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

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INTRODUCTION

The following recommendations are regarded as a minimum acceptable practice. The pathology department should have standard liver pathology texts available for reference, e.g. *Scheuer and Lefkowitz: Liver Biopsy Interpretation*¹ and *MacSween's Pathology of the Liver*.²

SECTION A LIVER NEEDLE CORE BIOPSIES FOR THE INVESTIGATION OF MEDICAL DISEASE

1 STAFFING AND WORKLOAD

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. For pathologists working outside hepatology centres, there should be the opportunity for consultation with a specialist liver pathologist, e.g. via a local network arrangement, so that biopsies may be referred when required either by pathologist or clinician.

Whilst not practicable for all pathologists reporting liver biopsies, it is recommended that those pathologists who report in excess of 100 liver biopsies per year participate in a specialist liver EQA scheme, as these people are likely to be perceived as 'local experts'.

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.

2 SPECIMEN SUBMISSION

Routine needle core or wedge biopsies are submitted in formalin. An adequate biopsy core is important, particularly for the assessment of liver architecture and fibrosis. In general, a liver biopsy should be at least 1 cm long and contain a minimum of six portal tracts. The diameter of the core is also important and broader gauge needles are preferable for medical biopsies. Clinicians and pathologists should be aware that assessment of disease stage is compromised in biopsies less than 20 mm long, 1.4 mm wide, or containing fewer than 11 portal tracts,^{3,4} although this amount of tissue is not always available in practice.

The specimen is treated with great care to minimise fragmentation and artefact introduced by handling. Biopsies from patients with hepatitis B or C should be labelled with risk of infection stickers, and fixed overnight before processing.

Additional specimens as clinically indicated, e.g. samples for biochemistry for heavy metals (contact the local department for advice on specimen submission); fresh tissue for frozen section detection of microvesicular steatosis (e.g. Reye's syndrome in children, acute fatty liver of pregnancy); fresh sample for freezing where metabolic abnormality is suspected (usually paediatric biopsies); sample in glutaraldehyde for electron microscopy (usually paediatric biopsies).

3 SPECIMEN DISSECTION AND BLOCK SELECTION

The whole biopsy is processed. Wedge biopsies may be sliced prior to embedding. The use of rigid foams to support the biopsy core during processing introduces artefact and should be avoided.

4 EMBEDDING OPTIONS

All tissue is embedded. Care is taken to avoid fragmentation.

5 SECTIONING

Sections are cut with the microtome set at 3–5 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 taken 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 deficiency, Wilson's 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 general rule, haematoxylin and eosin (H&E; at least two levels) and special stains for reticulin, collagen (such as van Gieson), 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.

For 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

Immunohistochemistry at the request of the pathologist, e.g. cytokeratins (7 or 19) for bile ducts, ubiquitin for Mallory bodies.

Sections in file from any previous biopsy may be required for comparison with the current biopsy.

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 EB virus.

8 REPORT CONTENT

The report includes the following:

- the **clinical information** received with the biopsy
- **biopsy size/adequacy**, i.e. the length of the core and/or an approximate maximum number of portal tracts per section
- an assessment of the overall **architecture** and the presence and severity of fibrosis. Subtle architectural abnormalities in the form of focal atrophy and nodularity alert to the possibility of portal venous insufficiency (non-cirrhotic portal hypertension)
- for chronic liver disease, an attempt is made to assimilate the features into one or more of the **main patterns of disease** (e.g. chronic hepatitis, chronic biliary disease, fatty liver disease, or as a result of vascular aberration). This may be achieved by 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 alpha-1-antitrypsin globules) are documented in the report
- 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 the 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
- 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. Comparison with previous biopsy samples may be important in refining the diagnosis and establishing the rate of progression of the disease or the response to treatment (see also Appendix 1)
- where appropriate, a concise **summary** to conclude the report
- a SNOMED code.

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.

SECTION B LIVER NEEDLE CORE BIOPSIES FOR THE INVESTIGATION OF FOCAL LESIONS

Needle core biopsy for the investigation of focal lesions detected on imaging is a more frequent indication for liver biopsy than medical disease in many district hospitals. These biopsies require different handling and this section is also included in the College's *Dataset for liver resection specimens and liver biopsies for primary and metastatic carcinoma*.⁵ Most hepatobiliary surgeons would advise against needle biopsy because of the risk of upstaging a malignant tumour where future surgical excision may be an option, e.g. in the appropriate clinical setting, imaging evidence of metastatic colorectal carcinoma or hepatocellular carcinoma.

Many aspects of the medical liver biopsy pathway are also applicable to targeted biopsies of focal lesions. Differences in the handling or reporting of these biopsies are described below.

1 SPECIMEN SUBMISSION

The request form should indicate that the biopsy is from a focal lesion and 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.

2 SECTIONING AND STAINING

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

3 FURTHER INVESTIGATIONS

Once the presence of tumour has been confirmed, discussion with the clinician at an early stage is recommended in order to guide the immunohistochemical investigations. For example, there may be a previous history of primary malignancy that was omitted from the request form or relevant information 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 panel of markers chosen will be tailored individually for each biopsy, depending on the tumour morphology, any clinically suggested site of origin for metastatic disease, the amount of tissue available in the biopsy and the need to exclude potentially treatable disease. See Appendix 2 for a guide to immunohistochemistry.

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 3 for a guide to special stains.

If no tumour tissue is seen in the initial sections, deeper levels are requested before reporting a negative biopsy. The possibility that the biopsy is from a well differentiated hepatocellular lesion (focal nodular hyperplasia, liver cell adenoma, well differentiated hepatocellular carcinoma or focal fatty change/sparing) is considered. 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.

4 REPORT CONTENT

The report includes 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.

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APPENDIX 1 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. Treatment is indicated for patients with raised ALT, high HBV DNA and histologically verified liver inflammation and/or fibrosis.⁶

Hepatitis C

The aim of the treatment is to achieve a sustained viral response (HCV RNA undetectable in serum 6 months after the end of treatment).⁷ Treatment is not 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.

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. There are a number of different scoring systems in current use in the UK; for a historical review and comparison of these, see reference 8.

Mild disease

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 2 IMMUNOHISTOCHEMISTRY FOR THE DIFFERENTIAL DIAGNOSIS OF LIVER-BIOPSIES CONTAINING TUMOUR

Tumours that resemble hepatocellular carcinoma (HCC): support HCC

Antibody	% in HCC	Comments
HepPar1	86	Well differentiated, rarer in metastasis
AFP	37	Poorly differentiated, usually also seropositive
pCEA	75	Canalicular pattern specific for HCC, cytoplasmic non-specific
CD10	61	Canalicular, clearer than pCEA
CAM5.2	90	If CK7-ve, suggests HCC due to CK8 & 18 in HCC
Care with: PGP – 87% HCC+ve; synaptophysin 9% +ve, CD56 14% TTF1 – 63% HCC cytoplasmic +ve, 0% nuclear +ve		

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
CK7, CK20	15, 9	Useful in conjunction with CAM5.2

Investigation of origin of adenocarcinoma⁸

Adenocarcinoma % positivity for each marker										
Primary site	PSA	TTF1	GCDFP15	CDX2	CK20	CK7	ER	Mesothelin	CA 125	Lysozyme
Breast	0	0	49	0	0	87	79	4	13	11
Colon	0	0	6	86	76	3	1	7	1	43
Lung	0	90	3	1	1	90	6	36	38	35
Ovary - serous	0	0	4	0	0	93	81	96	93	7
Ovary - mucinous	7	0	14	14	14	57	50	36	36	29
Pancreas	0	4	1	1	2	94	0	49	50	51
Stomach	2	2	0	0	21	50	0	19	8	81
Prostate	100	8	4	4	0	0	8	0	0	4
HCC		0	0	0	9	15	12	0	10	
Cholangio-carcinoma		0	0	22	49	95	0	44	56	

APPENDIX 3 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. alpha-1-antitrypsin).
Perls'	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. liver cell 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.