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

Tissue pathways for lymph node, spleen and bone marrow trephine biopsy specimens

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Comments	This document will replace the 2nd edition of *Tissue pathways for lymph node, spleen and bone marrow trephine biopsy specimens* published in 2008. In accordance with the Collegeôs pre-publications policy, this document was on the College website for consultation from 12 December 2016 to 20 January 2017. Responses and authorsôcomments will be available to view, following publication of this dataset. **Dr Lorna Williamson** Director of Publishing and Engagement

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NICE has accredited the process used by The Royal College of Pathologists to produce its tissue pathways. Accreditation is valid for 5 years from 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 stakeholder consulted for this document was the British Lymphoma Pathology Group.

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, local guidelines in the UK (including relevant guidelines published by NICE) and the *WHO Classification of Tumours of the Haematopoietic and Lymphoid Tissue* (2008; and the updated version published in 2017[1]). 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, this includes national and international referral practice as well as experience from evaluating responses in the UK Haematopathology EQA Scheme run by the British Lymphoma Pathology Group. 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 five-yearly basis. However, each year, the College will ask the authors of the tissue pathways, in conjunction with the relevant subspecialty adviser 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 membersô attention. If members 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.

The pathway has been reviewed by the Clinical Effectiveness department, the Working Group on Cancer Services and the Lay Governance Group. It was placed on the College website for consultation with the membership from 12 December 2016 to 20 January 2017. 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 Director of Publishing and Engagement.

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 Clinical Effectiveness 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 haematopathology specimens: lymph nodes, spleens and bone marrows. Lymph node specimens may consist of whole node excisions or, increasingly, needle biopsy cores. This guidance is not intended to encompass lymph nodes sampled or excised as part of staging for known cancers. However, many of the principles in this document can be of benefit in this context, and it should be remembered that tumour staging specimens many contain additional nodal pathology for which this guidance will be relevant. Spleens are generally received as resected laparoscopic or open splenectomy specimens, although needle core biopsy can also be carried out at this site. In most cellular pathology departments, bone marrows are received in the form of trephine biopsy cores.

Regional specialist review networks are well established in haematopathology, and it is essential that laboratory and diagnostic procedures are in place in both the site of origin of the specimen and in the reviewing laboratory, in order to optimise the clinical benefit obtained from the tissue.

Centralisation and integration is currently a major aim in haematopathology. This is particularly true of bone marrow specimens, where the production of a single report that combines the findings in the aspirate, the results of the flow cytometry analysis, the histopathology, cytogenetics and molecular genetics is regarded as ideal. Where histopathologists and haematologists are reporting separate aspects of haematopathological specimens, there should be formal procedures in place that enable communication between these groups in order to facilitate this integration.

The purpose of this guideline is to promote uniform good practice in initial specimen handling and reporting, either by the local histopathology department or for clinicopathological review within haematopathology networks. A separate RCPath cancer dataset contains specific details to support the histological reporting of lymphomas (see https://www.rcpath.org/resourceLibrary/dataset-for-the-histopathological-reporting-of-lymphomas.html).[2]

1.1 Target users of this guideline

The primary users of this tissue pathway are trainee and consultant histopathologists. The recommendations will also be of value to trainee and consultant haematologists and haemato-oncologists, histology laboratory managers and technical staff, users of a diagnostic haematopathology service and service commissioners.

1.2 Generic issues relating to staffing, workload and facilities

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

1. 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 RCPath.
2. pathologists involved in the diagnostic process should:
    - participate in audit
    - participate in the RCPathôs continuing professional development (CPD) scheme
    - participate in relevant external quality assessment (EQA) schemes of a general or specialist nature
    - via their pathology department have access to the WHO *Classification of Tumours of the Haematopoietic and Lymphoid Tissues*[1]

- have access to specialist referral opinions on a local network or national basis.

3. the laboratories involved should, as appropriate for their contributions:
   - 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 Bone Marrow Histology Technique
   - participate in the UK National EQA Schemes for immunohistochemistry, flow cytometric immunocytochemistry and fluorescence in-situ hybridisation (FISH)
   - participate in EQA for PCR clonality studies of BCR and TCR genes.
4. 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, D 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.
5. workload data should be recorded in a format that facilitates determination of the resources involved.

2 Lymph node specimens for suspected haematological malignancy

2.1 Staffing and workload

Ideally, two or more pathologists in a unit should be competent in reporting haematopathological biopsy specimens, in order to provide cover for periods of leave.[3] Where haematopathology specimens are reported in a general hospital setting (or in a non-haematopathological specialist unit), the pathologists must liaise closely with their local specialist haematological malignancy diagnostic service (SIHMDS) centre so that expert assistance is available.[3,4] Competency in reporting should extend to the interpretation of lymphoid tissues at non-nodal sites including spleen, bone marrow and mucosa-associated lymphoid tissue.

In each unit it is expected that lymph node and other haematopathology specimens, where the histological appearance is suggestive of a haematological malignancy, will be directed to the designated haematopathologist(s) who will liaise with colleagues at the SIHMDS as needed for detailed work-up and diagnosis. Specimens that are initially examined by a non-designated pathologist should be quality assured by a designated haematopathologist and timely referral of suspected haematological malignancies to the SIHMDS must not be hindered by such local reporting pathways.

Pathologists designated as haematopathology leads should attend specialist haemato-oncology multidisciplinary team (MDT) meetings (usually held at the SIHMDS), should participate in appropriate EQA schemes and should undertake appropriate CPD directed to haemato-oncology.

2.2 Specimen submission

In all cases, adequate clinical information is essential to assess any potential infective risk of the specimen and to help plan the pathological investigations required. Use of diagnostic material may not be efficient or appropriate if gathering of clinical information is left until the MDT meeting. Apart from the presenting features, this information should include a summary of the haematological status of the patient, including white blood cell count and differential

cell counts, and results of any preceding investigations such as peripheral blood analysis, bone marrow biopsy and flow cytometry. A detailed history of chemotherapy and allograft/autograft status should also be mentioned. If the lymph node biopsy is being performed at the request of a haematologist or oncologist, a member of that team is usually in a better position to write the request form than the surgeon, radiologist or other practitioner performing the biopsy. The diagnosis and subtyping of lymphoma is an integrated, multidisciplinary process often involving histopathological and molecular techniques.[5ï 8]

Consent for all laboratory investigations required for diagnosis, including DNA analysis, should be part of the initial consultation with the patient. Examination of the tissue generally takes the form of all investigations required to establish a diagnosis, rather than a specific list of tests that are to be performed. However, the patient may choose to limit the investigations to which they consent; for example, excluding human immunodeficiency virus (HIV) serology. It is the responsibility of the requesting clinician to ensure that the patientôs consent is documented appropriately. When investigations lead to suspicion of a diagnosis not initially expected (such as HIV infection), consideration might be given to seeking additional consent from the patient. Separate consent is required if fixed or frozen tissue is to be banked specifically as a future research resource.

Lymph nodes may be received as complete (or partial) nodes or as needle biopsy cores. Cytological procedures such as fine needle aspiration (FNA) and endoscopic bronchoscopy sampling (EBUS) are also an important part of the investigative pathway of patients with lymphadenopathy, who may subsequently be subject to biopsy or resection of tissue. The laboratory handling of cytological specimens in this context is covered in a separate document, *Tissue pathways for exfoliative cytology and fine needle aspiration cytology* (https://www.rcpath.org/resourceLibrary/tissue-pathways-exfoliative-cytology-fnacytology-jan10.html).[9] We would emphasise the importance of including flow cytometric immunophenotyping in the standard analysis of cytological specimens obtained from lymph nodes, where facilities permit.

Either fresh and/or fixed material can be submitted, depending upon local arrangements and facilities. Submitting fresh tissue facilitates the use of specialist techniques such as cytogenetic analysis, allows the storing of frozen material where appropriate and enables optimal fixation for paraffin embedding and processing (see below). If fresh tissue is to be used, there must be a clearly defined means of transporting the specimen from the operating theatre or clinic to the pathology laboratory in a timely fashion. Small fresh nodes (less than 1 cm in diameter) or nodal biopsy specimens are best lightly moistened with saline to prevent drying out. Larger nodes are best sent dry. The fresh specimen should reach the pathology laboratory and be dealt with within 60 minutes of its removal. Submission of fresh tissue requires close liaison with the operating theatre staff because lymph node biopsies are frequently added to the end of surgical lists and specimens may arrive in the laboratory at the end of the working day. It is therefore good practice to encourage surgeons and interventional radiologists to arrange lymph node biopsies early in the day, so that fresh material can be dealt with in working hours and further transport to specialist centres arranged when necessary. When tests such as cytogenetic analysis are to be carried out at a remote location, an appropriate transport or tissue culture medium is used to preserve the material for diagnosis; imprinting and sending of air-dried films for FISH can be a simple and effective approach prior to specimen fixation.

Since some cellular pathology laboratories are not equipped to handle high-risk pathogens, the possibility of tuberculosis, HIV or hepatitis B needs to be carefully considered by both the clinical team and the pathology laboratory. If there is a possible diagnosis of an infectious disease, such as tuberculosis, a fresh sample can be sent directly to a microbiology laboratory. Material for fixation is placed in formalin for 48 hours. Also, if HIV infection is suspected, most laboratories would expect the node to be fixed for 24ï 48 hours prior to processing.

With all specimens, the priority is for fixation in formalin to allow paraffin or plastic embedding and sectioning for morphological and immunophenotypical analysis. In small samples such as needle biopsy cores, there may be insufficient material for any additional investigations. Where a whole fresh lymph node of sufficient volume is received, the sample may be divided so that part of the specimen can be fixed for histological sections and part can be used fresh for other investigations and for research purposes where there is appropriate consent.

2.3 Specimen dissection, block selection, embedding and sectioning

The overall aim is to obtain óidealô material for reaching a diagnosis from the lymph node. This will include good-quality histological sections and well-fixed tissue for immunohistochemical analysis as well as material for additional investigations.

Where there is no documented risk of infection, the following general principles apply:

1. do not leave any lymph node tissue in the container, unless the node is particularly large (>5 cm diameter). Most smaller nodes should be processed entirely. With larger specimens it may be prudent to leave a small piece of tissue (0.5ï 1 cm in diameter) unprocessed in formalin as an emergency óreserveô in case of processor failure or other untoward events.
2. distribute the tissue between several paraffin blocks, to avoid depletion of diagnostic material while working up the case. This should usually yield one or more blocks with a single complete cross section of an excised lymph node per block (unless very large, in which case the full cross section should be divided between blocks and the macroscopic description annotated accordingly). For needle biopsy specimens, it is particularly important to ensure that only one core (+/- fragments) is placed in each block, to maximise the use that can be made of these small samples.
3. although additional molecular and genetic tests are important, prompt formalin fixation, routine histology and immunohistochemistry on paraffin sections are the most important for diagnosis. Hence, adequate material for paraffin embedding is the priority; only after this requirement is reasonably fulfilled is material saved for molecular and cytogenetic analysis.
4. where clinical information or macroscopic examination suggests that the lymph node is likely to show non-haematopathological disease, such as metastatic carcinoma or granulomatous inflammation, specialist haematopathology investigative techniques can be omitted in the first instance (but be mindful that there may be more than one pathology in the tissue).
5. if a frozen section is performed, the results of this will guide the subsequent approach. If a lymphoproliferative disorder is suspected at the time of frozen section, it is important to try to ensure that sufficient tissue is spared from freezing to use for routine histopathology ï the process of freezing and subsequent thawing for fixation and processing will interfere with both morphological and immunohistochemical analysis.

2.3.1 Protocol for lymph node specimens

The protocol is as follows:

1. where the node is smaller than 1.0 cm in diameter, it is bisected on arrival in the laboratory
2. where the node is larger than 1.0 cm in diameter, it is cut into slices, each of approximately 2 mm thickness, in a plane perpendicular (i.e. at 90 degrees) to the long axis of the node
3. record the size, colour and consistency and presence or absence of any visible nodularity, haemorrhage or necrosis in the cut slices
4. where appropriate, and where there is sufficient fresh tissue, a small piece from one of the slices may be sent for flow cytometric immunophenotyping and/or cytogenetic

studies (see below). Where facilities permit, small pieces may also be stored frozen for research (with consent) or possible future molecular genetic studies.

5. in the unlikely event that macroscopic examination of a fresh lymph node identifies a possible infective focus, and where there is no evidence of previous microbiological sampling, a small piece from the focus can be sent to microbiology for culture. Microbiology laboratory staff should be informed that the specimen was not taken under sterile conditions.
6. process the rest of the tissue into paraffin blocks. For nodes less than 1 cm in diameter each half of the bisected node is put into a separate block. With larger nodes, each block should contain sliced tissue no more than 2 mm thick, with only one piece of tissue placed in each cassette. Multiple pieces make immunostaining more difficult; very large slices may also interfere with even, high-quality immunostaining. To ensure that slices of lymph node remain flat, it may be helpful to incorporate sponges into the cassettes to prevent folding. The sampled tissue needs to fix for 24ï 48 hours; less than this leads to poor preservation of cytological detail and can make the tissue less amenable to histological interpretation. Standardisation of fixation makes immunochemistry and in-situ hybridisation (ISH) more reliable, since heat and protease recovery times will be similar. Prolonged fixation makes immunochemistry unreliable and can impair recovery of DNA from paraffin blocks.

2.3.2 Protocols for needle core biopsy specimens and other small samples of nodal/extranodal tissue

Needle core specimens of lymph nodes or small biopsy fragments of extranodal/nodal lesions are processed entirely for routine formalin fixation and paraffin embedding. Where multiple cores are sent, the RCPath *Dataset for the histopathological reporting of lymphomas* recommends separation of the cores, placing one or two cores at most into each separate paraffin block.[2]

2.3.3 Protocol regarding sectioning levels and ribbons of tissue

All sections are ideally 2 μm thick, as this renders the best morphological appearance. In cases of lymph node specimens which are small and appear uninvolved, but where there is a clinical suspicion of malignancy, the initial haematoxylin and eosin (H&E)-stained section may be followed up by examining H&E-stained sections from three deeper levels before interpreting the tissue as negative.

With regard to needle core biopsies, it is important to consider the need to preserve tissue for immunohistochemistry and molecular biology at the time of sectioning. Some laboratories prepare a single good-quality H&E-stained section for initial assessment, while others cut sections at two or three levels for H&E staining. The latter may be helpful where the disease process is patchy or heterogeneous, but producing excessive H&E-stained sections can limit tissue remaining for immunohistochemical and molecular analysis and prevent a definitive diagnosis. Where multiple levels are cut, some laboratories will preserve intervening sections from the ribbon on coated or charged glass slides. This allows subsequent immunohistochemical analysis of a focal infiltrate, if such is detected in one of the H&E-sections sections, and we recommend this as good practice.

[Level of evidence – GPP.]

2.4 Staining

Lymph node sections are stained using the standard H&E stain. Reticulin staining may be helpful in assessing the follicular architecture, but is rarely used and has been largely superseded by immunohistochemical demonstration of follicular dendritic cells using CD21 and/or CD23.

2.5 Further investigations

Additional investigations that may be carried out in suspected haematopathological disease include immunostaining, cytogenetic analysis, flow cytometric immunophenotyping and molecular biology analysis.

2.5.1 Immunostaining

All immunostaining should be requested as a panel of antibodies rather than individual tests so that appropriate comparisons can be made. If all tissue blocks are similar, immunostaining needs to be performed on one block only, otherwise blocks need to be selected to demonstrate all suspected diagnoses in a patient. When selecting panels for immunohistochemistry it is important to include antibodies that are expected to give negative as well as positive results. Most lymphomas are substantially defined by their immunoprofile.

Of note, discrepancies between the expected morphological and immunophenotypic findings can be related to technical failure, loss of antigens or expression of aberrant antigens; they can also indicate a potential erroneous initial diagnosis. In these circumstances further investigations are needed to clarify the situation and to confirm or refute the putative diagnosis.[10]

[Level of evidence – D].

The final report must highlight any such discrepancies and should suggest an explanation for any unusual or conflicting immunochemical findings.

All immunostaining must be supported by satisfactory laboratory performance and appropriate external quality control. Knowledge of the normal staining pattern and cross-reactions of an antibody is crucial for correct interpretation and diagnosis.[11,12]

2.5.2 Cytogenetic analysis

While a fresh sample of the specimen in tissue culture medium may be sent for cytogenetic analysis, currently the most cost-effective way to perform genetic analysis in tissue biopsy specimens is by targeted FISH for specific translocations. Most cytogenetic laboratories now offer a routine service for tissue section FISH using paraffin-embedded material and, hence, special arrangements for handling and sending fresh tissue are rarely needed.

2.5.3 Flow cytometric immunophenotyping

A small sample of fresh tissue can be sent, wrapped in saline-dampened gauze in a clean, dry container, if prompt analysis (<1 hour after removal from patient) in the appropriate laboratory is feasible. If longer transport times are anticipated, the tissue should be transferred in culture medium and consideration should be given to disaggregating it before dispatch.

2.5.4 Molecular biology

If required, and if facilities for frozen storage are available, a sample of fresh tissue can be snap-frozen and stored for subsequent investigations such as DNA analysis.

2.5.5 Retention of specimens

No diagnostic material is discarded until all investigations are complete. The RCPath recommends that paraffin blocks are stored for 30 years, in line with the legislation covering retention of patientsômedical records. Stained slides are stored for a minimum of 10 years, and preferably longer, especially in the case of small biopsy specimens where material permitting diagnosis may no longer be contained within the paraffin blocks. For those departments connected with research facilities, frozen tissue can be stored at ï 70ºC or lower for at least 10 years. Storage at ï 170ºC or lower is needed where viable cells are required, e.g. for subsequent tissue culture. Detailed advice is available from the RCPath and the Institute of Biomedical Science.[13]

2.6 Report content and MDT meetings

Reports should identify the pathological changes within the lymphoid specimen, whether intact node(s), needle biopsy core(s) or extranodal lymphoid tissue. The report may also include comments on the preservation or effacement of the lymph node architecture and an overall assessment of follicles, paracortex, sinuses, capsule and extracapsular tissue where relevant. In extranodal lymphoid tissue the relationship with associated epithelial structures may also be important. The cytomorphology of any abnormal infiltrate is normally described and in most cases the infiltrate is categorised as reactive or neoplastic (Appendix A). The results of additional tinctorial stains and the immunohistochemical findings should be summarised clearly. Where a lymphoproliferative condition is identified, the disease is classified using current WHO terminology, as detailed in the RCPath dataset for the histological reporting of lymphomas and the 2017 revision of the WHO classification.[1,2,13]

[Level of evidence – D].

The MDT meeting plays an important role in lymph node pathology. Data generated from all modes of investigation need to be collated and interpreted in a clinical context. The reporting pathologist remains responsible for his or her diagnosis and for ensuring that appropriate additional investigations are instituted to resolve discrepancies. An individual's experience of all of the different analytical methods (tinctorial stains, immunohistochemistry, FISH, PCR etc.) is useful in weighing up the contribution each investigation makes to the final diagnosis. All diagnoses of haematological malignancy should be discussed by the MDT, which records the pathological diagnosis and the clinical management decisions. The role of the pathologist(s) at MDT meetings is summarised in *The role of the lead pathologist and attending pathologists in the multidisciplinary team* (www.rcpath.org/resourceLibrary/g087_roleofleadpathinmdt_mar2014-pdf.html).[14]

It is important to note that the majority of MDT meetings focus almost exclusively on the management of neoplastic disease, and non-neoplastic lymphoid pathology will not be routinely considered. Haematopathologists should therefore be proactive in bringing patients with non-neoplastic pathology to the attention of the MDT where the findings may be relevant to clinical management.

3 Spleen specimens

3.1 Staffing and workload

See lymph node tissue pathway (section 2.1 above).[3,4]

3.2 Specimen submission

Splenic tissue may be received in the form of an intact spleen from open splenectomy, fragments from laparoscopic splenectomy or as needle core biopsy specimens. Occasionally (currently very rarely in the UK), partial/segmental splenectomy is undertaken. Ideally, as for lymph nodes, the material is sent to the laboratory without prior fixation, to enable sampling for flow cytometry, cytogenetic and FISH studies and frozen storage (where facilities permit) for possible future nucleic acid studies. Unless delivery of intact splenectomy specimens unfixed is genuinely unmanageable because of local arrangements and/or facilities, this is particularly strongly recommended because of the difficulty in achieving good fixation of spleen tissue. The organ is bulky and dense even at normal size and is often removed to investigate and/or alleviate substantial pathological enlargement. If not sliced and washed while fresh, adequate fixation may be restricted to a narrow rim of peripheral tissue less than 1 cm in thickness; accumulated blood and slow cooling of the intact organ encourage rapid central autolysis.

When laparoscopic splenectomy is undertaken, disruption of the tissue aids penetration of formalin and dispersal of blood, but surgeons should be encouraged to remove the tissue so that at least some fragments are large enough to yield histological sections free of surgical traumatic artefacts (5ï 10 cm^3). Even from small children, fragments of 5 cm^3 are straightforward for experienced laparoscopic surgeons to obtain as long as the requirement is made clear when the surgery is being planned. Laparoscopic splenectomy is practised with increasing frequency and it is essential to have good communication with the surgical teams undertaking these procedures to ensure that material suitable for diagnosis is received by the laboratory.

Needle core biopsy of the spleen has, historically in the UK, been relatively rarely undertaken because of the risks of haemorrhage. However, ultrasound- or CT-guided biopsy of solid lesions in the spleen is a useful and generally safe procedure in the hands of an appropriately trained radiologist.[15] Use of needle biopsy to sample solid intrasplenic lesions encountered incidentally during CT or PET scan is increasing as the use of such imaging investigations increases; it is important to consider whether such specimens are representative since many such lesions have complex, heterogeneous architecture with extensive (usually central) scarring. These specimens are handled as for lymph node needle cores.

As with lymph nodes, the risk of infectious disease should be considered with splenic specimens; see section 2.2. In all cases, adequate clinical information is essential to assess the risk of the specimen and plan the investigations. As for other haematopathology specimens, diagnostic material may not be used efficiently and appropriately if gathering of clinical information is left until the MDT meeting. Apart from the patientôs presenting features, the information should include a summary of their haematological status, including full blood cell counts and differential white cell counts, and the results of any preceding investigations such as peripheral blood film examination, bone marrow biopsy and flow cytometry. A detailed history of any prior chemotherapy or other haemato-oncological treatment should be available. Haematological information about the peripheral blood is particularly important for the interpretation of splenic pathology, which may include consideration of myeloid disorders and cytopenias, as well as the full spectrum of neoplastic and inflammatory diseases investigated in pathological lymph nodes. If splenectomy or spleen biopsy is being performed at the request of a haematologist or oncologist, a member of that team is usually better placed than the surgeon to complete a request form with the required information.

3.3 Specimen dissection, block selection, embedding and sectioning[16]

On receipt in the laboratory, the spleen is weighed, including any blood clot. It is usually convenient (especially if the spleen is large and received in an unfixed state) to do this without removing the organ from its container. Simply deduct the weight of an equivalent dry container.

Measurements are recorded of the vertical height, medio-lateral width and antero-posterior depth of the spleen. Any external abnormalities are noted (capsule, hilar lymph nodes [if present] and vessels).

For suspected lymphoma, if appropriate facilities are available, a piece of tissue approximately 1 cm^3 is removed under sterile conditions and cells are dispersed from this into culture medium (e.g. RPMI medium) for rapid immunostaining by flow cytometry. Samples can be taken for cytogenetic analysis and frozen storage at this stage, if required, as for fresh lymph nodes.

The spleen is then sliced like a loaf of bread, across its horizontal axis, making each slice 0.5ï 1 cm thick and separating them completely. After laying representative slices out on the cutting surface, the internal appearances are described (congestion, white pulp prominence, any focal lesions).

[Level of evidence – GPP.]

The slices are then placed back into the container and immersed in plenty of formalin. Swirling gently will rinse as much blood from the tissue as possible; the heavily blood-contaminated formalin can then be discarded and replaced with fresh formalin. Repeating this rinsing process can be helpful with particularly large and/or congested spleens, as blood within the tissue is a major obstacle to good fixation. The tissue can then be left to fix overnight before further sampling the following day. It can still be necessary to fix the samples for a further 24 hours before processing if the tissue is very congested with blood.

Alternatively, after initial slicing, selected small pieces may be sampled directly into cassettes for further fixation in abundant formalin; care should be taken not to slice these too thinly (<2 mm) or they may become distorted. Adding sponges above and below the tissue inside the cassettes, to prevent such distortion, can be helpful.

Blocks are best taken immediately adjacent to the capsule, where fixation is best even with the most careful attention to tissue received fresh in the laboratory, unless there is a need to sample a focal lesion deeper within the spleen.

As routine practice it is generally adequate to take four blocks from any macroscopically normal spleen (superior and inferior poles, hilar [medial] aspect and convex [lateral] aspect). Extra blocks are taken, as needed, if any focal lesions are present within the parenchyma or if the spleen is significantly enlarged (>500 g). Sample all hilar lymph nodes in cases of suspected lymphoma or other neoplasm.

3.4 Staining

Blocks from spleen and hilar nodes should be stained with H&E. Reticulin, Giemsa, periodic acid-Schiff and Perlsôstains can be added to assess architecture and iron load in more detail.

3.5 Further investigations

Additional investigations that may be carried out in suspected haematopathological disease include flow cytometry, immunohistochemistry, molecular analysis and cytogenetic analysis, as for lymph node specimens. If all tissue blocks are similar, immunostaining needs to be performed on one block only, but it may be helpful in some instances to stain a representative hilar lymph node block in parallel, since there is greater familiarity with lymphoid architecture. Criteria for selection and reporting of antibodies employed in immunohistochemical panels are the same as those applied in lymph nodes. It is important to be familiar with antigen expression patterns of normal lymphoid and stromal constituents of the spleen, since these differ in some respects from those in lymph nodes.

[Level of evidence – GPP.]

Staining for micro-organisms should be considered for all granulomatous lesions encountered in the spleen.

For retention of specimens, see section 2.5.5.

3.6 Report content and MDT meetings

Reports should identify pathological changes in each compartment within the white and red pulp of the spleen, and in hilar lymph nodes if present. These include preservation or effacement of architecture with an overall assessment of white pulp nodules, cords, sinusoids, capsule and trabecular connective tissue. The cytomorphology of any abnormal infiltrate is described and categorised as reactive or neoplastic. The results of additional tinctorial stains and the immunohistochemical findings are summarised clearly. Where a

lymphoproliferative condition is identified, the disease is classified using current WHO terminology.[1]

The MDT meeting plays an important role in splenic pathology. Data generated from all modes of investigation need to be collated and interpreted in a clinical context. The reporting pathologist remains responsible for his or her diagnosis and for ensuring that appropriate additional investigations are instituted to resolve discrepancies. An individualôs experience of all of the different analytical methods (tinctorial stains, immunohistochemistry, FISH, PCR) is useful in weighing up the contribution each investigation makes to the final diagnosis. All diagnoses of haematological malignancy should be discussed by the MDT. Both the diagnosis and the clinical management decisions are recorded at a formal MDT meeting or an equivalent clinico-pathological forum for discussion of non-neoplastic pathology.

4 Bone marrow trephine (BMT) biopsy specimens

4.1 Staffing and workload

See section 2.1.[3,4]

4.2 Specimen submission

BMT biopsy is carried out to assist in the diagnosis of various haematological problems. It is particularly useful for assessment of marrow cellularity, cell distribution and the spatial relationships between different cell types. BMT may be crucial in identifying disease processes in the marrow that are focal or that produce changes in the bone, blood vessels and other components of the marrow stroma.

In some disorders, the pattern of infiltration provides additional prognostic information. BMT specimens have a major role in diagnoses where immunohistochemistry is required and where antigen expression has to be evaluated in a spatial context. They are also invaluable in cases of ódry tapô, where examination of an aspirate has been unsuccessful owing to a fibrotic or an infiltrative process.

The BMT specimen is preferably taken from the posterior iliac crest and should be a minimum of 1.6 cm (ideally at least 2 cm) in length with multiple sections taken from various levels.[17ï19] Bilateral trephine samples have been recommended previously[20,21] but, as long as a single sample of sufficient length and quality has been obtained, and multiple sections are examined, there is little additional benefit in carrying out two painful procedures.[20]

[Level of evidence – D].

The exception to this is where staging bone marrow biopsies are performed in the context of childhood solid tumours. In this setting, the procedure is usually carried out under general anaesthesia, and samples may be collected from both anterior and posterior iliac crests on one side or from right and left (usually posterior) iliac crests. Metastases from these tumours can be extremely focal and there is evidence that sampling from two sites improves the detection rate.[22]

[*Level of evidence – D].*

The sample is collected either in 5% formalin or in aceto-zinc formalin (AZF) fixative, as these fixatives allow the use of most staining and molecular techniques.[23,24] Specimens collected into formalin are then delivered to the laboratory and transferred into decalcifying solution, the latter ideally taking place after 8ï 24 hours. AZF-fixed specimens are processed further following fixation for 20ï 24 hours.

Preparation after fixation will depend on the processing methods used in the laboratory. There are advocates for both paraffin and plastic (resin) embedding techniques, with advantages and disadvantages to each; some laboratories use a combination of the two.[25,26] The use of paraffin embedding after decalcification alone is cheaper and is most suitable for DNA extraction,[27] but plastic embedding avoids the requirement for decalcification, gives improved morphology and can now be used for immunohistochemistry with a wide variety of antibodies as well as for some DNA-based tests.[28–30]

Where decalcification is used, there must be a defined decalcification protocol and standard operating procedure. The most common methods employed are based on exposure to weak organic acid solutions (such as 5–10% formic acid) and are relatively rapid (12–24 hours). AZF-fixed tissues are decalcified in Gooding and Stewartôs decalcification fluid (10% formic acid and 5% formaldehyde) for about 6 hours before being processed and embedded in paraffin with procedures similar to other specimens.

Decalcification by calcium chelation using EDTA is a practical alternative, although generally slower (24–48 hours); the time required can be reduced by agitation and warming (up to 40°C). Chelation has the advantage of superior nucleic acid preservation in addition to good morphological and antigenic preservation. Inorganic acids such as nitric or sulphuric acid (as used with some large bone specimens) are avoided if at all possible, although their use may occasionally be justified if there is extreme urgency (4–8 hours). Inorganic acid decalcification results in marked impairment of haemopoietic tissue morphology and immunohistochemistry and leads to nucleic acid denaturation. Although usually employing organic acids, proprietary solutions for combined fixation and decalcification should also be avoided. They offer increased speed but the exposure of tissue to acid before proteins within it have been fixed by the aldehyde components has similar harmful effects to those of inorganic acid exposure.

In all cases, adequate clinical information is essential to assess any biological hazard associated with the specimen and plan the investigations required for diagnosis. Diagnostic material may be lost if clinical information is only made available at the MDT meeting. Apart from presenting features, the information provided should include a summary of the haematological status of the patient, including their full blood count, and results of any preceding investigations such as peripheral blood immunophenotyping by flow cytometry. If the biopsy is undertaken for post-treatment assessment of disease, it is important that details of the original diagnosis are supplied on the request form and that important details of the therapy used, including the timing of the biopsy relative to the most recent treatment, are included.

Consent issues are similar to those for lymph nodes and can be found in section 2.

4.3 Specimen embedding and sectioning

In all cases of BMT biopsy, the complete specimen must be embedded. No tissue is left in the container. Accompanying clotted material often includes useful amounts of fragmented marrow (equivalent to the particles in aspirated marrow specimens) and, therefore, this is processed too. In many laboratories, these fragments can be retrieved separately from the trephine core and processed without decalcification, since they usually do not contain any bone.

Bilateral or paired anterior and posterior marrow cores are identified and processed separately.

A variety of embedding and sectioning strategies are available for BMT specimens and there is no one recommended technique. Whatever methods are employed, the aims are to ensure that the sections examined are representative of the tissue specimen, sufficient material is examined to detect focal pathology, the cellular morphology of the haemopoietic tissue

allows diagnostic interpretation, and immunohistochemical and tinctorial staining, supplemented in some instances with molecular genetic tests (FISH and PCR), can be carried out if required.

When focal pathology is suspected (e.g. granulomas or metastases), it is helpful if sections are prepared and examined from multiple levels throughout the trephine core.[17, 19,31]

Many laboratories cut sections at 2–3 μm thickness and examine three levels, each separated by 50 μm. Sections from the first level are cut at approximately one-third of the distance between ôfirst faceô (the first point in the block at which the microtome blade touches the tissue edge) and the anticipated equator of the cylindrical core. Three to five (more in some laboratories where routine immunostaining is anticipated) spare sections are cut at each level and those from level 2 or level 3 used routinely for additional stains (see below).

An alternative method is to cut paraffin sections at a notional 1 μm thickness and to ensure that the full face of the block is represented on the slide.[24]

[Level of evidence – D].

Fixation in AZF and decalcification using Gooding and Stewartôs fluid enables slides to be cut in this manner; the morphology of haematopoietic tissue in such sections is similar to those obtained following plastic embedding.

Sections required for immunohistochemistry are cut at the time of initial sectioning, to avoid wasteful re-trimming of the block, and are placed onto glass slides coated with poly-L-lysine or APES, or with commercially prepared negatively charged surfaces.

In departments employing plastic embedding of BMT specimens, sections can generally be cut at 1–2 μm. Spares for immunohistochemistry are again best cut at the same time as initial sectioning, to avoid difficulties that may arise from further hardening of the plastic over time. The techniques used must be detailed in an appropriate standard operating procedure document.

4.4 Staining

BMT sections are stained with H&E and with a reticulin stain. A Giemsa stain is also very helpful[32] and routine Perlsô staining is used in some laboratories. However, it is not usually necessary or desirable to stain sections routinely with Perlsô stain for assessment of iron stores, since this is inaccurate in decalcified trephine specimens and should be undertaken using air-dried marrow aspirate films. If Giemsa staining is employed routinely, this can provide excellent staining of haemosiderin to demonstrate excess deposition (e.g. in inflammatory myelopathies, post-transfusional iron overload and in anaemia of chronic disease). Ziehl-Neelsen staining (including a modified method for atypical mycobacteria) is carried out routinely on bone marrows from patients with HIV infection. Any of these tinctorial methods may require minor modifications in individual laboratories to suit samples decalcified by different methods and for use with plastic-embedded sections.

4.5 Further investigations

Immunostaining is frequently required for assessing BMT specimens. All immunostains are requested as panels of antibodies rather than individual tests so that appropriate comparisons can be made. When selecting panels for immunohistochemistry, it is important to include antibodies that are expected to give negative as well as positive results. Most leukaemias and lymphomas are substantially defined by their immunoprofile, usually determined primarily in marrow aspirates or lymph node specimens. Where morphology and immunophenotype are discrepant, or where the findings from trephine biopsy sections do not agree with those of the marrow aspirate or lymph node results, further investigations may be

required to clarify the pathology.[2] The final report must highlight such discrepancies and suggest an explanation for any unexpected or conflicting findings.

In some laboratories, light chain expression is assessed by use of ISH techniques to detect kappa and lambda mRNA rather than immunohistochemistry to detect the proteins. PCR techniques can be used for clonality studies or to look for specific translocations; FISH techniques, however, are currently more sensitive for the latter.

For retention of specimens, see section 2.5.5.

4.6 Report content and MDT meetings

The report should include comment on the adequacy and integrity of the specimen, noting any significant artefacts caused by compression or shear injury during collection. There should be an overall assessment of marrow cellularity[32] and a comment on bone trabecular architecture. Any abnormal infiltrate is identified; the cellular morphology and pattern of infiltration are described. The pattern of infiltration (interstitial, paratrabecular, nodular or diffuse) may be of diagnostic or prognostic relevance.[33] Reticulin content is described, using a systematic grading scheme such as that espoused by the WHO,[34] and the grading system used must be identified. The report should summarise the results of any additional stains and all the immunohistochemical findings. Where a lymphoproliferative condition is identified, the disease is classified using the 2016 WHO terminology (see Appendix A).[1] Bone marrow specimens may also be reported using a synoptic report template.[35]

The report takes into account the patientôs haematological indices, bone marrow aspirate findings and the results of flow cytometric immunophenotyping and cytogenetic studies. An ideal report integrates all of these results, preferably after consensus discussion by the various individuals likely to have contributed the separate pieces of data.[36]

[Level of evidence – D].

Bone marrow pathology must be interpreted in the context of the MDT and the MDT meeting has a crucial role. Data generated from all modes of investigation are collated and interpreted in a clinical context. The reporting pathologist or haematologist remains responsible for the final diagnosis and for ensuring that appropriate additional investigations are pursued to resolve any difficulties in interpretation. An individualôs experience of all the different analytical methods (additional tinctorial stains, immunohistochemistry, FISH, PCR, etc.) is essential in weighing up the contribution that each investigation makes to the final diagnosis and assessing the significance of any apparently discrepant findings. All diagnoses of haematological malignancy are discussed by the MDT and incorporated into an integrated report. There should be an equivalent mechanism for multidisciplinary discussion and integration of non-malignant conditions. Details of the final agreed diagnosis and clinical management decisions are recorded at the MDT (or equivalent) meetings.

5 Criteria for audit

5.1 Staffing and workload

The following are required: annual review of numbers and types of specimens reported by each pathologist; EQA scheme compliance; and RCPath CPD compliance.

5.2 Report content

Auditing of the completeness of the data in the histopathology report. This would include the adequacy of the specimen, the presence of a WHO-defined diagnosis for haematological malignancies (or a reason why one could not be assigned), references to previous biopsy

specimens where appropriate (to track disease progression), the presence of SNOMED codes or equivalent (the RCPath key performance indicator [KPI] requires that 100% of cases have SNOMED or SNOMED-CT T, M and P codes) and details of the authorising pathologist(s).

5.3 Timeliness of report

Since bone marrow specimens need decalcification, and the majority of haematopathology specimens require immunohistochemical analysis, it is difficult to provide uniform guidance regarding the timeliness of the reports. The need for molecular investigation (FISH and PCR) in a subset of cases further complicates the issue.

The most recent RCPath dataset for the histopathological reporting of lymphomas recognises this, and sets out a staged timeline for the reporting of these specimens.[2] For lymph nodes, this is as follows (days are working days):

Day 1: Specimen received at cellular pathology department. Macroscopic description completed. Endoscopic and needle core biopsy specimens processed overnight; other specimens processed as per department protocols.

Day 2: H&E-stained section(s) from endoscopic and needle core biopsies are examined and decisions made regarding further investigations (immunohistochemistry, special stains, etc). A preliminary report may be available at this stage for some cases.

Day 3: H&E-stained section(s) from lymph node excisions and other incisional biopsy specimens are examined. A provisional report may be available and further investigations ordered as appropriate.

Day 4ï 5: Immunostains should be available for assessment. The definitive report will be available for uncomplicated cases but other, more complex, cases may require further immunohistochemistry and/or molecular investigations.

For BMT biopsy specimens, this timeline will be extended by at least an additional day depending upon local decalcification methods.

As well as the above timeline, the RCPath KPIs suggest the following overall end-to-end turnaround times (see https://www.rcpath.org/resourceLibrary/key-performance-indicators-in-pathology---recommendations-from-the-royal-college-of-pathologists-.html):

- histopathology diagnostic biopsy specimen turnaround times:
  - 90% of biopsy specimens reported within seven calendar days of the biopsy being undertaken. Diagnostic haematopathology biopsy specimens will include needle biopsy cores, endoscopic and punch biopsy specimens.
- overall histopathology reporting turnaround times:
  - 90% of all histopathology cases should be reported within ten calendar days of the specimen being taken. Molecular tests are excluded from this indicator.

For audit purposes, the lymphoma histology reporting dataset timelines can be used to monitor the different stages of the reporting process. It is also desirable that haematopathology turnaround times should be in line with those recommended for other specimen types, so the expectation should be that at least 90% of cases are reported within ten calendar days, complying with the RCPath KPI for overall reporting turnaround time for histopathology. With the routine requirement for immunohistochemical and other stains in haematopathology, and formalised networking arrangements with regional SIHMDS,

definitive reporting within a window of seven calendar days is often impossible with current facilities.

In cases where results are required more rapidly to support urgent decisions for patient management, a provisional report can be issued on the basis of the H&E-stained section(s), and key information may be transmitted by telephone or email. When this takes place, the content and time/date of the provisional report should be recorded in the final report.

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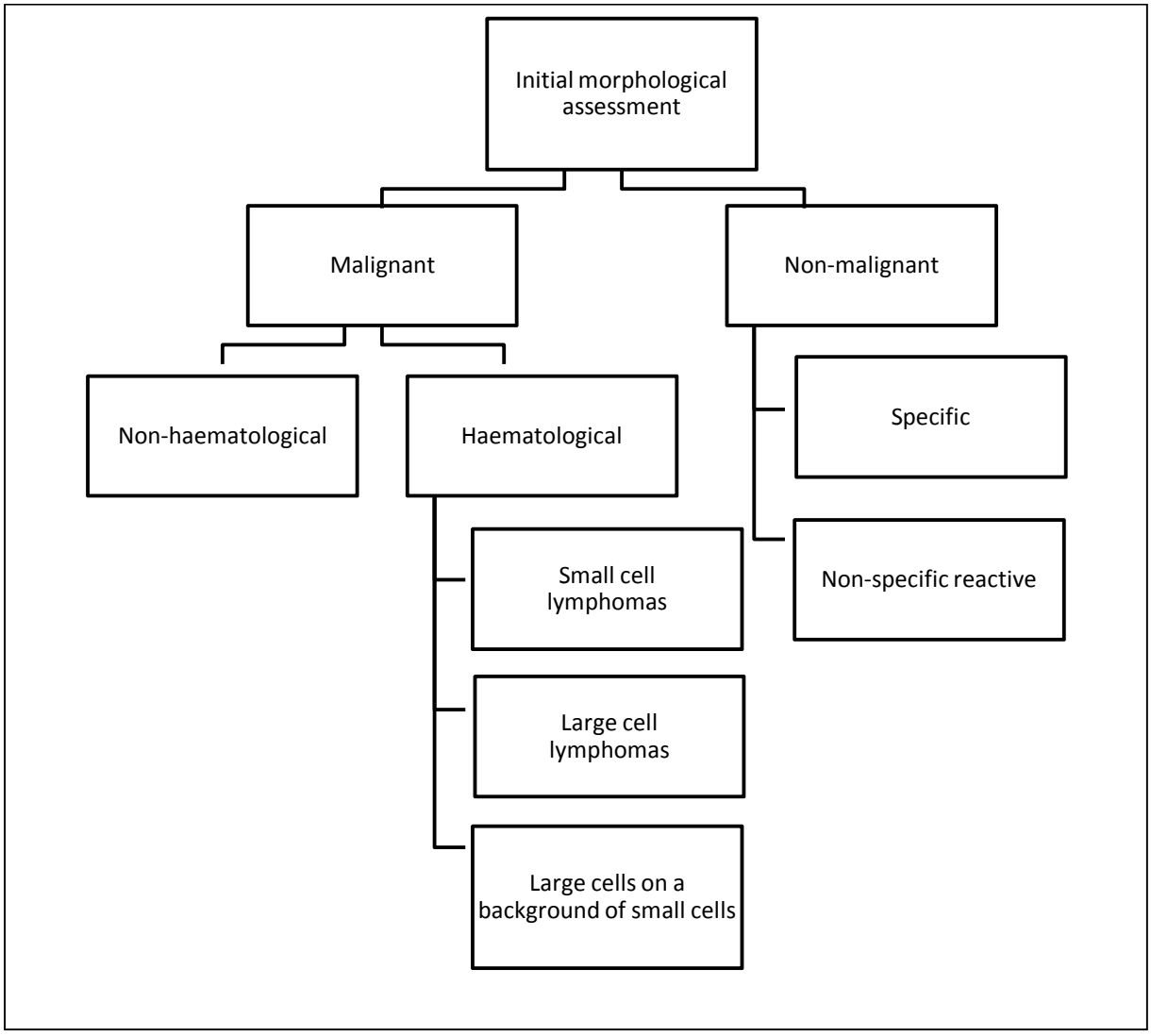
Appendix A Suggested diagnostic approach to lymph nodes

A1 Introduction

This document is designed to provide a simplified algorithmic approach to the histopathological diagnosis of lymph node specimens. This is an outline only, and is not intended to cover every situation or to be prescriptive about the immunohistochemistry and other investigative techniques that laboratories use in their routine practice. It is not practical or appropriate to separate the concepts underpinning the diagnosis of neoplastic and non-neoplastic lymph nodes; consequently, there is considerable reference to neoplastic pathology in what follows.

A2 Initial work-up

Morphological assessment is carried out on H&E-stained sections, and the pathology present is categorised in the first instance as either neoplastic or non-neoplastic.



A3 Non-neoplastic

Non-neoplastic cases are may be subdivided into those with specific pathological features (granulomas, acute inflammation, dermatopathic lymphadenopathy, Castleman's disease, etc) and those showing non-specific reactive changes: follicular hyperplasia, paracortical hyperplasia and sinus histiocytosis. Additional stains such as Ziehl-Neelsen, Grocott or Giemsa may be required in these cases.

A4 Neoplastic

Lymph nodes in which there is a neoplastic process are then separated morphologically into those in which the lesion appears to be lymphoma or other haematological malignancy, carcinoma, sarcoma, melanoma or germ cell tumour. In difficult and undifferentiated cases, an initial immunohistochemistry panel may be used to distinguish these broad neoplastic categories. The panel would contain a selection of antibodies that includes leucocyte common antigen (CD45), cytokeratins (e.g. AE1/AE3) and markers of melanoma (S100, HMB45, melan-A), sarcoma (desmin, myogenin, myoD1, etc.) and germ cell tumour (PLAP, OCT3/4, D2-40).

A5 Lymphoma suspected

In cases in which the morphology and clinical history support a diagnosis of lymphoma, the lesion is subcategorised into one of several broad diagnostic groups based on the cellular populations present. These groups include lesions composed predominantly of small mature cells, lesions composed predominantly of large pleomorphic cells and lesions in which there are large atypical cells in a background of smaller reactive cells. Nodularity and follicularity are also assessed at this stage.

The next stage is to identify the lineage of the neoplastic cells, categorise the lesion using the WHO 2016 classification (where possible) and to check for the presence or absence of prognostic biomarkers. Immunohistochemistry is critical at this stage and utilises lineage markers for B cells, T cells, myeloid cells, NK cells, histiocytes and plasma cells.

A6 Small cell lymphomas

The vast majority of small cell lymphomas seen in lymph nodes are of B-cell lineage. Small B-cell lymphomas are divided into CD5-positive and CD5-negative categories; the former contains mantle cell lymphoma (MCL) and chronic lymphocytic leukaemia (CLL)/small lymphocytic lymphoma and the latter contains follicular lymphoma (FL), marginal zone lymphoma (MZL) and lymphoplasmacytic lymphoma (LPL). Germinal centre cell markers, markers of follicular dendritic cells, bcl-2 staining, markers of plasmacytic differentiation and light chain staining are all used here. Rare small cell lymphomas of T-cell lineage are assessed using a full panel of T-cell lineage markers (CD3, CD2, CD5 and CD7) as well as subset markers (CD4, CD8 and antibodies reactive with T follicular helper cells). FISH can be used to detect *BCL2*, *BCL6, cMYC* and *CCND1* translocations and PCR can confirm clonal IGH or TCR rearrangements in difficult cases.

A7 Large cell lymphomas

Large cell lymphomas are categorised using B-cell and T-cell lineage markers, CD30, germinal centre markers, bcl-2, cytotoxic markers, ALK1, EMA, CD23 and MIB1. Potential prognostic information is provided with CD10, bcl-6, MUM1/IRF4, bcl-2 and MIB1/Ki67. FISH for *BCL2, BCL6* and *cMYC* translocations can also contribute and PCR can confirm clonality in poorly differentiated lesions. In rare cases with a histiocytic lineage, additional staining with macrophage markers such CD68/CD68R, CD11c, CD163 and lysozyme may be required.

A8 Lymphomas containing large cells in a small cell background

Lymphomas composed of large cells in a background of small cells are assessed in a similar manner to large cell lesions, but the immunohistochemistry panels used must include markers that will identify both classical and nodular lymphocyte predominant Hodgkin's lymphoma. Antibodies used in this setting are CD30, CD15, CD45, OCT2, BOB1, EMA and PAX5. ISH to demonstrate EBER should be included. T-cell/histiocyte-rich large B-cell lymphoma also enters into the differential diagnosis.

Appendix B Suggested diagnostic approach to bone marrows

B1 Introduction

This appendix is designed to provide a simplified guide to the pathological investigation of haematological malignancy in BMT biopsy specimens. The complexity and heterogeneity of the pathology seen in this setting means that it is not possible or desirable to be prescriptive about the exact investigations that should or should not be performed in a given clinical setting. It is implicit throughout that the histopathological findings need to be fully integrated with the wide range of other haematological investigations, including clinical features, peripheral blood picture, the aspirate morphology, flow cytometry and cytogenetics. As for lymph nodes, the diagnostic approach for non-neoplastic bone marrow conditions cannot be separated from that appropriate for neoplasms; hence, the following comments regarding neoplasia.

B2 Acute myeloid leukaemia (AML)

Suspected AML (new diagnosis)

A new diagnosis of AML is made primarily by haematological techniques. Clinical and morphological features (in the aspirate) are combined with flow cytometry, cytogenetics, FISH and molecular testing. The main priority is to rule out acute promyelocytic leukaemia. This is followed by lineage assignment based on immunophenotypic and cytochemical characteristics. The role of the BMT biopsy is to confirm disease burden using H&E and markers of mature and immature myeloid cells (typically MPO, CD117 and CD34).

Known AML – suspected relapse or treatment response

H&E morphology and CD34, CD117 and MPO, as above.

B3 Precursor lymphoid leukaemias

Suspected new diagnosis

Immunophenotyping on flow cytometry is required to assign a lineage: either B-lymphoblastic leukaemia (B-LL) or T-lymphoblastic leukaemia (T-LL). Cytogenetics and FISH are used to identify specific recurrent genetic abnormalities and molecular techniques are used for minimal residual disease monitoring. The role of the BMT biopsy is to confirm the lymphoid lineage with T-cell (CD2, CD3, CD5, CD7) and B-cell markers (CD19, CD22, CD20, CD79a, PAX5), to assess disease burden and to determine other diagnostic information (CD34, TdT, CD1a).

Known precursor lymphoid leukaemia – treatment response (B/T-LL) or relapse

H&E morphology and immunohistochemistry targeted to the phenotype of the initial infiltrate (TdT, CD34 and appropriate B- or T-lineage markers).

Mature lymphoproliferative disorders

The basis of the initial investigation of mature lymphoproliferative disorders is aspirate morphology and flow cytometry performed on the aspirate or peripheral blood. Based on these initial assessments, the following diagnostic subgroups should be considered.

CD5 positive mature B-lymphoproliferative disorder

The main aim here is to differentiate between CLL and MCL. The trephine specimen should be assessed with H&E and also stained for CD20, CD3, CD5, CD23 and cyclin D1. SOX11 and an alternative B-cell marker (CD79a, PAX5) can be added.

CD5 negative mature B-lymphoproliferative disorder

This category includes FL, MZL, LPL/Waldenstrom's macroglobulinemia and hairy cell leukaemia (HCL). Where LPL is suspected MYD88 molecular testing is recommended. The trephine specimen is assessed morphologically and stained for CD20, CD3, CD138, CD10, bcl-6, IgM and light chains. Note that when FL involves the bone marrow, the germinal centre cell markers (CD10 and bcl-6) are frequently downregulated. If the morphology and flow cytometry are consistent with a diagnosis of HCL, then the suggested panel is CD20, CD3, DBA44, CD25 and CD11c.

T-cell lymphoma

If flow cytometry demonstrates marrow involvement, then the immunohistochemistry performed will depend upon the flow findings and any index histology. If bone marrow is the primary diagnostic specimen, then a full work-up would be performed as with a lymph node specimen. The immunohistochemistry panel for T-cell neoplasms can include CD2, CD3, CD4, CD5, CD7, CD8, CD30, ALK1, cytotoxic markers and T follicular helper markers (for angioimmunoblastic T-cell lymphoma).

If bone marrow is not the primary site of disease, immunostaining should be limited and should confirm the flow cytometry findings. When there is no morphological evidence of involvement from H&E staining then a simple staging panel (CD3, CD20 and CD79a) can be used.

Large cell lymphoma and Burkitt lymphoma

If bone marrow is the primary diagnostic specimen, the work-up should be as for a lymph node specimen (the diffuse large B-cell lymphoma panel). If there is known disease elsewhere, a staging panel can be used (CD20, CD79a, CD3).

Classical Hodgkin's lymphoma

The bone marrow is rarely the site of primary diagnosis in classical Hodgkin's lymphoma; bone marrow aspirate and BMT biopsy are usually only performed for staging purposes. Immunophenotyping is not routinely required unless there is suspected involvement from H&E-stained sections, in which case CD30 staining is used.

Myelodysplasia and bone marrow failure syndromes

This category includes suspected or known myelodysplasia and aplastic anaemia. The morphological features of dysplasia are primarily assessed in aspirated marrow and peripheral blood. Megakaryocyte dysplasia may be assessed from both aspirate cytology and trephine histology. Reticulin staining can be helpful in this setting and immunohistochemistry can be used to assess the blast cell content. A routine panel used for assessing the haemopoietic compartment is typically CD34, CD117, MPO, CD71 (or glycophorin A/C) and CD61 (or CD42b).

Myeloproliferative neoplasms

Subclassification of the myeloproliferative disorders is predominantly based on peripheral blood counts (including historical blood counts), peripheral blood film appearances, presence of splenomegaly, clinical symptoms and molecular analysis. For example, the diagnosis of chronic myeloid leukaemia depends upon the molecular identification of the BCR-ABL1

translocation. The JAK2-V617F mutation is almost universally found in polycythaemia vera and is also common in primary myelofibrosis and essential thrombocythaemia. Other mutations involving CALR and MPL may also be informative in JAK2-V617F-negative cases. As with myelodysplasia, the haemopoietic compartment is assessed using CD34, CD117, MPO, CD71 and CD61 stains, or similar, in addition to H&E. Although the role of the trephine biopsy may be limited in the myeloproliferative setting, reticulin staining and systematic grading are essential to assess and monitor the disease, and documentation of the blast cell content with CD34 and CD117 is also important in these conditions.

B4 Plasma cell neoplasms

Suspected new plasma cell myeloma

Investigation of a suspected plasma cell neoplasm includes a combination of clinical and imaging findings, full blood count, serum paraprotein, light chain levels and immunofixation electrophoresis. The plasma cell content of aspirated bone marrow is assessed; karyotyping and FISH studies are also usually carried out. Where available, highly sensitive flow cytometry immunophenotyping with a specific myeloma antibody panel is conducted on CD138+ selected cells. The BMT biopsy specimen provides a better estimate of disease bulk than the aspirate. Morphological assessment is combined with immunohistochemistry for CD138 or IRF4/MUM1 (to detect plasma cells), CD20, CD56, CD117 and cyclin D1 (markers frequently expressed aberrantly by neoplastic plasma cells), and light chain staining. Loss of CD79a and/or EMA (expressed by normal plasma cells) provide additional indicators of a neoplastic immunophenotype. Heavy chain expression is generally best determined in peripheral blood and is not required in trephine assessment; it can be confirmed by immunohistochemistry, if needed. Light chain restriction in plasma cell populations is more sensitively determined by mRNA ISH than immunostaining.

Monoclonal gammopathy of uncertain significance (MGUS)

MGUS is defined as <10% plasma cells in the aspirate, a paraprotein level of <30 g/L and no evidence of end-organ damage. In this setting, the priority is to exclude a non-plasma cell chronic lymphoproliferative disorder and to confirm the only minimal extent of any plasmacytosis. H&E morphology is important and the immunohistochemical approach is essentially similar to that employed for suspected myeloma, plus additional B-cell immunophenotyping if lymphoid aggregates are present to suggest an alternative diagnosis.

POEMS and solitary plasmacytomas

In these conditions, there is often no excess of plasma cells in the marrow (i.e. <5% involvement). However, more sensitive methods such as flow cytometry immunophenotyping can detect the presence of clonal plasma cells with aberrant and abnormal cell surface markers. This may have prognostic and treatment implications. If there is minimal morphological involvement in H&E and sections immunostained for CD138 or IRF4/MUM1 (<5% plasma cells) with no clearly identifiable pathological plasma cell population and no abnormal plasma cells detected by flow cytometry and detection of this level of disease in the trephine specimen is considered of clinical relevance, then additional immunostains as above, plus immunostaining or ISH for kappa and lambda light chains, can be added.

Known plasma cell myeloma (restaging and relapse)

Restaging is usually performed after completion of treatment, before stem cell transplant or anticipating trial enrolment. Bone marrow investigations should be interpreted in conjunction with biochemical investigations such as immunofixation and paraprotein estimation. Relapsed plasma cell myeloma is detected by biochemical markers in the vast majority of patients. Bone marrow sampling is carried out to allow assessment of the disease extent and to investigate the evolution of cytogenetic abnormalities.

The aspirate is assessed morphologically for both restaging and relapsed disease. At first relapse FISH is carried out on CD138+ selected cells to detect 17p and 1p deletion and trisomy 1q. Trephine histology is assessed using H&E and CD138 or IRF4/MUM1 staining to confirm residual or relapsed disease. Where there is minimal morphological involvement (<5% plasma cells) with no clearly identifiable abnormal plasma cell population, further investigations (CD56, CD117, cyclin D1, kappa and lambda light chains) are only required when detection of this level of disease is considered to be clinically relevant.

Investigation of eosinophilia/mastocytosis

The differential diagnosis for underlying causes of eosinophilia is broad and depending on the selection criteria for bone marrow assessment, the yield of investigations can be low.

The main aims of testing are to:

- exclude T-cell lymphoma
- detect cytogenetic abnormalities that are amenable to treatment (e.g. FIP1L1-PDGFRA)
- exclude CML
- identify blast excess
- exclude mast cell disease.

Clinical history and additional tests are central to directing this investigation. The investigation and exclusion of mast cell disease overlaps with that of eosinophilia investigation. The aspirate is assessed using morphology, immunophenotyping, cytogenetics (conventional and FISH studies) and molecular testing. The latter is variable in extent, but can include JAK2 and CALR mutation studies, BCR-ABL1 RT-PCR, TCR gene rearrangement studies, FIP1L1-PDGFRA RT-PCR and demonstration of c-KIT D816V (in all cases of suspected mast cell disease). Trephine histology is assessed using H&E and reticulin staining, together with immunohistochemistry for CD117 and mast cell tryptase (which detect neoplastic and non-neoplastic mast cells) and CD25 and CD2 (which can selectively identify neoplastic mast cells).

Investigation of cytopenias

Chronicity of cytopenias and the morphological features seen in peripheral blood and aspirated bone marrow will direct investigation within this category. Patients with morphological dysplasia are likely ultimately, on consideration of all information at MDT discussion, to be appropriately diagnosed with a myelodysplastic syndrome. However, a variety of constitutional, inflammatory, immune and toxic conditions may produce cytopenias. It is often important to exclude an underlying neoplastic cause such as metastasis or lymphoma, which may be subtle and require immunohistochemical demonstration. When the cause of cytopenias(s) cannot clearly be identified, the BMT specimen should be examined with H&E and reticulin stains, supplemented by immunohistochemistry to assess the haemopoietic components (e.g. MPO, CD61, CD71, CD117, CD34) and any lymphoid components (CD3, CD20, CD79a). Including an anti-cytokeratin antibody provides a valuable screen to exclude subtle metastatic carcinoma in selected cases where there might be clinical suspicion from the patient's history or symptoms.

Appendix C Suggested diagnostic approach to spleens

1. Interpretation of splenic histology is much easier if you are confident that you know the range of appearances of normal and non-specifically reactive spleens. You may find it helpful to keep a representative H&E-stained section from a suitable, well-fixed, reactive case as an *aide memoire* (e.g. from an incidental splenectomy performed for access during renal or gastrointestinal surgery).

2. Reactive white pulp changes may resemble those of lymph nodes, with or without marginal zone expansion. However, regressing immunological reactions are seen more frequently in the spleen, characterised by hyalinosis within germinal centres. Germinal centres may show fibrinoid necrosis in fulminant septicaemia, particularly in neonates and young children. Florid marginal zone expansion may occur in the absence of reactive germinal centres. Make sure you are familiar with the normal cytological mixture within marginal zones; this will assist in recognising marginal zone differentiation in lymphomas. Reactive T-cell hyperplasia resembles dermatopathic change in lymph nodes; look for these appearances in the peri-arteriolar lymphoid sheaths (PALS).

3. Red pulp reactive changes predominantly involve sinusoidal lumina (intraluminal haemophagocytosis), sinusoidal endothelium (cuboidal/hobnail appearances), cordal macrophages (widening of cords with or without plump storage cells/foam cells; sequestration of red blood cells and platelets), capillaries (peri-capillary plasma cell clusters) and peri-follicular zones (accumulation of red blood cells and/or neutrophils).

4. Do not expect to find normal histology in spleens removed following trauma or incidentally during other surgery; they are often highly reactive.

5. Do not expect spleens in autoimmune conditions (e.g. autoimmune haemolytic anaemia, immune thrombocytopenic purpura) necessarily to look reactive. They may do, but patients have often been heavily treated with steroids, in which case the splenic white pulp may be atrophic. Do not forget that low-grade lymphoma may underlie these conditions.

6. In suspected lymphoma, marshal as much additional information as possible before even looking at the sections. If peripheral blood, bone marrow or lymph node findings are available (particularly immunophenotyping and cytogenetics) make use of them.

7. In suspected lymphomas, examine any hilar lymph nodes first; they will often be more familiar and hence easier to interpret than the spleen itself. However, remember that splenic MZL is rather nondescript in lymph nodes ï it rarely shows a marginal zone distribution at extrasplenic sites.

8. Do not expect to be able to diagnose splenic lymphomas of any type without immunostains. Morphological mimicry occurs between types and you can get caught out; immunophenotyping is highly discriminatory.

9. Extramedullary haemopoiesis (EMH) involves the red pulp. Scattered single megakaryocytes (often óbareô end-stage forms) are a normal component of red pulp and do not, on their own, indicate EMH. Any reactive or enlarged spleen may contain foci of óincidentalô EMH, of no pathological significance; these consist of clusters of maturing nucleated erythroid cells, usually within sinusoidal lumina. óSignificantô EMH (splenic involvement by acute or chronic myeloid neoplasia) is characterised by the presence of immature granulocytes and monocytes in the red pulp, often most concentrated around edges of PALS. Megakaryocytes in significant EMH are often clustered, hyperchromatic and clearly atypical in morphology (seen in chronic myeloid neoplasms; not usually present in acute leukaemic infiltrates).

10. Learn to recognise curiosities like Gamna-Gandy bodies, capsular sugar-icing, oleogranulomas and angiomas, to ensure you do not mistake them for more significant lesions.

    [Level of evidence – GPP.]

Appendix D 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.

Appendix E AGREE compliance monitoring sheet

The tissue pathways of The Royal College of Pathologists comply with the AGREE II standards for good quality clinical guidelines (www.agreetrust.org). 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	Foreword, 1
2 The health question(s) covered by the guideline is (are)specifically described	1
3 The population (patients, public, etc.) to whom the guideline is meant to apply is specifically described	Foreword,1
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	1
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	Throughout
12 There is an explicit link between the recommendations and the supporting evidence	2ï 4
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	Throughout
16 The different options for management of the condition or health issue are clearly presented	Throughout
17 Key recommendations are easily identifiable	2ï 4
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	Appendices Aï C
20 The potential resource implications of applying the recommendations have been considered	Foreword
21 The guideline presents monitoring and/or auditing criteria	5
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