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

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SECTION A TISSUE PATHWAY: LYMPH NODE SPECIMENS FOR SUSPECTED HAEMATOLOGICAL MALIGNANCY

1 STAFFING AND WORKLOAD

Ideally, two or more pathologists in a unit should be competent in reporting haematopathological biopsies, in order to provide cover for periods of leave.[1] It is recognised that in some smaller units only one pathologist may have specialist expertise; such individuals should develop networking links with designated haematopathologists in other local hospitals for support. Where haematopathology specimens are reported in a general hospital setting (or in a non-haematopathological specialist unit), it is recommended that the pathologists liaise with a specialist regional centre for additional expert assistance.[1] Competency 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 referred to the designated haematopathologist(s) for detailed work-up and diagnosis. Specimens that are initially examined by a non-designated pathologist should normally subsequently be quality assured by a designated haematopathologist.

Pathologists designated as haematopathology leads should attend specialist haemato-oncology multidisciplinary team (MDT) meetings at their local centre, participate in appropriate EQA assessment and undertake CPD directed to haemato-oncology.

2 SPECIMEN SUBMISSION

In all cases, adequate clinical information is essential to assess the risk of the specimen and plan the investigations. Diagnostic material may not be used efficiently and appropriately if gathering of clinical information is left until the MDT meeting. Apart from the presenting features, the 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 allo-/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 performing the biopsy. The diagnosis and sub-typing of lymphoma is an integrated, multidisciplinary process often involving histopathological and molecular techniques.[2–4]

Consent for all laboratory investigations essential for diagnosis, including DNA analysis, should be part of the initial consultation with the patient. This is generally in the form of an investigation to find what is wrong, rather than a specific list of tests that are to be performed. However, the patient may choose to limit the investigations; for example, excluding HIV serology. It is the responsibility of the

clinician to ensure that patient consent is appropriately documented. Where investigations lead to a diagnosis not initially expected, consideration should be given to obtaining additional consent from the patient.[5] Specific separate consent is required where frozen tissue is banked as a future research resource.

Lymph nodes may be received as complete nodes or as needle core biopsy specimens. Cytological procedures are an important part of the investigative pathway of patients who are also subject to biopsy or resection of tissue. The laboratory handling of cytological specimens in this context is covered in a separate document:
(www.rcpath.org/resources/pdf/G036-ConsentForProcessingAndAnalysisOfClinSamples-Jan05.pdf).
Either fresh and/or fixed material can be submitted, depending upon local arrangements and/or 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 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 their 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. Where 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 fluorescence in-situ hybridization (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 is suspected, most laboratories would expect the node to be fixed for 24–48 hours prior to processing.

With all specimens, the priority is for fixation in formalin to allow paraffin or plastic embedded sections for morphological and immunophenotypical analysis. In small samples, such as needle core biopsies, there may be insufficient material for any additional investigations. Where a whole fresh lymph node with sufficient volume of tissue is received, the sample is 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.

3 SPECIMEN DISSECTION, BLOCK SELECTION, EMBEDDING AND SECTIONING

The overall aim is to obtain ‘ideal’ material for reaching a diagnosis on the lymph node. This will require 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.

a) Unless the node is very large (over 3 cm in diameter), the specimen should be processed entirely. Since nodal involvement by lymphoma may be focal (e.g. nodular sclerosing Hodgkin lymphoma), for most lymph node specimens it is advisable that no tissue is left in the container (except see item d) below).

b) Prepare multiple paraffin blocks to avoid depletion of diagnostic material while working-up the case.

c) Although additional tests are important, prompt formalin fixation, routine histology and immunohistochemistry on paraffin sections are the most important for diagnosis. Obtaining

adequate material for paraffin embedding is the priority; only after this is reasonably fulfilled is material saved for molecular and cytogenetic analysis, or for consented research.

d) Where clinical information or macroscopic examination suggests that the node is likely to contain non-haematopathological disease, such as metastatic carcinoma or granulomatous inflammation, the node may be sampled and the specialist haematopathology investigative techniques omitted.

e) If a frozen section is performed, the results of frozen section 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 unfrozen tissue is kept for routine histopathology; the process of freezing and subsequent thawing for fixation and processing will interfere with both morphological and immunohistochemical analysis.

Protocol for lymph node specimens

a) Where the node is smaller than 1.0 cm in diameter, it is bisected on arrival in the laboratory.

b) Where the node is larger than 1.0 cm in diameter, it is cut into 2 mm thick slices in a plane perpendicular to the long axis of the node.

c) Record the size, colour and consistency and presence or absence of any visible nodularity, haemorrhage, or necrosis on the cut slices.

d) If the node is fresh, make up to five imprints from the lymph node and preserve for FISH analysis where available. The imprints are initially air-dried. The dried imprint can then be fixed in chilled methanol-acetic acid (3:1) for 15–30 minutes and stored at –20ºC in a desiccated chamber.

e) Where appropriate and where there is sufficient fresh tissue, a small piece from one of the slices is sent for cytogenetic studies (see below).

f) If the node is fresh and there is sufficient tissue, snap-freeze ¼ of one of the node halves (for nodes less than 1.0 cm in diameter) or one of the peripheral slices (for nodes over 1.0 cm in diameter) for molecular diagnostics. Freeze the tissue in 2–3 aliquots, each 2–3 mm^3 in size.

g) If macroscopic examination of a larger node leads to the detection of a possible infective focus, a small piece from one of the slices should be sent for microbiological culture.

h) Unless the node is very large, the remaining tissue should be processed into paraffin blocks. With nodes less than 1.0 cm in diameter, each half is put into a separate block. With larger nodes, each block should contain tissue no more than 2 mm thick, and only one piece of tissue placed in each cassette since multiple pieces make immunostaining more difficult. To ensure the slices of lymph node remain flat, incorporate sponges into the cassettes to help prevent folding. Proper tissue fixation is required to ensure good preservation of cytological detail and reliable immunohistochemistry. Different processing techniques will require different fixation schedules and it is important that laboratories optimise their immunohistochemical antigen retrieval methods to suit the local fixation and processing schedules. Prolonged fixation makes immunochemistry unreliable and can impair recovery of DNA from paraffin blocks.[6]

Protocols for needle core biopsy specimens and other 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, it is preferable to place each core in a separate paraffin block.

Protocol regarding sectioning levels and ribbons of tissue

All sections are ideally 3–4 um thick, as this renders the best morphological appearance. In cases of lymph node specimens that are small and appear uninvolved, but where there is a clinical suspicion of malignancy, it is prudent to prepare sections from at least three levels, stain with haematoxylin and eosin (H&E) and examine these sections carefully before interpreting the tissue as negative.

With regard to needle cores, a single section or sections from three levels are cut in the initial instance and stained with H&E before histological interpretation. If levels are cut, at least 10 intervening sections from the ribbon should be prepared, using coated or charged glass slides, and stored unstained. These are invaluable if a small infiltrate is noted in any one level, or unexpected features suspicious of lymphoma are found, requiring subsequent immunohistochemical analysis.

4 STAINING

Lymph node sections are stained using the standard H&E stain. Reticulin staining may be helpful in assessing the follicular architecture.

5 FURTHER INVESTIGATIONS

Additional investigations that may be carried out in suspected haematopathological disease include the following.

a) 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. Where there are discrepancies between the morphology and the immunophenotype these may represent technical failure, an incorrect diagnosis or a genuine aberrant finding. In these circumstances, further investigations are needed to clarify the results.[7] The final report must highlight such discrepancies and should suggest an explanation for abnormal 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 to the correct interpretation and diagnosis.[8,9]

b) Cytogenetics

While a fresh sample of the specimen in tissue culture medium may be sent for cytogenetic analysis, the most cost-effective way to perform genetic analysis in tissue biopsy specimens is by targeted FISH for specific translocations. Tissue imprints should therefore be made from all fresh specimens. Pathologists and geneticists should consult to ensure that these are made in a way that allows most efficient use for FISH. Most cytogenetic laboratories now offer a service for tissue section FISH. Imprints, frozen or fixed tissue sections can be sent for FISH when it is clear that molecular genetic information is required. If tissue samples are processed for karyotyping by G-banding or for metaphase FISH, these are stored and analysis only performed after the morphological assessment of the sample has indicated a need for cytogenetics.

c) Flow cytometry

If the sample is to be analysed promptly a dry, fresh sample is provided to the appropriate laboratory. If this is not possible due to transport times, consideration should be given to disaggregating and fixing the sample prior to transport for analysis.

d) Molecular biology

If required, a sample of fresh tissue is snap-frozen and stored for subsequent investigations such as DNA analysis. polymerase chain reaction (PCR) can usefully detect clonal lymphoid populations and specific translocations, and can be used on fixed tissue. FISH studies can also be carried out on fixed tissue sections (see b) above).

6 SAVING SPECIMENS

No diagnostic material should be discarded until all investigations are complete. The Royal College of Pathologists recommends that paraffin blocks are stored for 30 years. 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. Frozen tissue should be stored at –70ºC or lower for at least 10 years, preferably longer. Storage at –170ºC or lower is needed where viable cells are required, e.g. for tissue culture. Detailed advice is available from The Royal College of Pathologists and the Institute of Biomedical Science.[10]

7 REPORT CONTENT AND MDT MEETINGS

Reports should identify the pathological changes within the lymphoid specimen, whether intact node(s), needle core biopsy or extra-nodal lymphoid tissue. These include preservation or effacement of architecture with an overall assessment of follicles, paracortex, sinuses, capsule and extra-capsular tissue. In extra-nodal lymphoid tissue, the relationship with associated epithelial structures may also be important. The cytomorphology of any abnormal infiltrate is described and categorised as reactive or neoplastic. 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.[11]

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 analytic methods (tinctorial stains, immunohistochemistry, FISH) 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. 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.

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SECTION B TISSUE PATHWAY: SPLEEN SPECIMENS

1 STAFFING AND WORKLOAD

See lymph node tissue pathway (Section A).

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 for possible future nucleic acid studies. This is particularly strongly recommended because of the difficulty in achieving good fixation of spleen tissue, unless delivery of intact, unfixed splenectomy specimens is genuinely unmanageable because of local arrangements and/or facilities. 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 from surgical traumatic artefacts (5–10 cm). Even from small children, fragments of 5 cm size are straightforward for experienced laparoscopic surgeons to obtain as long as the requirement is made clear when the surgery is being planned.

Needle core biopsy of the spleen is relatively rarely undertaken because of the risks of haemorrhage, but 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. 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 lymph node tissue pathway). In all cases, adequate clinical information is essential to assess the risk of the specimen and plan the investigations. Diagnostic material may not be used efficiently and appropriately if the gathering of clinical information is left until the multidisciplinary (MDT) meeting. Apart from the patient's presenting features, the information should include a summary of their haematological status, including full blood cell counts, 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. This information is particularly important for interpretation of splenic pathology, which often reflects haematological disease and has a broader spectrum in this regard than lymph node assessment. 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 to complete a request form with the required information than is the surgeon.

Consent for appropriate investigations is essential (see lymph node tissue pathway).

3 SPECIMEN DISSECTION, BLOCK SELECTION, EMBEDDING AND SECTIONING[1]

On receipt in the laboratory, the spleen is weighed, including any blood clot. It is usually convenient (especially if the spleen is large) 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 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 dispersed from this into culture medium (e.g. RPMI) 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 1–2 cm thick and **separating them completely**. After laying the slices out on the cutting surface, the internal appearances are described (congestion, white pulp prominence, any focal lesions).

The slices are then placed back into the container and immersed in plenty of formalin. Swirling them around gently will rinse as much blood from the tissue as possible and the formalin can then be discarded. Repeat this rinsing once more and then refill the container with fresh formalin. It can be useful to reweigh the spleen slices before leaving the tissue to fix overnight; significant loss of weight compared to that before slicing is indicative of acute congestion and the final weight is more representative of the true spleen mass. As an alternative to fixing all of the spleen slices in a large volume, selected smaller pieces may be taken for further fixation but care should be taken not to slice these too thinly, to avoid tissue distortion.

On the following day, the tissue slices or selected pieces will be firm enough to re-cut at 2–5 mm thickness to encourage further fixation. At this stage, blocks can be taken and placed in cassettes. These will need at least 24 hours further fixation before processing.

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 a routine, from any macroscopically normal spleen, two to four blocks are generally adequate (hilum and centre of convex aspect, +/– superior and inferior poles, may provide convenient landmarks for routine sampling). Extra blocks are taken, as needed, if any focal lesions are present within the parenchyma or if the spleen is significantly enlarged (greater than 500 g). Sample **all** hilar lymph nodes in cases of suspected lymphoma or other neoplasm.

4 STAINING

In addition to haematoxylin and eosin (H&E) stains, one block may be selected (plus one block representing hilar nodes, in suspected lymphoma) for some or all of these additional stains:

PAS: shows cytological detail, vascular structure, stored ceroid

Giemsa: highlights white pulp, plasma cells, and extramedullary haemopoiesis

Reticulin: shows red pulp cord and sinusoid structure, capsule, trabeculae

Perls': shows haemosiderin in chronic haemolysis or iron overload.

5 FURTHER INVESTIGATIONS

Additional investigations that may be carried out in suspected haematopathological disease include flow cytometry, immunohistochemistry, molecular and cytogenetic investigations, 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 node. 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. Staining for micro-organisms should be considered for all granulomatous lesions encountered in the spleen.

6 SAVING SPECIMENS

See lymph node tissue pathway (Section A).

7 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.

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 individual's experience of all of the different analytic 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 clinicopathological forum for discussion of non-neoplastic pathology.

8 HINTS AND TIPS FOR REPORTING SPLEEN HISTOLOGY[2]

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 from a suitable, well fixed, reactive case near to hand as an *aide memoire* (e.g. from an incidental splenectomy performed for access during renal or GI 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 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 haemophagocyosis), sinusoidal endothelium (cuboidal/hobnail appearances), cordal macrophages (widening of cords with or without plump storage cells/foam cells; sequestration of red blood cells {RBCs} and platelets), capillaries (peri-capillary plasma cell clusters) and peri-follicular zones (accumulation of RBCs and/or neutrophils).

4) Do not expect incidental or traumatised splenectomies to be normal; they are usually highly reactive.

5) Do not expect spleens in autoimmune conditions (e.g., auto-immune haemolytic anaemia, idiopathic 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 histology. 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 marginal zone lymphoma is rather nondescript in lymph nodes; it rarely shows a marginal zone distribution at extra-splenic 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 'bare', 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 'incidental' EMH, of no pathological significance; these consist of clusters of maturing nucleated erythroid cells, usually within sinusoidal lumina. 'Significant' EMH (in effect, metastatic spread of 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 proliferations; not usually present in acute leukaemic infiltrates).

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

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SECTION C TISSUE PATHWAY: BONE MARROW TREPHINE BIOPSY

1 STAFFING AND WORKLOAD

See lymph node tissue pathway (Section A).[1]

If the local haematology service generates fewer than 10 bone marrow trephine specimens per month, then it is recommended that the processing of the specimens and the diagnostic service are contracted out to a larger neighbouring hospital or regional centre.

2 SPECIMEN SUBMISSION

Bone marrow trephine biopsy (BMT) is carried out to assist in 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 in nature, or which 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 'dry tap', where examination of an aspirate has been unsuccessful owing to a fibrotic or an infiltrative process.

The bone marrow trephine biopsy 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.[2–4] Bilateral trephine samples have been recommended previously[5,6] but, providing 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.[4] 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 both anterior and posterior iliac crest samples may be collected. Metastases from these tumours can be extremely focal and bilateral sampling improves the detection rate.[7]

The sample is collected either in 5% formalin or in aceto-zinc formalin (AZF) fixatives, as these fixatives allow the use of most staining and molecular techniques.[8,9] Specimens collected into formalin are then delivered to the laboratory and transferred into decalcifying solution, the latter ideally taking place after 8–24 hours. AZF-fixed specimens are processed further following fixation for 20–24 hours.

Preparation after fixation will depend on the preferred processing methods used in the laboratory. There are advocates for both paraffin and plastic embedding techniques, with advantages and disadvantages to each; some laboratories use a combination of the two.[10,11] The use of paraffin embedding after decalcification alone is cheaper and is most suitable for DNA extraction,[12] 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.[13–15]

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 Stewart's 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 is a practical alternative, although generally slower (24–48 hours). It has the advantage of superior nucleic acid preservation in addition to good morphological

and antigenic preservation. Inorganic acids such as nitric or sulphuric (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 the 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. 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 FBC and results of any preceding investigations such as peripheral blood flow cytometry. If the biopsy is undertaken for post-treatment assessment of disease, it is important that the original diagnosis is supplied on the request form and that important details of the therapy used are included.

Consent issues[16] are similar to those for lymph nodes, and can be found in the lymph node tissue pathway.

3 SPECIMEN DISSECTION, BLOCK SELECTION, EMBEDDING AND SECTIONING

In all cases of bone marrow trephine 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 these are 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 bone marrow trephine biopsies, and there is no one recommended technique. Whatever method is employed, the aims are to ensure that the sections examined are representative of the tissue specimen, that sufficient material is examined to detect focal pathology, that the cellular morphology of the haemopoietic tissue allows diagnostic interpretation and that immunohistochemical and tinctorial staining can be carried out if required.

In general, multiple sections from the trephine biopsy are prepared from several levels throughout the specimen. These are essential for the detection of focal pathology such as granulomas or metastases.[2–4,17]

Many laboratories cut sections at 3–4 μm thickness and examine three levels, each separated by 50 μm. The first level is cut at approximately one-third of the distance between ‘first face’ (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 spares 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 one-micron thickness and to ensure that the full face of the block is represented on the slide.[9] Fixation in AZF and decalcification using Gooding and Stewart’s fluid enables slides to be cut in this manner, and the morphology of haematopoietic tissue in such sections is similar to those obtained by plastic embedding. Sections required for immunohistochemistry are cut at the time of initial sectioning, and placed onto glass slides coated with poly-L-lysine or APES, or with commercially prepared negatively-charged surfaces.

In departments employing plastic embedding of bone marrow trephines, 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 STAINING

Bone marrow trephine sections are stained with haematoxylin and eosin and with a reticulin stain. A Giemsa stain is also helpful[2] and routine Perls' staining is used in some laboratories. However, it is not usually necessary or desirable to stain sections with Perls' stain for haemosiderin since assessment of iron stores is inaccurate in trephine specimens and should be undertaken using air-dried marrow aspirate films. If Giemsa staining is employed routinely, this provides excellent staining of haemosiderin to demonstrate excess deposition (e.g. in inflammatory myelopathies, post-transfusional iron overload and in anaemia of chronic disease). In laboratories using EDTA chelation for decalcification, Leder's stain (an enzymatic method to demonstrate chloroacetate esterase) as part of the routine work-up of trephines highlights the quantity and distribution of granulopoiesis and is helpful. Routine Ziehl-Neelsen staining (including a modified method for atypical mycobacteria) is usually carried out on bone marrows from patients with HIV disease. Any of these tinctorial methods may require minor modifications in individual laboratories for use with plastic-embedded sections.

5 FURTHER INVESTIGATIONS

Immunostaining is frequently required for assessing bone marrow trephine biopsies. 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. Where the morphology and immunophenotype are discrepant, or where the findings on the trephine biopsy sections do not agree with those of the marrow aspirate and/or flow cytometry results, further investigations may be required to clarify the pathology.[18] The final report must highlight such discrepancies and suggest an explanation for any unexpected or conflicting immunochemical findings.

In some laboratories, light chain expression is assessed by use of in-situ hybridisation (ISH) techniques to detect mRNA rather than immunohistochemistry. PCR techniques can be used for clonality studies or to look for specific translocations.

6 SAVING SPECIMENS

See lymph node tissue pathway (Section A).[19]

7 REPORT CONTENT AND MDT MEETINGS

The report includes 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[20] and a comment on bone trabecular architecture. Any abnormal infiltrate is identified and the cellular morphology and pattern of infiltration described. The pattern of infiltration (interstitial, paratrabecular, nodular or diffuse) may be of diagnostic or prognostic relevance.[21] Reticulin content is described and the results of additional stains, including all immunohistochemical findings, are summarised. Where a lymphoproliferative condition is identified, the disease is classified using the current WHO terminology.[22]

The report should take into account the patient's haematological indices, the bone marrow aspirate findings and the results of flow cytometry 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.

Bone marrow pathology must be interpreted in the context of the multidisciplinary team (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 instituted to resolve any difficulties in interpretation. An individual's experience of all the different analytic methods (additional tinctorial stains, immunohistochemistry, FISH, PCR, etc.) is useful in weighing up the contribution that each investigation makes to the final diagnosis. All diagnoses of haematological malignancy are discussed by the MDT and there should be an equivalent mechanism for multidisciplinary discussion of non-malignant diagnoses. Details of the final agreed diagnosis and clinical management decisions are recorded at the MDT (or equivalent) meetings.

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