serological and radiological investigations.

Histological grading and staging in chronic liver disease

Pathological grading and staging may be used to supplement the histological assessment of chronic liver diseases, including chronic viral hepatitis and fatty liver disease. Grading provides an indication of ongoing damage to the liver, which is potentially treatable — examples include inflammation in chronic viral hepatitis, steatosis and hepatocyte ballooning in fatty liver disease. Staging is a measure of progressive liver injury (usually fibrosis), which is less likely to be reversible.

Several semi-quantitative scoring systems have been devised that convert subjective assessments of inflammatory activity and fibrosis into numerical scores. These have a potentially useful role in the context of clinical trials but should not be used as a substitute for conventional liver biopsy reporting in routine clinical practice. Furthermore, scoring systems intended primarily to assess disease severity are sometimes used inappropriately to establish a primary diagnosis; one example is the Kleiner system, which was designed to assess disease severity in NAFLD, but has also been used wrongly to establish a diagnosis of non-alcoholic steatohepatitis.

Computer-assisted image analysis approaches have recently been used to determine accurately the amount of fibrous tissue in a liver biopsy, and can provide information that is more reproducible and more accurate prognostically than semi-quantitative scoring of fibrosis.[3] Similar techniques can also be used to quantify other histological features, such as steatosis. With ongoing developments in the field of digital pathology, which may ultimately replace conventional microscopy, the use of these approaches in routine practice is likely to increase.[4]

Several non-invasive methods have been developed as surrogate markers for the pathological changes that occur in liver disease, particularly hepatic fibrosis in chronic viral hepatitis and NAFLD. Non-invasive methods for assessing liver fibrosis include serological markers and various imaging techniques, such as transient elastography to measure liver stiffness. These alternative approaches have obvious attractions: they reduce the discomfort and potentially serious complications associated with liver biopsy and enable frequent assessments to be made.[5]

Non-invasive methods are most accurate in identifying patients with advanced or mild/minimal fibrosis and less useful in assessing intermediate levels of liver injury. The results need to be interpreted with caution in patients with alternative causes of changes in liver stiffness (e.g. inflammation, steatosis, cholestasis) or serum markers of fibrosis. Furthermore, liver biopsy frequently identifies coexistent lesions that may be relevant for prognosis and management (e.g. fatty liver disease in patients with chronic hepatitis C infection). Nevertheless, non-invasive methods for assessing liver fibrosis are increasingly used in clinical practice and have resulted in a changing role for liver biopsy in the management of patients with chronic liver disease. ◆

KEY REFERENCES

1 Lefkowitch JH. Scheuer's liver biopsy interpretation. 8th edn. Philadelphia: Saunders Elsevier, 2010.
2 Burt AD, Ferrell LD, Hübscher SG, eds. MacSween's pathology of the liver. 7th edn. Philadelphia: Elsevier, 2018.
3 Kendall TJ, Dolman GE, Duff CM, et al. Hepatic elastin content is predictive of adverse outcome in advanced fibrotic liver disease. *Histopathol* 2018; **73:** 90–100.
4 Griffin J, Treanor D. Digital pathology in clinical use: where are we now and what is holding us back? *Histopathol* 2017; **70:** 134–45.
5 Tapper EB, Lok AS-F. Use of liver imaging and biopsy in clinical practice. *N Engl J Med* 2017; **377:** 756–68.

TEST YOURSELF

To test your knowledge based on the article you have just read, please complete the questions below. The answers can be found at the end of the issue or online here.

Question 1

A 40-year-old man presented for review of deranged liver function tests. He had a history of diabetes mellitus and joint pain affecting the knees and hands.

On clinical examination, he had bronze-coloured skin without any stigmata of chronic liver disease.

Investigations

- Ferritin 1135 micrograms/litre (15–300)
- Transferrin saturation 63% (15–50).
- Ultrasonography of the liver showed slight irregularity of the contours

The patient proceeded to liver biopsy.

Which of the following special stains will identify the most likely aetiology?

A. Haematoxylin van Gieson
B. Orcein
C. Periodic acid–Schiff diastase
D. Perls'
E. Rhodanine

Question 2

A 23-year-old woman was referred with deranged liver function tests. She had no symptoms and clinical examination was normal.

Investigations

- Bilirubin 27 micromol/litre (<22)
- Alanine aminotransferase 178 U/litre (5–35),
- Alkaline phosphatase 110 U/litre (45–105)
- Immunoglobulin G 32.3 g/litre (6–13)
- Antinuclear antibodies positive 1:1600
- Smooth muscle antibody positive
- f-Actin antibody positive
- Mitochondrial antibodies negative
- Increased liver stiffness was detected on elastography

The patient proceeded to liver biopsy.

Which of the following features seen on the liver biopsy is likely to be of most prognostic importance?

A. Bridging fibrosis with incomplete nodule formation
B. Centrilobular necroinflammatory lesions
C. Hepatitic rosette formation
D. Plasma cell rich portal inflammation with interface activity
E. Spotty lobular inflammation

Question 3

A pathologist received a liver biopsy taken from a 37-year-old man. The request form stated only 'Abnormal liver function tests'. On assessment of the biopsy, the portal tracts showed bile duct loss and a marginal ductular reaction. There was prominent concentric 'onion-skin' fibrosis surrounding a medium size bile duct. Deposition of copper in periportal hepatocytes was seen on the rhodanine stain.

In the absence of any further clinical information, what is the most appropriate diagnosis?

A. Chronic autoimmune hepatitis of unknown aetiology
B. Drug-induced chronic hepatitis
C. Primary biliary cholangitis
D. Primary sclerosing cholangitis
E. Sclerosing cholangitis