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

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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.

It may rarely be necessary or even desirable to depart from the guidelines in the interests of specific patients and special circumstances. The clinical risk of departing from the guidelines should be carefully considered by the reporting pathologist; just as adherence to the guidelines may not constitute defence against a claim of negligence, so a decision to deviate from them should not necessarily be deemed negligent.

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

The stakeholders consulted for this document were:

- the members of UK Liver Histopathology EQA Scheme
- British Society of Gastroenterology
- British Association for the Study of the Liver
- The Royal College of Radiologists.

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

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 United Kingdom. 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 EQA Scheme (evidence corresponding to 'good practice point' in Appendix J). Published evidence to support the recommendations has been identified by a PubMed search and referenced where appropriate. The evidence was evaluated using modified SIGN guidance. 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.

A formal revision cycle for all tissue pathways takes place on a four-yearly basis. However, each year, the College will ask the authors 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 Fellows' attention. If Fellows do not object to the changes, the short notice of change will be incorporated into the pathways and the full revised version (incorporating the changes) will replace the existing version on the Publications page of the College website.

This pathway has been reviewed by the Working Group on Cancer Services and was placed on the College website for consultation with the membership from 21 October to 18 November 2013. 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 Vice-President for Advocacy and Communications.

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 Director of Professional Standards and are available on request. The authors of this document have declared that there are no conflicts of interest.

1 Introduction

This document provides guidance on the specimen handling and reporting of liver biopsies. This relates primarily to those biopsies taken for the investigation of medical liver disease (referred to as 'medical liver biopsies' in the document). For convenience, the section on targeted liver biopsy of focal lesions from the liver cancer dataset is reproduced here, since most such biopsies are reported outside specialist hepatobiliary cancer centres. A recent audit of UK practice showed that 67% of needle core liver biopsies in the UK in 2008 were for the investigation of diffuse parenchymal liver disease and 33% for diagnosis of focal lesions.[1]

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.

The previous guidelines, *Tissue pathways for liver biopsies for the investigation of medical disease and for focal lesions,* were published in 2008. These have now been revised to ensure that all recommendations are up to date and that the document complies with the revised format of the tissue pathway series.

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 (fatty liver diseases and viral hepatitis) and its detection at an early stage is also increasing.[2] 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. The effect of these opposing trends on biopsy numbers is uncertain, but there is likely to be a shift towards an increasing proportion being carried out in hepatology centres.

However, currently medical liver biopsies are reported by nearly all UK hospital histopathology departments. The purpose of these guidelines is to promote a uniform good practice of initial biopsy handling and reporting, either by the local histopathology department or for clinicopathological review within hepatology networks.

Target users of this guideline

The primary users of the tissue pathway documents are trainee 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.

i. The diagnostic laboratory should have sufficient pathologists, biomedical scientists and clerical staff to cover all of its functions. In general, staffing levels will follow the workload guidelines of The Royal College of Pathologists.

ii. Pathologists should:

- participate in audit
- participate in The Royal College of Pathologists' Continuing Professional Development (CPD) scheme
- participate in relevant external quality assessment (EQA) schemes of a general or specialist nature

- via their pathology department, have standard liver pathology texts available for reference, e.g. Scheuer and Lefkowitch's *Liver Biopsy Interpretation*,[3] MacSween's *Pathology of the Liver*[4] and *Practical Hepatic Pathology* (edited by Romil Saxena)[5]
- have access to specialist referral opinions on a local network or national basis.

iii. The laboratory should:

- be equipped to allow the recommended technical procedures to be performed safely
- be accredited by Clinical Pathology Accreditation (UK) Ltd, or equivalent
- participate in the UK National External Quality Assurance Scheme for Cellular Pathology Technique
- participate in the UK National External Quality Assurance Scheme for immunocyto-chemistry and fluorescent *in-situ* hybridisation (when these techniques are used in the diagnostic pathway).

iv. Reports should be held on an electronic database that has facilities to search and retrieve specific data items, and that is indexed according to Systematised Nomenclature of Medicine Clinical Terms (SNOMED) T, M and P codes or SNOMED-CT.

It is acknowledged that existing laboratory information systems may not meet this standard, however the ability to store data in this way should be considered when laboratory systems are replaced or upgraded.

v. Workload data should be recorded in a format that facilitates the determination of the resources involved.

3 Liver needle core biopsies for the investigation of medical disease

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'), where there is no clear medical diagnosis after routine 'liver screen' investigations, or when such investigations result in more than one possible diagnosis
- assessment of severity/stage of a known disease, and to monitor change over time or with treatment.

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

3.1 Staffing and workload

Laboratory staffing

Laboratories are required to produce 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.

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 posed by the clinician. In many cases, it may be appropriate to discuss pathological findings with the requesting clinician before the final report is sent out. 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.

In the UK, hepatology networks are being developed as part of the National Strategy 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), which include seven transplant centres.[2] 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).

For pathologists working outside hepatology centres

For pathologists in this situation, there should be an identified **local lead histopathologist responsible for liver biopsies**, ensuring quality of laboratory work, opportunity for clinicopathological discussion (as part of a formal multidisciplinary team [MDT] meeting, or informally) and communication 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, these should be discussed within the department to ensure sufficient numbers to maintain expertise. Biopsies may be referred to the hepatology centre when required, either by the pathologist or clinician (see section 8 below). Local lead pathologists for liver should participate either in a liver EQA scheme or other regular CPD activity in liver pathology.

An evidence-based minimum workload is, as yet, not clearly defined. However, pathologists must bear in mind their 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. When it is very low, passing the liver workload to a larger unit should 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.

For pathologists working within hepatology centres

Liver biopsies are 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, participate in a specialist EQA scheme and provide a referral service within the hepatology network.

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 diagnostic liver biopsies are needle core biopsies obtained in radiology departments under image guidance (liver directly visualised during procedure) or assistance (ultrasound used to locate biopsy site prior to procedure). Trans-jugular biopsies are indicated for patients with poor blood clotting. Occasionally small diagnostic wedge biopsies are taken during laparoscopy or surgery. Routine needle core or wedge biopsies are submitted in formalin, and are best sent free-floating rather than attached to blotting paper. Biopsies from patients with hepatitis B or C should be labelled with 'risk of infection' stickers, and fixed overnight before processing. Clinical guidelines on the use of liver biopsy in clinical practice, including indications, contra-indications and techniques, are published by the British Society of Gastroenterology.[6]

Size of biopsy

An adequate biopsy core is important. Biopsies using Menghini needles, as generally used by hepatologists on the ward, produce longer specimens than Tru-cut needles which have a fixed size trough.[1] However, most biopsies are now taken in radiology departments, using triggered gun devices that produce cores up to 25 mm long. While specific recommendations on biopsy technique are beyond the scope of these histopathological guidelines, clinicians and pathologists must be aware of sampling error and the impact of biopsy size, particularly for the assessment of liver architecture and fibrosis. Quantitative studies have shown that assessment of disease stage is compromised in biopsies shorter than 25 mm or containing fewer than 11 portal tracts,[7] or if tissue area is less than 22 mm^2.[2,8.] To fulfill this standard, the AASLD position paper on liver biopsy recommends "long and wide (an ideal size is 3 cm long after formalin fixation obtained with a 16 gauge needle)".[9] However, the clinical benefit of the biopsy must be balanced against the risks. Transjugular biopsies use a narrower, 19 g needles, and it has been shown that four passes are required for optimal specimens.[10]

In general, a biopsy with at least six portal tracts is considered sufficient for routine diagnosis *[Level of evidence D].*[11,2] The number of portal tracts in a given length of biopsy is variable and cannot be predicted from the biopsy length *[Level of evidence D].*[12,13] Wider gauge needles produce more robust samples with less fragmentation and more portal tracts per cm *[Level of evidence D].*[13] The biopsy report should include both 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. 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.

The specimen must be treated with great care to minimise fragmentation and artefact introduced by handling.

Additional specimens may be sent as clinically indicated, e.g. 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 done 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 where metabolic abnormality is suspected (usually paediatric biopsies). A sample in glutaraldehyde is occasionally sent for electron microscopy (usually paediatric biopsies).

3.3 Specimen dissection and block selection

The whole biopsy is processed. 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 artefact and should be avoided *[Level of evidence D].*[14]

4 Embedding options

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

5 Sectioning

Sections are cut with the microtome set at 3 µm. The number of sections routinely cut varies, but as a minimum will include serial sections as required for the stains below. The needle core may be very thin, and care is needed not to trim too far into the block.

6 Staining

A panel of histochemical stains is used to demonstrate tissue architecture, and screen for metabolic diseases (haemochromatosis, alpha-1-antitrypsin [α1-AT] deficiency, Wilson disease). The stains used vary among laboratories according to local preference, but are guided by what is sufficient to allow a retrospective specialist assessment of the biopsy, should this be required. As a minimum, haematoxylin and eosin (H&E, at least two levels) and special stains for reticulin, collagen (such as van Gieson, Masson trichrome or picro Sirius red), PAS with and without diastase, a stain for copper binding protein (such as Orcein) and Perls' stain for iron are provided routinely on all medical biopsies. Additional histochemical stains may be requested as appropriate, e.g. stains for copper (rhodanine, rubeanic acid), acid fast bacilli, amyloid, etc. Suitable control tissue blocks must be available, and appropriate control sections should be performed with each staining run where the substance stained is not normally present in the liver (e.g. Perls', Orcein) *[Level of evidence D].*[3,4]

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 unstained spares kept for further stains as required.

7 Further investigations

Retrieve sections in file from any previous biopsy, for comparison with the current biopsy.

Immunohistochemistry at the request of the pathologist, e.g. keratin 7 (K7) or 19 (K19) for bile ducts, ubiquitin, keratin 8/18 (K8/18) or p62 for Mallory-Denk bodies. Hepatitis B core and surface antigen (HBcAg, HBsAg respectively) in selected biopsies for hepatitis B.

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

Availability of facilities providing the following techniques may occasionally be required, e.g. by referral to an appropriate centre:

- electron microscopy for investigation of metabolic/storage disease
- in-situ hybridisation, e.g. for Epstein Barr virus.

8 Report content

8.1 Primary reporting of medical liver biopsies

Reporting style and order of items are influenced by 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 *[Level of evidence D].*[15]

- Any additional clinical information obtained prior to reporting, e.g. from electronic patient record or discussion with clinician, which provide context available to the histopathologist at the time of reporting the biopsy.

- **Biopsy size/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** 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.

  The report should indicate 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
  - fibrosis with bridging between vascular structures without parenchymal nodularity
  - advanced fibrosis with bridging and parenchymal nodularity – indicating definite or probable cirrhosis, and an indication for clinical management by the cirrhosis pathway.
  - subtle architectural abnormalities in the form of focal liver cell plate atrophy and nodularity without bridging fibrosis alert to the possibility of 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**, either:

  - **parenchymal abnormality** without progressive chronic liver disease, e.g. lobular hepatitis, cholestasis, steatosis or:
  - **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 features and parenchymal features and results of special stains in sequence, and integrating these into an overall histological diagnosis. Appropriate negative findings (e.g. lack of iron overload or α1-AT globules) are documented in the report. Further guidance is provided in Appendix C.

- A definite diagnosis where possible, or a discussion of the differential diagnosis. This is usefully incorporated in a clear **clinicopathological comment** following the morphological description. This will include the aetiological agents to consider (e.g. virus, drug, autoimmune, metabolic, obstructive) and relevance of the histological features to the clinical scenario, with an indication of diagnoses suggested or excluded. It might include suggestions for further investigations or indications for treatment. In

cases where a dual pathology is suspected, the report indicates if possible the dominant pathological process.

- In formulating comments on the likely diagnosis, a distinction should be made between:
  - biopsy performed for **abnormality of liver biochemistry without known clinical diagnosis;** based on the pattern of disease, the pathologist proposes one or more clinicopathological diagnoses to guide further clinical investigation
  - *pitfall: 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)*
  - **clinical details suggest one or more specific diagnosis;** the report should indicate the extent to which histopathology supports/excludes one or more of the suspected diagnosis and/or favours an alternative diagnosis. In cases where histological findings support more than one diagnosis (e.g. chronic hepatitis C virus (HCV) infection and non-alcoholic fatty liver disease), an attempt should be made to identify the predominant cause of liver injury
  - *pitfall: the pathologist should not simply report a biopsy as 'consistent with' a proposed clinical diagnosis without considering alternative diagnoses.*

- For chronic liver disease, there should be an indication of the **severity of the disease** in terms of grade/stage. This can be achieved either by descriptive text or a semi-quantitative scoring system, as agreed locally between pathologist and clinician. Scoring systems developed for use in clinical trials (e.g. Ishak[16] for chronic viral hepatitis, Kleiner[17] for non-alcoholic fatty liver disease) are poorly reproducible in routine practice. If used, clinicians and pathologists should be aware of their limitations. For the current role of liver biopsy in the management of chronic viral hepatitis, see Appendix D.

- Comparison with previous liver biopsy samples is important in refining the diagnosis and establishing the rate of progression of the disease.

- 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 is considered. A further biopsy may be indicated in some cases.

8.2 Indications for referral of case for second opinion

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

- pathologist request – diagnostic uncertainty in initial interpretation of unusual or complicated case

- clinician request – when the patient is referred to the hepatology centre for further clinical management, or for advice, e.g. when disease progress/response to treatment differs from what is anticipated.

- clinician and pathologist request – diagnostic uncertainty identified during clinicopathological discussion/meeting.

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 guidelines for handling and reporting are therefore transcribed from the liver biopsy section of The Royal College of Pathologists' liver cancer dataset.[18]

It should be noted that most hepatobiliary surgeons advise against needle biopsy to confirm a diagnosis of metastatic colorectal carcinoma or hepatocellular carcinoma where future surgical excision may be an option because of the risk of upstaging the disease, as a consequence of needle biopsy seeding *[Level of evidence D].*[19] The diagnoses in these cases are made on the basis of imaging and the appropriate clinical setting.

9.1 Specimen submission

The request form should indicate that the biopsy is from a focal lesion and should also include other relevant clinical information such as a previous history of malignant disease or imaging results.

Unlike medical liver biopsies, there is no minimum recommended specimen size. A biopsy containing diagnostic tumour tissue can be regarded as adequate, although small samples may not contain sufficient tissue for full immunohistochemical evaluation.

9.2 Sectioning and staining

Initially one or two shallow levels stained with H&E should be examined. The pathologist can then determine whether tumour is present and what further investigations are required, based on the morphology of the tumour in the biopsy and clinical circumstances.

9.3 Further investigations

Discussion with the clinician at an early stage is recommended if the presence of tumour is confirmed 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 clinical management decisions.

Immunohistochemical evaluation is usually required to investigate the nature of the tumour. The selected panel of markers will be tailored for each individual biopsy depending on the tumour morphology any clinically suggested site of origin for metastatic disease, the amount of tissue available in the biopsy, and for the exclusion of potentially treatable disease.

The guidelines from the National Institute for Health and Clinical Excellence (NICE) for investigating patients who present with metastatic carcinoma of unknown primary origin recommend the use of immunohistochemistry to investigate the likely primary site of origin *[Level of evidence D].*[20] In patients with a biopsy showing a malignancy of unknown origin, a simple panel of immunohistochemistry tests is essential to exclude melanoma, lymphoma or sarcoma.

For biopsies that show carcinoma of trabecular or hepatoid pattern in which the morphological differential is with primary hepatocellular carcinoma (HCC), immunohistochemistry is often helpful (see Appendix E).

For biopsies that show features of adenocarcinoma, the use of immunohistochemistry is specifically addressed in the NICE recommendations[20] as follows:

"Use a panel of antibodies comprising K7, K20, TTF-1, PLAP, ER (women only) and PSA (men only) in all patients with adenocarcinoma of unknown origin. Use additional immunohistochemistry to refine the differential diagnosis, guided by the results of the panel of antibodies in the previous recommendation and the clinical picture."

Appendix 2 of the NICE guidelines consists of two tables of immunohistochemistry in metastatic carcinoma:

1. a table of the various combinations of K7 and K 20 according to primary sites
2. a table of frequency of positivity for individual markers K7, K20, ER, PR, TTF-1 and PSA in 13 primary sites.

These are provided, in slightly amended format, in Appendix F.

Further markers can be used depending on the clinical circumstances; in particular, placental alkaline phosphatase (PLAP) is a useful marker for germ cell tumours, some of which have the appearance of adenocarcinoma. The clinical evidence base for these recommendations is included in the NICE guidelines.[20] Immunohistochemical evidence of neuroendocrine differentiation is worth seeking in non-small cell carcinoma of otherwise undetermined origin, as this can influence chemotherapy; in those carcinomas, supplementary immunohistochemistry can further indicate a likely primary site *[Level of evidence D]*.[21,22] A wider range of immunohistochemical markers is described in an earlier evidence-based study by Dennis *et al [Level of evidence D].*[23]

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

If no tumour tissue is seen in the initial sections, deeper levels should be requested before reporting a negative biopsy. The possibility that the biopsy is from a well-differentiated hepatocellular lesion (focal nodular hyperplasia, hepatocellular adenoma, well-differentiated hepatocellular carcinoma or focal fatty change/sparing) should be considered. The classification of benign focal hepatocellular lesions based on morphology and immunohistochemistry is evolving and is summarised in Appendix H *[Level of evidence C].*[24,25]

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

9.4 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 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 background liver, if sufficient is included

- a definite diagnosis of the focal lesion where possible, or a discussion of the differential diagnosis. This would include a discussion of tumours compatible with or excluded by immunohistochemistry
- an appropriate SNOMED code.

10 Criteria for audit of the tissue pathway

Staffing and workload

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

Report content

Audit of the completeness of recording each of the data items in the histopathology report. This tissue pathway lends itself to identifying criteria for an audit template, which should encourage good practice for liver biopsy handling, reporting and clinical liaison. A detailed audit template will be available on the RCPath website, www.rcpath.org/clinical-effectiveness/clinical-audit/clinical-audit-templates/

Timeliness of report

Audit recommended by the RCPath as KPIs (see *Key Performance Indicators – Proposals for implementation*, 2013, on www.rcpath.org/clinical-effectiveness/kpi/KPI) is as follows.

- For histopathology cases that are reported, confirmed and authorised within seven and ten calendar days of the biopsy being taken:

  Standard: 80% of cases must be reported within seven calendar days and 90% within ten calendar days.

For diagnostic biopsies cases where the report is required more urgently (e.g. acute liver failure, transplant biopsies), a preliminary report should be given by telephone or email; the diagnosis and time/date of the provisional report 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, best method for showing nodular regenerative hyperplasia. Collapse of reticulin framework occurs in areas of recent liver cell necrosis – can resemble fibrous septa occurring in chronic liver disease (other connective tissue stains are required to distinguish recent collapse from longstanding fibrosis).
Collagen stains: • Van Gieson • Masson trichrome • Picro Sirius red	Type 1 collagen fibres	Portal tracts Walls of hepatic veins	Increased in hepatic fibrosis – can be periportal, perisinusoidal or pericellular. Presence implies chronic liver disease. Three main stages of fibrosis recognised: • mild/early – fibrosis without bridging • bridging fibrosis without nodules • late – bridging fibrosis with nodules (implies early or established cirrhosis). Amount of collagen in portal tracts increases with size of tract and age. Longitudinally sectioned tracts may be mistaken as fibrous septa (can be identified by vessel/duct orientation).
PAS	Glycogen (in well-fixed biopsies) Glycoprotein	Hepatocytes Basement membrane of bile ducts	Highlights presence of hepatocytes, e.g. in interface hepatitis, or absence of hepatocytes, e.g. parenchymal granulomas or confluent necrosis.
PAS diastase	Glycoprotein Mucin Ceroid pigment Alpha-1-antitrypsin (α1-AT)	Basement membrane (BM) of bile ducts Bile duct lumen N/A N/A	Bile duct damage in chronic cholestatic disease (e.g. disrupted BM in Primary biliary cirrhosis [PBC], thickened BM in primary sclerosing cholangitis [PSC]). Ceroid pigment in Kupffer cells – non-specific marker of previous hepato-cellular injury, prominent in cholestasis. Screening test for α1-AT deficiency (can be confirmed by immunostaining).

Stain	Material demonstrated	Distribution in normal liver	Use in assessment of liver disease/ other comments
Orcein	Proteins with disulphide bonds:		
	• elastin	Portal tracts Walls of hepatic veins Internal elastica of arteries	Presence of elastic fibres in septa indicates longstanding fibrosis.
	• copper-associated protein	N/A	Presence of copper-associated protein – very important in: • non-cirrhotic liver any amount is abnormal (except in neonates) – sensitive indicator of chronic biliary disease (or rarely Wilson disease or vascular disorder) • cirrhosis small amounts non-specific; larger amounts as above.
	• hepatitis B surface antigen (HBsAg)	N/A	Screening test for HBsAg (can be confirmed by immunostaining).
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 possibility of haemochromatosis (especially in the absence of significant fibrosis). Secondary iron overload common in cirrhosis (especially alcoholic and HCV) – typically has mixed hepatocellular/ sinusoidal pattern. Pure sinusoidal cell siderosis (Kupffer cells and/or endothelial cells) seen following haemolysis or blood transfusion, systemic chronic inflammatory disease and in acute hepatitis.
Rhodanine	Copper	Absent	Confirms copper accumulation in cases with copper-associated protein. May show cytoplasmic copper in Wilson disease (diffuse rather granular periportal staining).

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 (CK 7) K19 (CK19)	Keratin 7 Keratin 19	Biliary epithelium – bile ducts and ductules Biliary epithelium – bile ducts and ductules	K7 and K19 – useful in biliary disease to assess duct loss and to demonstrate/ confirm presence of ductular reaction. K7 – identification of intermediate hepatobiliary cells in hepatocyte regeneration and biliary disease. 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.
K8/18	Keratin 8/18	Weak diffuse cytoplasmic staining (with submembranous accentuation) in hepatocytes Also present in bile ducts	Identifying features of steatohepatitis – alternative to ubiquitin and p62 for demonstrating Mallory-Denk bodies (MDBs). Ballooned hepatocytes also show loss of normal cytoplasmic/ submembranous staining.
Ubiquitin p 62	Mallory-Denk bodies (MDBs)	Negative	Component of MDBs. Helpful in confirming presence of MDBs in cases suspected to have steatohepatitis (versus simple steatosis).
HBsAg HBcAg	Hepatitis B surface antigen Hepatitis B core antigen	Negative Negative	Cytoplasmic positivity corresponds to ground glass hepatocytes, in chronic hepatitis B. More sensitive for demonstrating cytoplasmic HBsAg than orcein staining. Also demonstrates membranous HBsAg – not shown by orcein method. Negative in acute hepatitis B. Nuclei positive in immune tolerant 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	Alpha-1-antitrypsin	Negative in hepatocytes May be present in Kupffer cells	Confirms presence of α1-AT accumulation (more sensitive and specific than PAS-diastase).

Appendix C Main patterns of liver disease

Full diagnosis is dependent on correlating histological findings with clinical context. The table below is an initial checklist of main morphological patterns and their possible clinical significance, based on the authors' experience from referral cases.

(a) 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.	Dominant pattern of injury in acute hepatitis. Viral hepatitis, drugs and auto-immune 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 – 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, presence of plugs within canaliculi indicates bile) Activated Kupffer cells containing PAS-diastase positive ceroid usually present (unless very recent).
Steatosis	Fat vacuoles in hepatocyte cytoplasm, more than 5% parenchymal area.	Consider alcoholic and non-alcoholic causes (histology usually unable to distinguish these).	Distinguish bland steatosis from steatohepatitis – characterised by ballooned hepatocytes and inflammation. Presence of steatohepatitis implies transition to chronicity.
Acute venous outflow obstruction	Sinusoidal dilatation, perivenular hepato-cytes replaced by extravasated red blood cells.	Investigate for causes of venous outflow obstruction, including imaging of hepatic veins.	Rare but important. Changes often patchy in distribution and easy to miss. May be clinically unsuspected prior to biopsy.

(b) Chronic liver disease – four main types

Features may overlap; identify 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 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. lympho-cytic/granulomatous cholangitis in PBC, fibrous cholangitis 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 liver biopsies in medical liver biopsies.[26] 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 – should prompt a careful assessment of bile ducts and other features of chronic biliary disease, even if these are not immediately apparent. Hepatocytes may show other features of chronic cholestasis, e.g. ballooning, feathery degeneration and Mallory-Denk bodies ('cholate stasis') – usually seen in later stages. Ductopenia best assessed by matching ducts to hepatic arteries. Diagnostic duct lesions rarely seen in needle biopsy specimens. Neutrophil rich infiltration of bile ducts, including luminal aggregates of neutrophils, raises possibility of ascending cholangitis.

Pattern	Histological features	Clinical significance	Comments
Fatty liver disease with fibrosis	Steatohepatitis usually present. Fibrosis typically pericellular in early stages. May also be periportal, particularly in later stages.	Main causes are alcoholic and non-alcoholic (metabolic) fatty liver disease. Mild steatosis common in other types of liver disease, e.g. hepatitis C. Less frequently, HCV may lead to development of steatohepatitis – 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 typical features and present as 'cryptogenic' cirrhosis. Mild portal inflammation common in progressing steatohepatitis – 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 – large vessels, obliterative portal venopathy – small portal veins), chronic venous outflow obstruction or sinusoidal endothelial injury (usually drug-related – '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)

Appendix D The role of liver biopsy in the management of chronic viral hepatitis

Hepatitis B

The aim of the treatment is to prevent cirrhosis and hepatocellular carcinoma. In NICE guidance from 2013, liver biopsy is considered for adults with a transient elastography score of 6–10 kPa, and offered to adults aged <30 with a score of <6 kPa and HBV DNA >2000 and raised alanine amino transferase (ALT) persists over three months. Treatment is indicated for patients with raised ALT, high HBV DNA and histologically verified liver inflammation and/or fibrosis. Patients with a transient elastography score less than 6 kPa who have normal ALT and HBV DNA <2000 IU/ml are not offered liver biopsy.[27]

Hepatitis C

The aim of the treatment is to achieve a sustained viral response (HCV RNA undetectable in serum six months after the end of treatment).[28] There are many new antiviral agents currently in development, and the field is advancing rapidly. Treatment is not generally dependent on severity of disease, and so liver biopsy is no longer a requirement for treatment. Biopsy is performed to determine disease severity if management is by 'watchful waiting', or if clinical investigations indicate there may be dual pathology, or to confirm the presence of clinically suspected cirrhosis/late stage disease if this is unclear from transient elastography or other non-invasive tests for fibrosis.

The biopsy report includes the severity (stage and grade) of chronic hepatitis and presence/absence of any additional liver pathology. The stage/grade may be indicated by text or the use of a scoring system as agreed between pathologist and clinician.

Mild disease, for which treatment may be deferred in either hepatitis B or C, broadly corresponds to the absence of bridging fibrosis and only focal interface hepatitis in some portal tracts and less than two foci of necroinflammatory activity per acinus.

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

Tumours that resemble hepatocellular carcinoma (HCC): support HCC

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[29]
CD13	80	Well/moderately differentiated; useful with HepPar1 as some HCC only stain with one. Only a canalicular pattern staining is specific (like CD10 but more sensitive).
AFP	37	Poorly differentiated, usually also seropositive. Complements HepPar and CD13 which tend to stain better differentiated HCC.
pCEA	75	Canalicular pattern specific for HCC, cytoplasmic non-specific.
CD10	61	Canalicular, clearer than pCEA; less sensitive than CD13.
CAM5.2	90	If K7-ve, suggests HCC due to K8 & 18 in HCC.
Glypican 3	>70	Staining may be weak/focal in well-differentiated HCC; not a hepatocyte-specific marker, more an oncofoetal antigen, so can be seen with a number of non-HCC malignancies.
Care with: PGP 9.5 – 87% HCC+ve; synaptophysin 9%+ve, CD56 14% TTF1 – 93% HCC cytoplasmic +ve, 0%nuclear +ve; stains normal liver – depends on antibody clone (hepatocyte staining with clone 8G7/G3/1[30] K19 – stains a minority of HCC that is associated with a poorer prognosis Glutamine synthetase – diffusely stains some HCC but also beta-catenin mutated hepatocellular adenoma		

Tumours that resemble HCC: support metastasis

Antibody	% in HCC	Comments
mCEA	3	Positive in adenocarcinoma including cholangiocarcinoma
S100	0	Differential v. melanoma
Vimentin	7	Differential v. renal cell carcinomas
RCC	0	Differential v. renal cell carcinoma
K7, K20	15, 9	Useful in conjunction with CAM5.2

* Data in these tables is derived with permission from the previous immunohistochemistry online database, Statdxpathiq immunohistochemistry literature database query system, supported by the experience of the authors. The table was originally devised for the first edition of the liver cancer dataset and has been retained here because of its practical value, although the online link is no longer available.

Appendix F Immunohistochemical investigations for liver biopsies containing metastatic tumour: tumours with morphological features of adenocarcinoma[20]

These tables are adapted from Appendix 2 of *Diagnosis and management of metastatic malignant disease of unknown primary origin, Full Guideline* (July 2010), developed for NICE by the National Collaborating Centre for Cancer.

	K7+ K20+	K7+ K20-	K7- K20+	K7- K20-
Oesophagus	81	12	0	6
Ovary mucinous	74	23	3	0
Urothelium	61	21	4	14
Pancreas	55	36	4	4
Stomach	48	25	20	6
Biliary	33	62	5	0
Endometrium	9	92	0	0
Salivary gland	0	100	0	0
Ovary non-mucinous	6	91	0	3
Breast	6	86	1	6
Lung	9	85	0	6
Colon	11	2	79	9
Kidney	0	9	0	91
Prostate	1	9	11	79

The figures in each box represent the percentage of tumours of a particular type that are positive for that particular K7/K20 combination.

The squares are a grey scale, with black being 100% and white 0%.

Row totals do not always sum to 100% as not all studies reported all possible combinations of K7 and K20.

Individual antibodies

	K7	K20	ER	PR	TTF-1	PSA
Biliary	81	33	4	29	0	
Breast	83	7	63	43	0	0
Colon	16	88	2	20	0	0
Endometrium	85	5	31	100*	0	0
Kidney	9	2	3	8	0*	0*
Lung	85	13	5	21	85	0
Oesophagus	50	29	0	100*	0	0
Ovary	73	23	53	32	1	2
Pancreas	95	32	0	2	1	0
Prostate	11	14	10	0*	9	91
Salivary gland	100*	50*	25*	25*	0	
Stomach	49	37	0	5	1	3
Urothelium	74	72				0

The figures in each square represent the percentage of a particular tumour type that is positive for each of the six antibodies.

The squares are a grey scale, with black being 100% and white 0%.

Hash boxes indicate no data available.

* Sparse data (N<5).

Appendix G Other special stains which 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-D positive material and/or cytoplasmic mucin vacuoles favours a diagnosis of adenocarcinoma. Hepatocellular carcinomas may contain PAS-diastase positive globules (e.g. α1-AT).
Perls' or van Gieson	Bile retains green colour and may be more easily recognised than in an H&E stained section. Presence of intracellular or canalicular bile pigment favours diagnosis of hepatocellular neoplasm.
Reticulin	Normal reticulin fibre content retained in dysplastic nodules and benign hepatocellular lesions (e.g. hepatocellular adenoma, focal nodular hyperplasia). Reticulin fibres usually reduced or absent in hepatocellular carcinoma (but may be focally retained in some well differentiated HCCs).

Note: Adenocarcinoma includes primary cholangiocarcinoma as well as metastatic adenocarcinoma

Appendix H Benign focal liver cell lesions: morphology and immunophenotype[31,32]

Lesion	Clinicopathological features	Immunophenotype	Genetic alteration
Focal nodular hyperplasia (FNH)	Central scar, vascular septa with ductular reaction and inflammation	'Map-like' staining pattern for glutamine synthetase (GS)	Polyclonal
Inflammatory hepatocellular adenoma (HCA) (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 (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
HNF 1a inactivated (H-HCA)	Marked steatosis (rare in men; commonest type in women – can be multiple)	Lack of normal cytoplasmic staining for LFABP	HNF1a inactivation
Beta catenin mutated (b-HCA)	May show cytological atypia, 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
Unclassified	Usually solitary	No abnormalities	

Abbreviations

FNH	Focal nodular hyperplasia
LFABP	Liver fatty acid binding protein
HNF-1a	Hepatocyte nuclear factor 1 alpha
JAK/STAT	Janus kinase – signal transducer and activator of transcription
IL-6/STAT3	Interleukin-6/signal transducer and activator of transcription

Appendix I AGREE compliance monitoring sheet

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

AGREE standard	Section
SCOPE AND PURPOSE	
1 The overall objective(s) of the guideline is (are) specifically described	1
2 The clinical question(s) covered by the guidelines is (are) specifically described	1
3 The patients to whom the guideline is meant to apply are specifically described	1
STAKEHOLDER INVOLVEMENT	
4 The guideline development group includes individuals from all the relevant professional groups	Foreword
5 The patients' views and preferences have been sought	N/A *
6 The target users of the guideline are clearly defined	1
7 The guideline has been piloted among target users	Foreword
RIGOUR OF DEVELOPMENT	
8 Systematic methods were used to search for evidence	Foreword
9 The criteria for selecting the evidence are clearly described	Foreword
10 The methods used for formulating the recommendations are clearly described	Foreword
11 The health benefits, side effects and risks have been considered in formulating the recommendations	Foreword
12 There is an explicit link between the recommendations and the supporting evidence	Throughout
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	2–10
16 The different options for management of the condition are clearly presented	Throughout
17 Key recommendations are easily identifiable	Throughout
18 The guideline is supported with tools for application	Appendices
APPLICABILITY	
19 The potential organisational barriers in applying the recommendations have been discussed	Foreword
20 The potential cost implications of applying the recommendations have been considered	Foreword
21 The guideline presents key review criteria for monitoring and/audit purposes	10
EDITORIAL INDEPENDENCE	
22 The guideline is editorially independent from the funding body	Foreword
23 Conflicts of interest of guideline development members have been recorded	Foreword

* The Lay Advisory Committee (LAC) of The Royal College of Pathologists has advised that there is no reason to consult directly with patients or the public regarding this tissue pathway because it is technical in nature and intended to guide pathologists in their practice. The authors will refer to the LAC for further advice if necessary.

Appendix J 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 cancer type 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 cancer type.
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 cancer type. 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 cancer type 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.