The Royal College of Pathologists
Pathology: the science behind the cure

Tissue pathways for liver biopsies for the investigation of medical disease and focal lesions

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Comments	This document replaces the 2nd edition of *Tissue pathways for liver biopsies for the investigation of medical disease and for focal lesions* published in 2014. In accordance with the College's pre-publications policy, this document was on the Royal College of Pathologists' website for consultation from 22 July to 19 August 2020. Responses and authors' comments are available to view on publication of the final document. **Dr Brian Rous** **Clinical Lead for Guideline Review**

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Contents

NICE has accredited the process used by the Royal College of Pathologists to produce its tissue pathways. Accreditation is valid for five years from 25 July 2017. More information on accreditation can be viewed at www.nice.org.uk/accreditation.

For full details on our accreditation visit: www.nice.org.uk/accreditation.

Foreword

The tissue pathways published by the Royal College of Pathologists (RCPath) are guidelines that enable pathologists to deal with routine surgical specimens in a consistent manner and to a high standard. This ensures that accurate diagnostic and prognostic information is available to clinicians for optimal patient care and ensures appropriate management for specific clinical circumstances. This guideline has been developed to cover most common circumstances. However, we recognise that guidelines cannot anticipate every pathological specimen type and clinical scenario. Occasional variation from the practice recommended in this guideline may therefore be required to report a specimen in a way that maximises benefit to the patient.

The guidelines themselves constitute the tools for implementation and dissemination of good practice.

The following stakeholders were contacted to consult on this document:

- UK Liver Pathology Group
- British Society of Gastroenterology
- British Association for the Study of the Liver
- The Royal College of Radiologists.

The information used to develop this tissue pathway was collected from electronic searches of the medical literature, previous recommendations of the RCPath and local guidelines in the UK. Published evidence to support the recommendations was identified by a PubMed search (terms 'Liver' AND 'Biopsy', searched 2000−2018) and referenced where appropriate. Much of the content of the tissue pathways represents custom and practice, and is based on the substantial clinical experience of the authors. For the reporting guidance and related appendices, this includes referral practice and experience from the evaluation of responses in the UK Liver Pathology external quality assessment (EQA) scheme (evidence corresponding to ‘good practice point’ in Appendix J). Published evidence to support the recommendations was identified by a PubMed search and referenced where appropriate. The evidence was evaluated using modified SIGN guidance (see Appendix H). Consensus of evidence in the tissue pathways was achieved by expert review. Gaps in the evidence were identified by College Fellows via feedback received from consultation. The sections of this tissue pathway that indicate compliance with each of the AGREE II standards are indicated in Appendix I.

No major organisational changes or cost implications have been identified that would hinder the implementation of the tissue pathways.

A formal revision cycle for all tissue pathways takes place on a five-yearly basis. However, each year, the College will ask the author(s) of the tissue pathways, in conjunction with the relevant subspecialty advisor to the College, to consider whether or not the document needs to be updated or revised. A full consultation process will be undertaken if major revisions are required. If minor revisions are required, an abridged consultation process will be undertaken, whereby a short note of the proposed changes will be placed on the College website for two weeks for members’ attention. If members do not object to the changes, the changes will be incorporated into the pathways and the full revised version (incorporating the changes) will replace the existing version on the College website.

This tissue pathway has been reviewed by the Clinical Effectiveness department, Working Group on Cancer Services (WGCS) and Lay Governance Group. It was placed on the College website for consultation with the membership from 22 July to 19 August 2020. All comments received from the Working Group and membership were addressed by the authors to the satisfaction of the Chair of the Working Group and the Clinical Lead for Guideline Review.

This pathway was developed without external funding to the writing group. The College requires the authors of tissue pathways to provide a list of potential conflicts of interest; these are monitored by the Clinical Effectiveness department and are available on request. One author is Chair of the UK Liver Pathology Group. All other authors have no conflicts of interest.

1 Introduction

This document provides guidance on the specimen handling and reporting of liver biopsies. The term 'biopsy' is used to refer to the tissue specimen itself rather than the broader clinical usage that encompasses the procedure used to procure that specimen. This document relates primarily to those biopsies taken for the investigation of medical liver diseases (sections 3–8, referred to as 'medical liver biopsies'). The term 'medical liver diseases' is used to describe conditions that typically affect the liver diffusely and are generally managed medically (as opposed to focal liver lesions, which require alternative therapeutic approaches, usually surgical). For convenience, the section on targeted liver biopsy of focal lesions from the liver cancer dataset is reproduced here (section 9) since most of these biopsies are reported outside specialist hepatobiliary cancer centres. An audit of UK practice in 2008 showed that 67% of needle core liver biopsies in the UK were for the investigation of diffuse parenchymal liver disease and 33% for the diagnosis of focal lesions. This proportion varied widely among departments.[1]

The first guideline *Tissue pathways for liver biopsies for the investigation of medical disease and for focal lesions* was published in 2008 and updated in 2014. These have now been revised to ensure that all recommendations are up to date.

This third edition has been produced to coincide with the *Joint Clinical Guidelines on the Use of Liver Biopsy*, a joint publication by the British Society of Gastroenterology, Royal College of Radiologists and Royal College of Pathologists (referred to in the following text of the tissue pathway as the Joint Clinical Guidelines).[2]

Liver biopsy is an invasive procedure associated with a small risk of serious and potentially life-threatening complications. The decision to perform a liver biopsy is based on a careful risk-benefit assessment. Once the decision to perform a liver biopsy has been made, it is essential that laboratory and diagnostic procedures are in place to optimise the clinical benefit obtained from the biopsy. This document will consider the 'test' component of the liver biopsy; for pre-test and post-test issues, the reader is referred to the Joint Clinical Guidelines document.[2]

The Joint Clinical Guidelines also include a section on the use of biopsy tissue purely for research purposes.[2] Any division of biopsy tissue between research and clinical investigation should be the subject of informed local governance and ethics panel decisions that take account of any impact on clinical adequacy. This is distinct from the study of routinely fixed and processed liver biopsies, including residual tissue superfluous to diagnosis, which studied over decades forms the basis of the contribution of histopathology to patient management.

This is a time of change in the use of medical liver biopsies. Regional hepatology networks are becoming established, the prevalence of liver disease is increasing in the population (particularly fatty liver diseases, both alcohol and non-alcohol related, and viral hepatitis) and its detection at an early stage is also increasing.[3] At the same time, the increasing use of non-invasive methods for the assessment of liver fibrosis is reducing the use of medical liver biopsy purely for staging purposes. Furthermore, liver biopsies are now rarely obtained from people with chronic hepatitis C virus (HCV) infection owing to the advent of antiviral agents that are effective in treating people with HCV infection irrespective of disease stage. The effect of these opposing trends on biopsy numbers is uncertain. There is likely to be an overall increase in the complexity of liver biopsies obtained due to the presence of multiple aetiological risk factors. There is a detailed description of disease-specific indications for liver biopsy in the online supplementary material appendix of the BSG Joint Clinical Guidelines.[2]

Medical liver biopsies are currently reported by nearly all UK hospital histopathology departments. The purpose of this document is to promote uniform good practice of laboratory biopsy handling and primary reporting. Indications for biopsy specialist review/second opinion within hepatology networks are also considered. The following recommendations are regarded as the minimum acceptable practice for liver biopsies.

1.1 Target users of these guidelines

The primary users of this tissue pathway are trainees and consultant cellular pathologists. The recommendations will also be of value to histology laboratory managers, users of a liver pathology service and service commissioners.

2 Generic issues relating to staffing, workload and facilities

The following recommendations should be met for a general level of acceptable practice:

- the laboratory should have sufficient pathologists, biomedical scientists and clerical staff to cover all of its functions. In general, staffing levels should follow the workload guidelines of the RCPath.
- pathologists should:
  - participate in audits
  - participate in the RCPath's continuing professional development (CPD) scheme
  - participate in pathology EQA schemes (see sections 3.1.3 and 3.1.4)
  - have standard liver pathology texts available for reference via the pathology document, e.g. Scheuer and Lefkowitch's *Liver Biopsy Interpretation*, MacSween's *Pathology of the Liver* and Torbenson's *Atlas of Liver Pathology*[4–6]
  - have access to specialist referral opinions on a regional network or national basis.
- the laboratories should:
  - be equipped to allow the recommended technical procedures to be performed safely
  - be accredited by United Kingdom Accreditation Service (UKAS) or equivalent
  - participate in the UK national EQA scheme for cellular pathology technique
  - participate in the UK national EQA scheme for immunocyto-chemistry and fluorescent in situ hybridisation (when these techniques are used in the diagnostic pathway).
- reports should be held on an electronic database that has facilities to search and retrieve specific data items, and is indexed according to SNOMED-CT
- workload data should be recorded and monitored in a format that facilitates determination of the resources involved.

3 Liver needle core biopsies for the investigation of medical disease

For detailed information on all pre-test aspects of liver biopsy including clinical indications, procedure, precautions and complications of liver biopsy, please refer to the Joint Clinical Guidelines.[2]

In summary, these are biopsies taken for the investigation of diffuse parenchymal liver disease. The common indications for biopsy include:

- persistent unexplained abnormality of liver biochemistry (abnormal liver function tests), when there is no clear medical diagnosis after routine non-invasive liver screen investigations, or when such investigations suggest more than one possible diagnosis
- assessment of severity/stage of a known disease to monitor change over time or with treatment, when this cannot be achieved from non-invasive tests or when there are atypical clinical or serological features.

In hepatology centres, further indications include the need for urgent diagnosis in the context of acute liver failure and post-transplant liver biopsies. A panel of special stains (see section 6) is used routinely for all of these medical liver biopsies.

3.1 Staffing and workload

3.1.1 Laboratory staffing

Laboratories must provide the range of liver special stains listed in section 6. They should have sufficient staffing and expertise to produce these stains to a high standard.

3.1.2 Medical staffing

Medical liver biopsies should be reported by pathologists who have sufficient knowledge of hepatology to formulate a report that addresses the clinical question(s) posed by the clinician. The clinical indication for performing the biopsy and/or specific clinical question to be addressed must be clearly stated on the request form. In many cases, it may be appropriate to discuss pathological findings with the requesting clinician before the final report is issued. Access to electronic patient data, which may include clinic letters, radiology reports and results of other investigations, helps to improve clinicopathological correlation and should be encouraged as much as possible, but this does not detract from the responsibility of the requesting clinician to specify the clinical indication for the biopsy.

In the UK, hepatology networks are being developed as part of the national strategy Improving Quality in Liver Services to formalise the sharing of clinical management of medical liver disease between consultants working in secondary care (gastroenterologist with hepatology interest) and tertiary care centres (consultant hepatologist), including seven transplant centres.[3] The number of liver biopsies and range of diagnoses, as well as the experience of local histopathologists in liver pathology, varies between these settings.

There should be commissioning arrangements that facilitate the referral of biopsies to the network centre whenever this is considered desirable for good patient care. This may be at the instigation of either the pathologist or the clinician (see section 8.2).

3.1.3 For pathologists working outside hepatology centres

For pathologists in this situation, there should be an identified local lead histopathologist responsible for liver biopsies, ensuring the quality of laboratory work, engaging in opportunities for clinicopathological discussion (as part of a formal multidisciplinary team [MDT] meeting or informally) and communicating with the hepatology centre pathology department. This responsibility should be formally identified in the pathologist's job plan. Where more than one local pathologist reports liver biopsies, cases should be discussed within the department to ensure exposure to sufficient numbers to maintain expertise, as well as to unify diagnostic criteria and terminology. Biopsies may be referred to the hepatology centre for review/second opinion when required, either by the pathologist or clinician (see section 8 below). Local lead pathologists for liver biopsies should participate either in a liver EQA scheme or other regular CPD activities in liver pathology.

An evidence-based minimum workload is not yet clearly defined. However, pathologists must bear in mind their previous diagnostic experience, ongoing CPD activity and EQA outcomes in assessing their ability to maintain an acceptable level of reporting expertise. When the liver workload is low (fewer than 40 biopsies per year), no more than two pathologists should report the biopsies. Passing the liver workload to a larger unit could also be considered, as

maintaining an acceptable level of expertise may be difficult if only small numbers of biopsies are reported. This should be through a contractual arrangement with another hospital, normally within the same network, and should include arrangements for clinicopathological dialogue.

3.1.4 For pathologists working within hepatology centres

Liver biopsies in this setting should be reported by consultants with a specialist interest in liver pathology. There should be at least two consultants to ensure specialist cover. There should be a regular formal clinical meeting for case discussion with hepatologists, and sufficient consultant time to maintain CPD in liver pathology and provide a referral service within the hepatology network.

All pathologists who work within hepatology centres should participate in a specialist liver EQA scheme.

The interpretation of post-transplant biopsies usually requires discussion with transplant clinicians, awareness of other clinical investigations and comparison with previous biopsies. It is therefore recommended that late post-transplant biopsies performed in local hospitals are referred to the relevant transplant centre for review.

3.2 Specimen submission

Most medical liver biopsies are needle core biopsies obtained in radiology departments under image guidance. Other types of specimen include transjugular biopsies (narrower cores), small wedge biopsies (at laparoscopy or laparotomy) and fine needle aspiration (FNA) biopsies taken under endoscopic ultrasound guidance.

The accompanying request form should clearly state the primary indication for the biopsy and relevant clinical investigations so that the pathologist's report can address the clinical question. Pathologists frequently supplement this with further information from the electronic patient record, but this does not detract from the responsibility of the requesting clinician to communicate the specific issue(s) to be addressed. The clinical indications for different biopsy types are considered in the Joint Clinical Guidelines.[2] Pathologists cannot refuse to process a biopsy received without clinical information, but if this is a recurring problem this would need to be addressed with the clinicians.

Routine needle core or wedge biopsies are submitted in formalin and should be sent free-floating rather than attached to blotting paper. Biopsies from patients with known risk of infection, including hepatitis B and C viruses, HIV and tuberculosis, should be labelled according to the local generic policy for danger of infection specimens. Owing to the small specimen size, needle core biopsies do not require additional time in fixative before processing. Clinical guidelines on the use of liver biopsy in clinical practice, including indications, contra-indications and techniques, are discussed in the Joint Clinical Guidelines.[2]

Additional specimens may be sent as clinically indicated, for example unfixed, dry tissue for measurement of iron or copper (although iron can be measured in remaining tissue from the paraffin embedded biopsy); these measurements are performed in chemical pathology departments. Fresh tissue is required for frozen section detection of microvesicular steatosis (e.g. Reye's syndrome in children, acute fatty liver of pregnancy) or for freezing when metabolic abnormality is suspected (usually paediatric biopsies). A sample in glutaraldehyde is occasionally sent for electron microscopy (usually paediatric biopsies).

3.3 Size of biopsy

The risks and benefits of liver biopsy are considered in detail in the Joint Clinical Guidelines.[2]

To interpret the biopsy, the pathologist needs to be able to analyse the pattern of disease over several acini/lobules. The core of tissue should be intact and sufficient to demonstrate the

lobular architecture of the liver over several portal tracts. Portal tracts and hepatic veins are around 0.8 mm apart and are therefore better seen in a biopsy with a diameter of approximately 0.8−1 mm, which can be obtained using a 16 gauge needle.[7]

Small biopsies reduce the likelihood of recognising the patterns of disease and increase the risk of underestimating the severity of fibrosis. The size and integrity of the sample is particularly important when being taken to investigate the stage of fibrosis in chronic liver disease or in diseases that affect the liver in a variable way, for example biliary disease.[8,9] Evidence suggests that a biopsy containing ten or fewer portal tracts results in under-estimation of both the fibrosis stage and the inflammatory grade in chronic viral hepatitis.[10]

Medical liver biopsy samples are regarded as adequate for clinical trial purposes if they measure at least 20 mm in length and contain more than ten portal tracts per section, which is achieved in most biopsies measuring >20 mm taken with a 16 gauge needle.[7,11] This represents a sufficient standard for routine diagnostic setting, since the same principles and limitations apply. It is a pragmatic compromise with respect to the ideal size of 3 cm long, as advocated in the American Association for the Study of Liver Disease (AASLD) position paper. This would require two needle passes with a 16 gauge neede.[12] A biopsy of less than 10 mm is of limited diagnostic value and a second pass should be considered. If the biopsy core is between 10 and 20 mm in length, the diagnosis may be compromised. A second pass should be considered, especially if the main indications for the biopsy are staging fibrosis or investigating biliary disease.[13] There is concern that more than one pass for the purposes of medical liver biopsy increases the complication rate, although there is currently no clear evidence to support this.[14,15]

Transjugular biopsies are indicated for patients with an increased risk of complications and/or when venous pressure studies are also required. Transjugular biopsy devices have a narrower gauge, usually 19 gauge needles, and therefore require multiple passes. Four passes are required for optimal specimens.[16]

The number of portal tracts in a given length of biopsy is variable and cannot be predicted from the biopsy length.[7,17] Wider gauge needles produce more robust samples with less fragmentation and more portal tracts per centimetre.[7] The biopsy report should include the length of the biopsy specimen, whether there is fragmentation and an approximate number of portal tracts. This gives an objective measure of the reliability of the sample. The quality of the biopsy can be summarised as:

- good: total core length >20 mm
- compromised: total core length 10−20 mm
- inadequate: total core length <10 mm.

While some comment on the liver tissue submitted is possible even for the smallest biopsies, if the pathologist considers that the biopsy sample is insufficient and limits their assessment this should be stated in the report.

[Level of evidence – D.]

Recently, FNA specimens from the liver obtained under endoscopic ultrasound guidance have been introduced. These are usually taken in the context of endoscopic biliary investigation for painful jaundice and tend to generate narrow fragmented specimens that may be sufficient to suggest the cause of jaundice (e.g. obstructive, hepatitic, late-stage chronic disease) when no specific obstruction by stone or stricture has been identified by the examination.[18,19] FNA specimens are not usually suitable for full histological assessment of diagnosis or disease stage.

3.4 Specimen processing

The specimen must be treated with great care to minimise fragmentation and reduce the risk of an artefact being introduced by handling.

The whole biopsy should be processed; multiple passes received in one container can be processed together in a single block. Wedge biopsies may be sliced prior to embedding. Specimens can be wrapped in tissue paper during processing to reduce fragmentation. The use of rigid foams to support the biopsy core during processing introduces an artefact and should be avoided.[20]

[Level of evidence – D.]

4 Embedding options

All tissue should be embedded and care taken to avoid fragmentation. Narrow needle cores can be gently held flat during embedding to ensure that the whole core is included in the plane of the sections.

5 Sectioning

Sections cut with the microtome set at about 3 µm are generally suitable for haematoxylin and eosin (H&E) stained sections. The number of sections routinely cut varies, but as a minimum will include serial sections as required for the stains listed in the section below. The needle core may be very thin and care should be taken not to trim too far into the block.

6 Staining

A panel of histochemical stains is used to demonstrate liver architecture and identify pigment and intracellular deposits, some of which are associated with metabolic diseases (e.g. haemochromatosis, alpha-1-antitrypsin [α1-AT] deficiency, Wilson disease). The stains used vary among laboratories according to local preference; however, they are guided by what is sufficient to allow a retrospective specialist assessment of the biopsy should this be required. The following stains should be routinely provided for all medical biopsies as a minimum: H&E on at least two levels, special stains for reticulin (toned or untoned), collagen (such as van Gieson, Masson trichrome or picro sirius red) and copper-binding protein (such as orcein), periodic acid-Schiff (PAS) with diastase (most also include PAS without diastase) and Perls' stain for iron. Additional histochemical stains may be requested as appropriate, e.g. stains for copper (rhodanine, rubeanic acid), acid fast bacilli and amyloid. Suitable control tissue blocks must be available, and appropriate control sections should be performed with each staining run when the substance stained is not normally present in the liver (e.g. Perls', orcein, copper stains). These should be made available to the pathologist for evaluation at the time of reporting.[4,5]

[Level of evidence – D.]

Appendix A summarises the routine special stains, material demonstrated and application in assessment of liver disease.

For acute liver failure and transplant biopsies, rapidly processed H&E sections may be required for urgent reporting, with a preliminary report given in advance of the other stains being available.

7 Further investigations

Sections from any previous liver biopsies should be retrieved for comparison with the current biopsy.

Immunohistochemistry may be carried out at the request of the reporting pathologist, e.g. keratin 7 (K7) or 19 (K19) for bile ducts, bile ductules and intermediate hepatobiliary cells, ubiquitin, keratin 8/18 (K8/18), CAM5.2 or p62 for Mallory-Denk bodies. The use of antibodies to detect hepatitis B core and surface antigen has decreased massively owing to the ready availability of full hepatitis B virus (HBV) serological testing.

Appendix B summarises the use of immunohistochemistry in medical liver biopsy interpretation.

Facilities providing electron microscopy for investigation of metabolic/storage disease and in situ hybridisation (e.g. for Epstein Barr virus) may occasionally be required. Referral should be made to an appropriate centre.

8 Report content

8.1 Primary reporting of medical liver biopsies

Reporting style and order of items are influenced by the personal preference of the reporting pathologist/clinician. As a guide, the following items are recommended to be included in all medical liver biopsy reports.

- The clinical information received with the biopsy. This should include the indication/purpose of the biopsy, details of other relevant investigations and a summary of any previous liver biopsy findings. There is a detailed description of disease-specific indications for liver biopsy in the appendix of the BSG Joint Clinical Guidelines.[2]

  [Level of evidence – D.]
- Any additional clinical information obtained prior to reporting, e.g. from electronic patient records or discussion with the clinician, which provide context to the histopathologist at the time of reporting the biopsy.
- Biopsy size and adequacy. This should be indicated by the length of the biopsy core (measured either on receipt before processing or in tissue sections) and an approximate maximum number of portal tracts per section.
- An initial overview of the architecture (including portal-central vascular relationships) and the presence and severity of fibrosis, as an indication of the absence/presence of progressive chronic liver disease. A disease-specific stage may also be included (see below) as an indication of disease severity. Interobserver variation in the assessment of fibrosis stage can be improved by the use of reference images available online (www.virtualpathology.leeds.ac.uk/eqa/specialist/liver/liverdocs/2017/Fibrosis%20stage%20reference%20images.v%20June.pdf).[21]
- Indication of whether there is:
  - no fibrosis/equivocal fibrosis (i.e. no evidence of progressive chronic liver disease)
  - mild/early fibrosis without bridging; this may involve portal tracts and/or sinusoids, particularly in perivenular regions
  - fibrosis with bridging between vascular structures without parenchymal nodularity
  - advanced fibrosis with bridging and incomplete or complete parenchymal nodularity, which is suggestive of definite or probable cirrhosis; this would be an important indication for clinical management by the cirrhosis pathway

- subtle lobular architectural abnormalities in the form of focal liver cell plate atrophy and nodularity or regenerative plates without bridging fibrosis, which could suggest portal venous insufficiency (non-cirrhotic portal hypertension).

- A description of the histological abnormalities, and an attempt to assimilate the features into one or more of the main patterns of disease:
  - parenchymal abnormality without progressive chronic liver disease, e.g. lobular hepatitis, cholestasis, steatosis (without additional features of steatohepatitis or fibrosis) or post-inflammatory features
  - chronic liver disease, e.g. chronic hepatitis, chronic biliary disease, fatty liver disease, or changes reflecting vascular disease.

  This may be achieved by systematically describing portal tract and parenchymal features together with the results of the special stains and integrating these into an overall histological diagnosis. Appropriate negative findings (e.g. lack of iron overload or α1-AT globules) should also be documented in the report. Further guidance is provided in Appendix C.

  A definite diagnosis should be included when possible or discussion of the differential diagnosis. This is usually incorporated in a clear clinicopathological comment following the morphological description, which includes the aetiological agents to consider (e.g. viral, drug, autoimmune, metabolic, obstructive), the relevance of the histological features to the clinical scenario, and suggested or excluded diagnoses. The comment might include suggestions for further investigations or indications for treatment.

  For biopsies from patients without a known clinical diagnosis, the pathologist may propose one or more clinicopathological diagnoses to guide further clinical investigation. Note: the unqualified diagnosis ‘chronic hepatitis’ as a morphological description for any biopsy with unexplained portal inflammation should be avoided, as this may be interpreted clinically as a specific disease (i.e. implying chronic autoimmune or viral hepatitis).

  When the patient's clinical details suggest one or more specific diagnoses, the report should indicate the extent to which histopathological findings support/exclude one or more of the suspected diagnoses and/or favour an alternative diagnosis. In cases where histological findings support more than one diagnosis (e.g. chronic HBV infection and non-alcoholic fatty liver disease), an attempt should be made to identify the predominant cause of liver injury. Note: the pathologist should not simply report a biopsy as ‘consistent with’ a proposed clinical diagnosis without considering alternative diagnoses.

- There should be an indication of the severity of chronic liver disease in terms of grade/stage. This can be achieved either by descriptive text or using a semi-quantitative scoring system, as agreed locally between the pathologist and clinician. Scoring systems developed for use in clinical trials (e.g. Ishak for chronic viral hepatitis, Kleiner for non-alcoholic fatty liver disease) are poorly reproducible in routine practice.[22,23] If used, clinicians and pathologists should be aware of their limitations.

- Comparison with previous liver biopsy samples since these are important in refining the diagnosis and establishing the rate of progression of the disease or response to treatment.

- A concise, single-line summary to conclude the report.

- An appropriate SNOMED code.

- A record (including names) of any intra-departmental consultation, outside referral for second opinion and/or discussion with clinician that has contributed to the histopathology report. This may be achieved by adding a supplementary report when the diagnosis is later refined or revised as a result of discussion at a clinical meeting or outside review.

Many diseases have an uneven distribution within the liver. In any case where there is a disparity between the clinical and histological findings, the possibility of sampling variation should be considered. A further biopsy may be indicated in some cases.

8.2 Indications for referral of the case for a second opinion

The liver biopsy may be referred for a second opinion, normally to the hepatology network centre in the following circumstances:

- the patient is being referred to the hepatology centre for further clinical management or for transplant assessment, and biopsy review forms part of the clinical assessment
- the patient is being managed locally but there is uncertainty concerning the interpretation of liver biopsy findings or there appears to be a discrepancy between the clinical and pathological assessments. In some cases, diagnostic uncertainty is identified during clinicopathological discussion at a local MDT meeting. These referrals may be initiated by the local pathologist and/or the clinician managing the patient locally.

There have been studies of circumstances when the original diagnosis on a liver biopsy is amended through specialist review as a result of clinical referral. Bejarano and colleagues found major discrepancies that would have affected clinical management in 28% of reviewed cases and minor discrepancies in 37% of biopsies. Interpretation errors were more common for chronic cholestatic disease and cirrhosis.[24] More recently, Paterson and colleagues found discrepancies in 59% of reviewed cases, although this included those sent for a second opinion and clinical referral. In agreement with Bejarano and colleagues, discrepancies were more common for diagnoses of biliary disease; however, issues around diagnoses for autoimmune disease and vascular abnormalities were also highlighted.[25] A detailed clinical review of these discrepancies found that 61% affected clinical management. A third review found a discrepancy rate of 38%, of which 70% were regarded as having a major clinical impact.[26]

Many specialist groups and societies have published free-access guidelines on the role of liver biopsy in various diseases, and awareness of these will help pathologists in constructing relevant reports. It is strongly suggested/advised that, owing to the difficulties in diagnosis and the management implications, specialist review be considered in new diagnoses of autoimmune disease, problematic biliary disease, including overlap entities, and vascular abnormalities as a matter of routine.[27,28] When requesting specialist review or a second opinion, it is valuable where possible to include any additional relevant clinical information to that provided on the request form, such as a copy of a recent clinic letter summarising the history, investigations and clinical questions.

9 Liver needle core biopsies for the investigation of focal lesions

Targeted needle core biopsies are commonly obtained during the investigation of focal liver lesions detected by ultrasound scanning or other imaging. Outside hepatology centres, these may outnumber medical liver biopsies. The following section for handling and reporting needle core biopsies is therefore also included in the liver biopsy section of the Royal College of Pathologists' liver cancer dataset.[29]

Targeted liver biopsies from focal lesions are taken for the diagnosis of:

- suspected metastatic malignancy, especially in the context of malignancy of unknown origin. The Royal College of Pathologists' dataset on malignancy of unknown origin describes a stepwise approach recommended for the immunohistochemical investigation of these biopsies.[30]
- suspected primary liver neoplasms in the following situations:

- in normal or non-cirrhotic liver when imaging shows features suggesting hepatocellular adenoma (HCA), hepatocellular carcinoma (HCC) or cholangiocarcinoma
- in the context of advanced stage chronic liver disease/cirrhosis when the diagnosis of a focal lesion cannot be made from its imaging characteristics
- when histological confirmation of advanced HCC, which is not amenable to curative treatment, is required following the identification of radiological features and prior to considering systemic treatment options.

It should be noted that hepatobiliary surgeons advise against needle biopsy to confirm a diagnosis of metastatic colorectal carcinoma when future surgical excision may be an option. This is because of the risk of chest wall recurrence at the biopsy site as a consequence of seeding.[31] The diagnosis in these cases is made on the basis of imaging and the appropriate clinical setting.

[Level of evidence –D.]

For cirrhotic patients under surveillance for HCC, diagnostic biopsy is recommended for lesions >10 mm that do not show characteristic features of HCC using specific radiological techniques.[32] The assessment of dysplasia and neoplasia can be challenging in this context, and biopsies are normally taken in hepatology centres where ancillary antibodies are available to help clarify the diagnosis.

9.1 Specimen submission

The request form should clearly indicate that the biopsy is from a focal lesion. It should include the size and intrahepatic site (segment) of the lesion targeted with other relevant clinical information, such as a previous history of malignant disease and imaging findings, and specify whether primary or metastatic disease is suspected. It is often helpful if the operator indicates on the request form if there was doubt or difficulty achieving successful targeting of the lesion.

Unlike medical liver biopsies, there is no minimum recommended specimen size. The risk of complications is higher for targeted tumour biopsies and an 18 gauge needle is usually used.[33] A small biopsy containing diagnostic tumour tissue can still be regarded as adequate, although such samples may not contain sufficient tissue for full immunohistochemical evaluation. A considered stepwise approach to investigation is especially important when tissue is limited. If the biopsy is small and fragmented, consideration may be given to embedding the tissue in separate blocks to maximise the number of tissue sections available.

9.2 Sectioning and staining

Initially, one or two shallow levels stained with H&E should be examined; if two levels are cut, intervening unstained sections should be kept on slides suitable for immunohistochemistry. Once the presence of lesional tissue is confirmed, further investigations may be requested based on the tumour morphology and clinical circumstances. If no tumour tissue is seen initially, deeper levels should be requested before reporting a biopsy as being negative for tumour.

The possibility that the biopsy is from a well-differentiated hepatocellular lesion (focal nodular hyperplasia, HCA, well-differentiated HCC or focal fatty change/sparing) should be considered if hepatocellular tissue is present without normal architectural landmarks (portal tracts and hepatic veins). The interpretation of well-differentiated hepatocellular lesions is complex and challenging, particularly in small needle biopsy specimens. A range of immunohistochemical stains may be used to further characterise these lesions (see Appendices E and F), although these may not all be available outside of hepatology centres.

Alternatively, the biopsy may show abnormalities owing to an adjacent focal lesion. If there is no lesional tissue present, the report should indicate that additional biopsies/investigations are required for diagnosis.

9.3 Further investigations

When a tumour is found to be present, further information from clinical discussions or electronic patient records should be accessed at an early stage to guide the immunohistochemical investigations. For example, details of a previous history of primary malignancy may have been omitted from the request form or from imaging studies. If the patient is extremely ill, a tissue diagnosis of malignancy may be sufficient to allow immediate clinical management decisions.

Immunohistochemical evaluation is usually required to investigate the nature of the tumour. The selected panel of markers will depend on tumour morphology, any clinically suggested sites of primary origin, past medical history, the amount of tissue available in the biopsy and, in certain circumstances, the ability to identify tumours that may respond to a specific form of chemotherapy. However, if there is a history of previous malignancy or radiological features of a primary tumour, a compatible morphology is often sufficient without immunohistochemistry, especially when previous histology is available for review.

Histological work up of a potentially primary liver lesion has a different strategy from the differential diagnosis of metastatic disease; this possibility should be considered before requesting a panel of immunohistochemistry routinely used for diagnosing metastatic adenocarcinoma.

Other special stains may also be useful. These include PAS and PAS diastase to distinguish between hepatocellular and glandular neoplasms, and reticulin staining for the differential diagnosis of dysplastic and neoplastic hepatocellular lesions. For a guide to special stains in tumour biopsies, other than immunostains, see Appendix D.

9.3.1 Metastatic malignancy

When the clinical suspicion and/or initial morphology is suggestive of metastatic disease, the RCPath dataset for cancer of unknown primary and malignancy of unknown primary origin should be followed.[30] It provides a detailed stepwise approach to diagnosis, and so this will not be considered further here.

9.3.2 Primary liver lesions

When a primary liver lesion is suspected on imaging and/or the biopsy has a morphological pattern of a ‘solid organ’ carcinoma (liver, kidney, adrenal, thyroid or neuroendocrine carcinoma), the next step depends on whether or not the patient has advanced stage chronic liver disease.

For a patient with no history, or clinical or imaging signs of chronic liver disease, the choice of immunohistochemistry will depend on whether:

- the lesion is clearly malignant histologically and the differential diagnosis lies between a primary hepatic neoplasm (HCC or cholangiocarcinoma) and metastatic carcinoma
- the lesion is clearly hepatocellular and the differential diagnosis lies between a benign hepatocellular lesion (HCA or focal nodular hyperplasia) and well-differentiated HCC.

For a patient who develops a focal liver lesion in the setting of chronic liver disease with advanced fibrosis/cirrhosis, immunohistochemistry is usually used to distinguish premalignant hepatocellular lesions from early/well-differentiated HCC. The immunohistochemical stains that are useful in this setting are broadly similar to those used to differentiate benign hepatocellular lesions from well-differentiated HCC in the non-cirrhotic liver.

Immunohistochemistry for the diagnosis of primary liver malignancy in a patient without advanced stage chronic liver disease

For biopsies that show carcinoma of trabecular or hepatoid pattern in which the morphological differential is between primary HCC and metastatic carcinoma, immunohistochemistry is often helpful. Appendix E summarises the immunohistochemical stains useful in this situation. Most primary HCCs are positive for hepatocyte-specific antigen (HepPar1). Poorly differentiated HCC may be HepPar1 negative but is more often positive for alpha-fetoprotein (serum levels may be raised and/or tumour cells immunopositive). Glypican 3 is an alternative oncofetal antigen expressed in most HCCs, but it is also expressed in some other tumours. Arginase-1 has been suggested to be the most specific/sensitive marker to demonstrate hepatocellular differentiation.[34] Canalicular staining patterns for CD10, CD13, BSEP or polyclonal CEA can be useful second-line indicators of hepatocellular differentiation.

Immunohistochemistry for the diagnosis of well-differentiated hepatocellular lesions in a patient without advanced stage chronic liver disease

The classification of well-differentiated/histologically benign focal hepatocellular lesions based on morphology and immunohistochemistry is summarised in Appendix F.

[Level of evidence – C.]

Focal nodular hyperplasia can usually be diagnosed on the basis of imaging and only requires biopsy diagnosis if it lacks typical features.

HCA is now classified according to morphological and immunohistochemical features, which broadly correspond to the molecular pathology of different subtypes that have been described. The classification of HCAs is still evolving, with the recent additions of sonic hedgehog mutated adenoma (which has a high risk of haemorrhage) and the molecular classification of beta-catenin mutated adenomas based on the mutated exon, which correlates with a degree of risk of malignant transformation. However, current practice in the UK is to classify the lesion as inflammatory, steatotic, beta-catenin activated or unclassified HCA based on morphology and immunohistochemistry.[35–37]

Well-differentiated HCC can be very difficult to distinguish from HCA on biopsy.[37] Deficiency of reticulin (although reticulin may be lost in steatotic adenoma), positivity for glypican 3, diffuse positivity for glutamine synthetase (also seen in beta-catenin activated HCA), or nodule-in-nodule appearance are features suggestive of HCC. The term ‘hepatocellular neoplasm of uncertain malignant potential (HUMP)’ has been proposed for lesions with some suspicion of HCC but that lack definitive features, based on resection specimens.[38] The morphological atypia in these lesions is focal and therefore may be absent from biopsies (reticulin loss, cytological atypia or pseudogland formation). Other terms that have been used to describe these difficult-to-classify lesions include ‘atypical HCA-like neoplasms’ and 'atypical hepatocellular neoplasms’.[39,40] In addition to morphological criteria, other features used to identify atypical lesions at increased risk of malignancy include genetic abnormalities (e.g. beta-catenin activation) or an unusual clinical context (e.g. male, or female aged >50 or <15 years).

Immunohistochemistry for the diagnosis of focal lesions in a patient with advanced chronic liver disease/cirrhosis

Patients with known advanced chronic liver disease/cirrhosis who are eligible for treatment of liver malignancy are usually enrolled into a surveillance programme. Lesions identified by ultrasound are further investigated with magnetic resonance imaging and/or computed tomography to establish a diagnosis of HCC. The Liver Imaging Reporting and Data System (LI-RADS) classification is commonly used for this assessment.[41] The likelihood of a lesion being HCC is scored on a scale of 1 to 5, where 1 is definitely benign and 5 is definitely malignant.[41] If the diagnosis is uncertain (LI-RADS score 4) and confirmation is important for patient management, a biopsy is recommended (European Association for the Study of the Liver [EASL] guidelines).[32] In this situation, the differential diagnosis lies between a premalignant lesion (large regenerative nodule or dysplastic nodule) and an early/well-

differentiated HCC. Such lesions represent a continuum of neoplastic progression and there may be intra-lesional variation; a definitive diagnosis may not be possible from a biopsy. For further details, see the Joint Clinical Guidelines.[2]

Immunohistochemical stains useful in this situation include glutamine synthetase, glypican 3 and heat shock protein 70. Positivity for at least two of these favours HCC.[42] Abnormality or loss of reticulin and an infiltrative growth pattern may enable a definite diagnosis of HCC to be made, although the latter is rarely seen in biopsies. Diffuse positivity of the sinusoidal endothelium for CD34 corresponds to 'capillarisation of sinusoids' in arterialised hepatocellular lesions and can provide useful confirmation that the lesion identified on imaging has been sampled; however, it does not distinguish between a high-grade dysplastic nodule and a well-differentiated HCC. Other features supportive of a diagnosis of malignancy are a high Ki67 labelling index compared with non-lesional liver and positive staining for AFP, although the latter is rarely seen in small well-differentiated HCCs.

9.4 Report content

The report should include the following:

- the clinical information received with the biopsy
- a macroscopic description, including biopsy size
- the presence or absence of tissue from the focal lesion, and of non-lesional liver tissue (hepatocytes, bile ducts) as histological confirmation that the specimen is indeed from the liver
- a morphological description of the lesion
- the results of any additional stains carried out, including immunohistochemistry
- a comment on the non-lesional liver, if a sufficient amount is included
- a definite diagnosis of the focal lesion when possible, or a discussion of the differential diagnosis. This would include a discussion of tumours compatible with or excluded by immunohistochemistry.
- a concise, single-line summary to conclude the report
- an appropriate SNOMED code
- a record (including names) of any intra-departmental consultation, outside referral for second opinion and/or discussion with clinician that has contributed to the histopathology report. This may be achieved by adding a supplementary report when the diagnosis is later refined or revised as a result of discussion at a clinical meeting or outside review.

10 Criteria for audit

10.1 Staffing and workload

- Annual review of numbers and types of specimens reported by each pathologist.
- EQA and RCPath CPD compliance.

10.2 Report content

The clinical value of the biopsy is dependent on the quality of the biopsy sample and clinical information provided. All reports should include the total length of biopsy core(s), fragmentation and approximate number of portal tracts.

For audit purposes, the quality of the biopsy taken to investigate medical liver disease can be categorised as:

- good >20 mm
- compromised: 10−20 mm
- inadequate: <10 mm long.

The quality of clinical information provided can be audited, especially if inadequately completed request forms is an issue.

The following audit templates addressing these issues in more detail are available on the RCPath website (www.rcpath.org/profession/patient-safety-and-quality-improvement/conducting-a-clinical-audit/clinical-audit-templates.html):

- an audit of the specimen quality and reporting of medical liver biopsies
- a clinicopathological audit of the effect of medical liver biopsies on patient management.

10.3 Communication and timeliness of the report

Key assurance indicators regarding turnaround times are provided by RCPath (www.rcpath.org/profession/guidelines/kpis-for-laboratory-services.html). Provisional expectations are that 80% of cases would be reported within seven calendar days and 90% of all cases are reported within ten calendar days. There should be an agreement between the laboratory and users regarding the turnaround times specific for liver biopsies, related to the patient pathway, if this differs from the requirements for the laboratory overall.

For non-urgent biopsies, turnaround times need to take into consideration the time required for all investigations to be completed, the opportunity for discussion (e.g. in a MDT discussion) and/or for the biopsy to been referred elsewhere if necessary. An interim report can be issued if clinically required to indicate sample adequacy and/or a preliminary differential diagnosis.

Arrangements for communication of urgent results should be specified. For diagnostic biopsies from cases when the report is required more urgently (e.g. acute liver failure, transplant biopsies), a preliminary report should be given by telephone or email, normally within one working day of the receipt of the biopsy. The diagnosis and time/date of the provisional report, including a summary of any clinicopathological discussion, should be recorded in the final report.

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Appendix A Routine special stains used in medical liver biopsy interpretation

Stain	Material demonstrated	Distribution in normal liver	Use in assessment of liver disease/other comments
Reticulin	• Type III collagen fibres	• Portal tracts • Hepatic sinusoids • Walls of hepatic veins	Useful for assessing overall architecture: • low magnification – vascular relationships and septa • high magnification – liver cell plate arrangement and best method for showing nodular regenerative hyperplasia Collapse of reticulin framework occurs in areas of recent liver cell necrosis. It can resemble fibrous septa, which occurs in chronic liver disease (other connective tissue stains are required to distinguish recent collapse from long-standing fibrosis)
Collagen stains: • van Gieson • Masson trichrome • picro sirius red	• Type I collagen fibres	• Portal tracts • Walls of hepatic veins	Increased in hepatic fibrosis and it can be periportal, perisinusoidal or pericellular. The presence of increased mature collagen implies chronic liver disease. Three main stages of fibrosis are recognised: • fibrosis without bridging – mild/early stage fibrosis • bridging fibrosis without nodules – moderate/intermediate fibrosis • bridging fibrosis with nodules, appearance of early or established cirrhosis – severe or late-stage fibrosis The amount of collagen in portal tracts increases with the size of tract and age. Longitudinally sectioned tracts may be mistaken for fibrous septa. They can be identified by vessel/duct orientation
PAS	• Glycogen (in well-fixed biopsies) • Glycoprotein	• Hepatocytes • BM of bile ducts	Highlights the presence of hepatocytes, e.g. in interface hepatitis, or absence of hepatocytes, e.g. parenchymal granulomas or confluent necrosis. PAS can also be helpful in identifying storage cells in some inborn errors of metabolism
PAS diastase	• Glycoprotein	• BM of bile ducts	Bile duct damage in chronic cholestatic disease (e.g. disrupted BM in primary biliary cholangitis, thickened BM in primary sclerosing cholangitis)
	• Mucin	• Bile duct lumen	
	• Ceroid pigment	• N/A	Ceroid pigment in Kupffer cells is a non-specific marker of previous hepatocellular injury, often prominent in biopsies with moderate/severe bilirubinostasis or lobular hepatitis
	• α1-AT	• N/A	Intracytoplasmic globules in α1-AT deficiency (can be confirmed by immunostaining)
	• Copper-associated protein	• N/A	Abundant copper-associated protein may be positive on PAS diastase; correlates with orcein stain

Stain	Material demonstrated	Distribution in normal liver	Use in assessment of liver disease/other comments
Orcein or Victoria blue	Proteins with disulphide bonds: • Elastin	Elastin in: • portal tracts • walls of hepatic veins • internal elastic of arteries	Presence of elastic fibres in septa indicates long-standing fibrosis
	• Copper-associated protein	• N/A	Presence of copper-associated protein:* • in non-cirrhotic liver: any amount is abnormal (except in neonates). It is a sensitive indicator of chronic biliary disease (or rarely Wilson disease or vascular disorder) • in cirrhosis: small amounts are non-specific; larger amounts as above
	• HBsAg	• N/A	Cytoplasmic positivity due to HBsAg; however, this is less sensitive than immunostaining. Clinically, hepatitis B serology and PCR for HBV DNA are more helpful
Perls' Prussian blue	• Haemosiderin (ferric iron)	• Usually absent • Small amounts in hepatocytes	May be present in hepatocytes (usually graded 0–4) or sinusoidal cells Hepatocyte siderosis grade 2+ raises the possibility of haemochromatosis (especially in the absence of significant fibrosis) in which there is also typically a gradient of deposition, initially periportal Secondary iron overload is common in cirrhosis (especially alcoholic and HCV). It typically has a mixed hepatocellular/ sinusoidal pattern Pure sinusoidal cell siderosis (Kupffer cells and/or endothelial cells) is seen following haemolysis or blood transfusion, and in systemic chronic inflammatory diseases and acute hepatitis A 'blush' of ferritin staining is not considered to be significant
Rhodanine	• Copper	• Absent	Copper accumulation is a sensitive indicator of chronic biliary disease (or rarely Wilson disease or vascular disorder). Cytoplasmic copper may be seen in Wilson disease (diffuse rather granular periportal staining)

*Orcein stain for copper-associated protein may be technically problematic. Rhodanine together with K7 can be used as alternatives for investigation of chronic biliary disease.[43]

α1-AT: Alpha-1-antitrypsin; BM: Basement membrane; HBsAg: Hepatitis B surface antigen; HBV: Hepatitis B virus; HCV: Hepatitis C virus.

Appendix B Use of immunostains in medical liver biopsy reporting

Stain	Material demonstrated	Distribution in normal liver	Use in assessment of liver disease/other comments
K7 (CK7)	Keratin 7	Biliary epithelium – bile ducts and ductules	K7 and K19 are useful in biliary disease to assess duct loss and demonstrate or confirm the presence of ductular reaction K7 (but not usually K19) can identify intermediate hepatobiliary cells in hepatocyte regeneration and biliary disease The presence of periportal cells with this morphology may be a manifestation of early 'ductular metaplasia', which can be a sensitive early marker of chronic cholestasis before a well-formed ductular reaction is present K7 may also be seen in ischaemic perivenular hepatocytes in diseases associated with impaired intrahepatic blood flow (e.g. portal or hepatic venous insufficiency)
K19 (CK19)	Keratin 19	Biliary epithelium – bile ducts and ductules	
K8/18	Keratin 8/18	Weak diffuse cytoplasmic staining (with submembranous accentuation) in hepatocytes. It is also present in bile ducts	K8/18 can identify features of steatohepatitis and is an alternative to ubiquitin and p62 for demonstrating MDBs. Ballooned hepatocytes also show loss of normal cytoplasmic/ submembranous staining
Ubiquitin p62	MDBs	Negative	Component of MDBs. Helpful in confirming presence of MDBs in cases suspected to have steatohepatitis (versus simple steatosis)
HBsAg	HBsAg	Negative	Cytoplasmic positivity corresponds to ground glass hepatocytes in chronic hepatitis B. More sensitive for demonstrating cytoplasmic HBsAg than orcein staining. It also demonstrates membranous HBsAg, which is not shown with orcein staining. Typically negative in acute hepatitis B
HBcAg	HBeAg	Negative	Nuclei positive in patients with HBeAg-positive infection. Cytoplasmic or membrane positive during seroconversion, lobular activity Note: reliable methods for HBV-associated antigens/antibodies and HBV DNA in serum have largely replaced the need to carry out HBV immunostains
α1-AT	α1-AT	Usually negative Diffuse cytoplasmic staining may be seen in normal hepatocytes	Coarsely granular immunoreactivity confirms presence of α1-AT accumulation (more sensitive and specific than PAS diastase) The PiZ antibody, which is more specific in identifying abnormal genotypes, is not readily available outside of hepatology centres

α1-AT: Alpha-1-antitrypsin; HBeAg: Hepatitis B core antigen; HBsAg: Hepatitis B surface antigen; HBV: Hepatitis B virus; MDB: Mallory-Denk bodies.

Appendix C Main patterns of liver disease

Full diagnosis is dependent on correlating histological findings with the clinical context. The table below is an initial checklist of the main morphological patterns and their possible clinical significance.

Lobular abnormalities without evidence of chronic liver disease

Pattern	Histological features	Clinical significance	Comments
Lobular hepatitis	• Inflammation, typically diffuse and spotty, sometimes with perivenular accentuation • Associated with lobular disarray, irregular size of hepatocytes, acidophil bodies, activated Kupffer cells, frequently containing ceroid pigment	• Dominant pattern of injury in acute hepatitis • Viral hepatitis, drugs and autoimmune hepatitis are three main possibilities to consider in the differential diagnosis • Cases with no identifiable cause may be labelled as 'seronegative hepatitis'	• Varying degrees of portal inflammation may also be present • More severe cases may be associated with confluent, bridging or panacinar necrosis • Reticulin collapse occurs in areas with confluent loss of hepatocytes. This may be mistaken for fibrous septa occurring in cirrhosis (see Appendix A)
Cholestasis ('bilirubinostasis')	• Bile plugs in canaliculi or cytoplasm of hepatocytes	• Drugs are the commonest cause of 'pure' or 'bland' cholestasis • Differential diagnosis includes early large duct obstruction, resolving cholestatic hepatitis, bile transporter protein defects, sepsis and occult malignancy (especially lymphoma)	• Pigment in cytoplasm of perivenular hepatocytes needs to be distinguished from ceroid/lipofuscin (lipofuscin typically has pericanalicular distribution; the presence of plugs within canaliculi indicates bile) • Activated Kupffer cells containing PAS-diastase-positive ceroid are usually present (unless cholestasis is very recent)
Steatosis	• Fat vacuoles in hepatocyte cytoplasm (more than 5% in the parenchymal area)	• Consider alcoholic and non-alcoholic causes (histology is usually unable to distinguish these)	• Distinguish bland steatosis from steatohepatitis, which is characterised by ballooned hepatocytes and inflammation • Presence of steatohepatitis implies the potential for transition to chronicity
Acute venous outflow obstruction	• Sinusoidal dilatation • Perivenular hepatocytes replaced by extravasated red blood cells	• Investigate for causes of venous outflow obstruction, including imaging of hepatic veins	• Rare but important • Changes are often patchy in distribution and easy to miss • May be clinically unsuspected prior to biopsy

Chronic liver disease – the four main types

It should be noted that features may overlap; identify the dominant pathology, or presence of more than one main pattern (e.g. chronic hepatitis and fatty liver in hepatitis C; chronic hepatitis and biliary features in autoimmune liver disease). For complex cases and post-transplant biopsies, consider referral to specialist centre.

Pattern	Histological features	Clinical significance	Comments
Chronic hepatitis	• Predominantly portal inflammation, with enlargement of portal tracts +/- interface hepatitis	• Dominant abnormality in autoimmune hepatitis (unless acute onset) and chronic viral hepatitis B and C • Less common causes include metabolic diseases (e.g. Wilson disease, α1-AT deficiency) and drugs	• Varying degrees of portal inflammation and interface hepatitis may also be seen in other chronic liver diseases, including chronic biliary disease (e.g. PBC, PSC) and fatty liver disease (alcoholic and non-alcoholic) • In cases where PBC or PSC is suspected and portal inflammation is unusually dense or plasma cell rich and/or associated with moderate/severe interface hepatitis, consider the possibility of an 'overlap syndrome' with autoimmune hepatitis. Such cases require clinicopathological discussion and consideration of referral to hepatology centre
Chronic biliary disease	• Portal expansion due to ductular reaction +/- oedema and fibrosis • Typically associated with bile duct loss • Bile duct lesions may point to the likely cause (e.g. lymphocytic/ granulomatous cholangitis in PBC and fibrosing cholangiopathy in PSC)	• Biopsy evidence of biliary disease is indication for further investigation/imaging • Two most common causes in adults are PBC and PSC	• Chronic biliary disease is the most often overlooked diagnosis in medical liver biopsies • Adequate biopsy size is particularly important in assessing chronic biliary disease • Copper-associated protein and K7 expression in periportal hepatocytes are sensitive early markers of biliary disease. Their presence should prompt a careful assessment of bile ducts and other features of chronic biliary disease, even if these are not immediately apparent • Periportal hepatocytes may show other features of chronic cholestasis, e.g. ballooning, feathery degeneration and Mallory-Denk bodies ('cholate stasis'). They are usually seen in later stages • Ductopenia best assessed by matching ducts to hepatic arteries • Diagnostic duct lesions are rarely seen in needle biopsy specimens • Neutrophil-rich infiltration of bile ducts, including luminal aggregates of neutrophils, raises the possibility of ascending cholangitis

Fatty liver disease with fibrosis	• Steatohepatitis is usually present • Fibrosis is typically pericellular in the early stages. It may also be periportal, particularly in later stages	• Main causes are alcoholic and non-alcoholic (metabolic) fatty liver disease • Mild steatosis is common in other types of liver disease, e.g. hepatitis C • Less frequently, HCV may lead to the development of steatohepatitis, either directly (HCV genotype 3) or indirectly (e.g. genotype 1, by predisposing to metabolic syndrome)	• Steatosis tends to diminish as fibrosis becomes advanced • Cases of end-stage fatty liver disease may lack the typical features and present as 'cryptogenic' cirrhosis • Mild portal inflammation is common in progressing steatohepatitis. It may also be associated with low titre autoantibodies, without necessarily indicating autoimmune hepatitis
Vascular diseases	• Nodular regeneration without fibrous septa (nodular regenerative hyperplasia) • Liver cell plate atrophy, with sinusoidal dilatation or sinusoidal fibrosis	• Causes include portal venous insufficiency (e.g. portal vein thrombosis in large vessels, obliterative portal venopathy in small portal veins), chronic venous outflow obstruction or sinusoidal endothelial injury (usually drug related, known as 'sinusoidal obstruction syndrome') • May present with portal hypertension in the absence of significant fibrosis or cirrhosis ('non-cirrhotic portal hypertension')	• May be associated with abnormalities of portal veins (e.g. obliteration and ectatic shunt vessels in obliterative portal venopathy) or hepatic veins (e.g. occlusion in chronic venous outflow obstruction)

α1-AT: Alpha-1-antitrypsin; PBC: Primary biliary cholangitis; PSC: Primary sclerosing cholangitis.

Appendix D Other special stains that may be useful for the differential diagnosis of liver biopsies containing tumour

Stain	Comment
Periodic acid-Schiff (PAS)	Glycogen commonly present in hepatocellular neoplasms, rarely in adenocarcinoma
PAS diastase	Presence of luminal PAS-diastase-positive material and/or cytoplasmic mucin vacuoles favours a diagnosis of adenocarcinoma Hepatocellular carcinomas may contain PAS-diastase-positive globules (e.g. alpha-1-antitrypsin)
Perls' or van Gieson	Bile retains green colour and may be more easily recognised than in a haematoxylin and eosin-stained section. Presence of intracellular or canalicular bile pigment favours a diagnosis of hepatocellular neoplasm
Reticulin	Normal reticulin fibre content retained in dysplastic nodules and benign hepatocellular lesions, e.g. hepatocellular adenoma (except steatotic hepatocellular adenoma), focal nodular hyperplasia Reticulin fibres are usually reduced or absent in hepatocellular carcinoma (but may be focally retained in some well-differentiated hepatocellular carcinomas)
Orcein/rhodanine	Copper/copper-associated protein present in focal nodular hyperplasia and some adenomas

Note: Adenocarcinoma includes primary cholangiocarcinoma as well as metastatic adenocarcinoma.

Appendix E Immunohistochemistry for the differential diagnosis of liver biopsies containing tumour

Tumours that resemble hepatocellular carcinoma (HCC): support HCC

This table provides more detail on the identification of HCC than is included in the cancer of unknown primary dataset.[18]

Antigen	% in HCC	Comments
HepPar1	86	Well/moderately differentiated, rarer in metastasis Granular staining pattern. Can be seen with hepatoid adenocarcinoma metastasis
Arginase-1	>85%	More sensitive than HepPar1, especially for poorly differentiated HCC[44]
Canalicular antigen expression	60−80	Demonstration of biliary canaliculi between tumour cells with antibodies such as polyclonal CEA, CD10, CD13 and BSEP, as available. Diffuse staining is non-specific
AFP	37	Poorly differentiated, usually also seropositive and very focal. Complements HepPar and canalicular stains, which tend to stain better differentiated HCC
pCEA	75	Canalicular pattern is specific for HCC. Cytoplasmic staining is non-specific
CD10	61	Canalicular pattern is clearer than with pCEA. It is less sensitive than CD13
CAM5.2	90	If K7 negative, suggests HCC owing to the presence of K8/18 in HCC
Glypican 3	>70	Staining may be weak/focal in well-differentiated HCC. It is not a hepatocyte-specific marker, more an oncofoetal antigen, so it can be seen with a number of non-HCC malignancies

Note: PGP 9.5 – 87% HCC positive; 9% synaptophysin positive, CD56 14%. TTF1 – 93% HCC cytoplasmic positive, 0% nuclear positive; stains normal liver but depends on antibody clone (hepatocyte staining with clone 8G7/G3/130).[45] K19 stains a minority of HCC and is associated with a poorer prognosis. Glutamine synthetase diffusely stains some HCC but also stains beta-catenin activated hepatocellular adenoma.

HCC: Hepatocellular carcinoma.

Tumours that resemble HCC: support metastasis

Please refer to the dataset for cancer of unknown primary, Table 5 pages 18−19.[18]

Appendix F Benign focal hepatocellular lesions: morphology and immunophenotype [36–38,46,47]

Lesion	Clinicopathological features	Immunophenotype	Genetic alteration
FNH	• Central scar with abnormal vessels • Fibrovascular septa with ductular reaction and inflammation	• 'Map-like' staining pattern for GS	• Polyclonal
I-HCA	• Sinusoidal dilatation away from arterioles (telangiectasia) • Ductular reaction (FNH-like) • Inflammation • Mild steatosis may also be present • Some associated with metabolic syndrome/alcohol excess and steatosis in background liver; can be multiple	• Serum amyloid A • C-reactive protein (Note: both of these are occasionally diffusely positive in non-lesional liver, in which case they are non-informative within the lesion)	• Oncogene-induced inflammation JAK/STAT pathway (IL-6/STAT3 activation) • Up to 10–20% may also be beta-catenin mutated
H-HCA	• Marked steatosis (Note: they are rare in men, but the most common type of HCA in women. They can be multiple)	• Lack of normal cytoplasmic staining for LFABP	• HNF-1a inactivation
b-HCA	• May show cytological atypia and pseudoglandular formation • Increased risk of malignant transformation (up to 40%)	• Diffuse staining for GS • Beta-catenin nuclear expression (usually sparse cells only)	• Wnt/beta-catenin • Further characterised by exon 3 or 7/8 mutations. Increased risk of malignant transformation confined to exon 3 mutated lesions[48]
Unclassified	• Usually solitary	No abnormalities	N/A

Note: The 'unclassified' lesions are likely to reduce as new subtypes are being described; for example, sonic hedgehog, which has GLI1 activation.[49]

b-HCA: Beta-catenin mutated hepatocellular adenoma; FNH: Focal nodular hyperplasia; GLI1: Gli family zinc finger 1; GS: Glutamine synthetase; HCA: Hepatocellular carcinoma; H-HCA: HNF-1a inactivated hepatocellular adenoma; HNF-1a: Hepatocyte nuclear factor 1 alpha; I-HCA: Inflammatory hepatocellular adenoma; IL-6/STAT3: Interleukin-6/signal transducer and activator of transcription; JAK/STAT: Janus kinase – signal transducer and activator of transcription; LFABP: Liver fatty acid binding protein.

Appendix G Suggested SNOMED-CT codes

This is not intended to be an exhaustive list of codes but aims to promote consistent use of codes that are applicable to the vast majority of cases. A more detailed list of tumour codes may be available in the relevant cancer dataset.

Topographical codes	SNOMED-CT code	Synonym
Liver structure (body structure)	10200004	
Structure of transplanted liver (body structure)	3860006	

Procedure codes	SNOMED-CT code	Synonym
Biopsy (procedure)	86273004	
Wedge resection of liver (procedure)	174431000	
Liver excision (procedure)	107963000	
Tissue frozen section technique, complete (procedure)	27204007	

General morphology codes	SNOMED-CT code	Synonym
Abscess (morphologic abnormality)	44132006	
Acute and chronic inflammation (morphologic abnormality)	75889009	
Acute inflammation (morphologic abnormality)	4532008	
Amyloid deposition (morphologic abnormality)	68790008	
Artefact (finding)	47973001	
Chronic inflammation (morphologic abnormality)	84499006	
Cyst (morphologic abnormality)	367643001	
Disorder related to transplantation (disorder)	429054002	
Extramedullary haematopoiesis (finding)	42952007	
Fibrosis (morphologic abnormality)	112674009	
Granuloma (morphologic abnormality)	45647009	
Granulomatous inflammation (morphologic abnormality)	6266001	
Hamartoma (morphologic abnormality)	51398009	
Haemorrhage (morphologic abnormality)	50960005	
Infarct (morphologic abnormality)	55641003	
Inflammation (morphologic abnormality)	23583003	
Morphologically abnormal structure (morphologic abnormality)	49755003	Abnormal tissue appearance
Morphologic description only (finding)	85728002	
Necrosis (morphologic abnormality)	6574001	
Normal tissue (finding)	30389008	
Specimen unsatisfactory for diagnosis (finding)	112631006	Inadequate
Structure showing abnormal deposition of pigment (morphologic abnormality)	51083003	
Thrombus (morphologic abnormality)	396339007	
Unknown (origin) (qualifier value)	54690008	

Infection codes	SNOMED-CT code	Synonym
Mycosis (disorder)	3218000	Fungal infection
Human immunodeficiency virus (organism)	19030005	HIV
Disease caused by parasite (disorder)	17322007	Parasitic infection
Family *Adenoviridae* (organism)	424470006	Adenovirus
Genus *Cytomegalovirus* (organism)	407444007	CMV (Cytomegalovirus)
Human herpes simplex virus (organism)	19965007	
Human herpesvirus 4 (organism)	40168006	EBV (Epstein-Barr virus)
Infection caused by *Schistosoma* (disorder)	10087007	Schistosomiasis
Tuberculosis (disorder)	56717001	
Viral disease (disorder)	34014006	Viral infection
Viral hepatitis, type A (disorder)	40468003	Hepatitis A
Viral hepatitis, type B (disorder)	66071002	Hepatitis B
Viral hepatitis, type C (disorder)	50711007	Hepatitis C
Viral hepatitis, type D (disorder)	707341005	Hepatitis D
Viral hepatitis, type E (disorder)	7.111E+12	Hepatitis E

Tumour codes	SNOMED-CT code	Synonym
Adenocarcinoma, no subtype (morphologic abnormality)	35917007	Adenocarcinoma
Adenoma, liver cell (morphologic abnormality)	78058005	Hepatocellular adenoma
Bile duct adenoma (morphologic abnormality)	39471001	
Biliary hamartoma (morphologic abnormality)	27721004	von Meyenburg complex
Carcinoma, metastatic (morphologic abnormality)	79282002	Metastatic carcinoma
Carcinoma, no subtype (morphologic abnormality)	68453008	Carcinoma
Cholangiocarcinoma (morphologic abnormality)	70179006	
Dysplasia (morphologic abnormality)	25723000	Dysplasia
Fibrolamellar hepatocellular carcinoma (disorder)	253018005	
Focal nodular hyperplasia (morphologic abnormality)	22995004	
Haemangioendothelioma (morphologic abnormality)	66229009	
Haemangioma, no International Classification of Diseases for Oncology subtype (morphologic abnormality)	2099007	Haemangioma
Heamangiosarcoma (morphologic abnormality)	39000009	Angiosarcoma
Hepatocellular carcinoma (morphologic abnormality)	25370001	
Malignant lymphoma, no International Classification of Diseases for Oncology subtype (morphologic abnormality)	21964009	Lymphoma
Mesenchymal hamartoma (morphologic abnormality)	80656004	
Multiple myeloma, no International Classification of Diseases for Oncology subtype (morphologic abnormality)	55921005	Myeloma
Neuroendocrine tumour (morphologic abnormality)	55937004	
Sarcoma, no International Classification of Diseases for Oncology subtype (morphologic abnormality)	2424003	Sarcoma

Site-specific disease codes	SNOMED-CT code	Synonym
Acute hepatitis (disorder)	37871000	
Alpha-1-antitrypsin deficiency (disorder)	30188007	Abnormal alpha-1-antitrypsin phenotype
Alcoholic liver damage (disorder)	41309000	Alcohol-related liver disease
Autoimmune hepatitis (disorder)	408335007	
Cholestasis (finding)	33688009	Bilirubinostasis
Cirrhosis of liver (disorder)	19943007	Cirrhosis
Congenital cystic disease of liver (disorder)	72925005	Polycystic liver disease
Congenital malformation (disorder)	276654001	Congenital abnormality
Deposition of iron (morphologic abnormality)	84182002	Siderosis
Drug-induced disorder of liver (disorder)	427399008	Drug-induced liver injury
Fatty degeneration (morphologic abnormality)	29185008	Steatosis
Gilbert's syndrome (disorder)	27503000	
Inflammatory disease of liver (disorder)	128241005	Hepatitis NOS
Non-alcoholic fatty liver (disorder)	197315008	
Nodular regenerative hyperplasia (morphologic abnormality)	715141007	
Peliosis hepatis (disorder)	58008004	
Metabolic disease (disorder)	75934005	
Neonatal hepatitis (disorder)	69800000	Giant cell hepatitis
Obstruction of vein (disorder)	766955008	Veno-occlusive disease
Primary biliary cholangitis (disorder)	31712002	
Primary sclerosing cholangitis (disorder)	197441003	
Sarcoidosis (disorder)	31541009	
Sickling disorder due to haemoglobin S (disorder)	417357006	Sickle cell disease
Steatohepatitis (disorder)	442191002	
Storage disease (disorder)	34420000	Storage disease
Wilson's disease (disorder)	88518009	

Transplant codes	SNOMED-CT code	Synonym
Acute rejection of liver transplant (disorder)	431222008	
Chronic rejection of liver transplant (disorder)	432908002	
Hyperacute graft rejection (finding)	26522000	Rejection, antibody-mediated

Appendix H Summary table – explanation of grades of evidence

(modified from Palmer K *et al. BMJ* 2008;337:1832)

Grade (level) of evidence	Nature of evidence
Grade A	At least one high-quality meta-analysis, systematic review of randomised controlled trials or a randomised controlled trial with a very low risk of bias and directly attributable to the target population or A body of evidence demonstrating consistency of results and comprising mainly well-conducted meta-analyses, systematic reviews of randomised controlled trials or randomised controlled trials with a low risk of bias, directly applicable to the target population.
Grade B	A body of evidence demonstrating consistency of results and comprising mainly high-quality systematic reviews of case-control or cohort studies and high-quality case-control or cohort studies with a very low risk of confounding or bias and a high probability that the relation is causal and which are directly applicable to the target population or Extrapolation evidence from studies described in A.
Grade C	A body of evidence demonstrating consistency of results and including well-conducted case-control or cohort studies and high quality case-control or cohort studies with a low risk of confounding or bias and a moderate probability that the relation is causal and which are directly applicable to the target population or Extrapolation evidence from studies described in B.
Grade D	Non-analytic studies such as case reports, case series or expert opinion or Extrapolation evidence from studies described in C.
Good practice point (GPP)	Recommended best practice based on the clinical experience of the authors of the writing group.

Appendix I AGREE II compliance monitoring sheet

The tissue pathways of the Royal College of Pathologists comply with the AGREE II standards for good quality clinical guidelines. The sections of this tissue pathway that indicate compliance with each of the AGREE II standards are indicated in the table.

AGREE standard	Section of guideline
Scope and purpose	
1 The overall objective(s) of the guideline is (are) specifically described	Introduction
2 The health question(s) covered by the guideline is (are) specifically described	Introduction
3 The population (patients, public, etc.) to whom the guideline is meant to apply is specifically described	Foreword
Stakeholder involvement	
4 The guideline development group includes individuals from all the relevant professional groups	Foreword
5 The views and preferences of the target population (patients, public, etc.) have been sought	Foreword
6 The target users of the guideline are clearly defined	Introduction
Rigour of development	
7 Systematic methods were used to search for evidence	Foreword
8 The criteria for selecting the evidence are clearly described	Foreword
9 The strengths and limitations of the body of evidence are clearly described	Foreword
10 The methods for formulating the recommendations are clearly described	Foreword
11 The health benefits, side effects and risks have been considered in formulating the recommendations	Foreword and Introduction
12 There is an explicit link between the recommendations and the supporting evidence	2−9
13 The guideline has been externally reviewed by experts prior to its publication	Foreword
14 A procedure for updating the guideline is provided	Foreword
Clarity of presentation	
15 The recommendations are specific and unambiguous	2−9
16 The different options for management of the condition or health issue are clearly presented	2−9
17 Key recommendations are easily identifiable	2−9
Applicability	
18 The guideline describes facilitators and barriers to its application	Foreword
19 The guideline provides advice and/or tools on how the recommendations can be put into practice	
20 The potential resource implications of applying the recommendations have been considered	Foreword
21 The guideline presents monitoring and/or auditing criteria	10
Editorial independence	
22 The views of the funding body have not influenced the content of the guideline	Foreword
23 Competing interest of guideline development group members have been recorded and addressed	Foreword