

# Leaders

## Bone marrow trephine biopsy

B J Bain

### Abstract

**Trephine biopsies of the bone marrow should be carried out, when clinically indicated, by trained individuals following a standard operating procedure. A bone marrow aspiration should be performed as part of the same procedure. For patient safety and convenience, biopsies are usually performed on the posterior iliac crest. The biopsy specimen should measure at least 1.6 cm and, if it does not, consideration should be given to repeating the procedure, possibly on the contralateral iliac crest. If bone marrow aspiration is found to be impossible, imprints from the biopsy specimen should be obtained. Otherwise, the specimen is placed immediately into fixative and after fixation is embedded in a resin or, more usually, decalcified and embedded in paraffin wax. Thin sections are cut and are stained, as a minimum, with haematoxylin and eosin and with a reticulin stain. A Giemsa stain is also desirable. A Perls' stain does not often give useful information and is not essential in every patient. The need for other histochemical or immunohistochemical stains is determined by the clinical circumstances and the preliminary findings. Trephine biopsy sections should be examined and reported in a systematic manner, assessment being made of the bones, the vessels and stroma, and the haemopoietic and any lymphoid or other tissue. Assessment should begin with a very low power objective, the entire section being examined. Further examination is then done with an intermediate and high power objective. Ideally, reporting of trephine biopsy sections should be done by an individual who is competent in both histopathology and haematology, and who is able to make an appropriate assessment of both the bone marrow aspirate and the trephine biopsy sections. When this is not possible, there should be close consultation between a haematologist and a histopathologist. The report should both describe the histological findings and give an interpretation of their importance. A signed or computer authorised report should be issued in a timely manner. If the report is a preliminary, this must be clearly stated.**

(*J Clin Pathol* 2001;54:737-742)

**Keywords:** trephine biopsy; bone marrow; haematological diagnosis

*Table 1 Indications for performing a trephine biopsy*

|   |  |
|---|--|
| <i>Definite indications</i>   |  |
| Investigation of suspected Hodgkin's disease and non-Hodgkin's lymphoma   |  |
| Staging of non-Hodgkin's lymphoma   |  |
| Diagnosis and follow up of hairy cell leukaemia   |  |
| Evaluation and follow up of chronic lymphocytic leukaemia   |  |
| Diagnosis of suspected metastatic carcinoma   |  |
| Diagnosis, staging, and follow up of small cell tumours of childhood  |  |
| Investigation of suspected myeloproliferative disorders (polycythaemia rubra vera, essential thrombocythaemia, idiopathic myelofibrosis, and systemic mastocytosis)       |  |
| Diagnosis of aplastic anaemia, hypoplastic myelodysplastic syndromes, and hypoplastic acute myeloid leukaemia   |  |
| Investigation of an unexplained leucoerythroblastic blood film  |  |
| Investigation of a fever of unknown origin  |  |
| Investigation of suspected bone disease   |  |
| Evaluation of any patient in whom an adequate bone marrow aspirate cannot be obtained   |  |
| Investigation of patients in whom multiple myeloma is suspected and investigation of selected patients with serum paraproteins without other evidence of multiple myeloma |  |
| <i>Possible indications</i>   |  |
| Investigation of suspected acute myeloid leukaemia  |  |
| Investigation of suspected myelodysplastic syndrome   |  |
| Staging of Hodgkin's disease  |  |
| Evaluation of chronic myeloid (granulocytic) leukaemia  |  |
| Investigation of suspected primary amyloidosis  |  |

A bone marrow trephine biopsy is an uncomfortable procedure for the patient and carries some risks; therefore, it should be performed only when there is a clear clinical indication. At least five patients have been reported who suffered considerable haemorrhage, sometimes with long term morbidity, as a result of trephine biopsy.<sup>1,2</sup> In addition, I am aware of two haemorrhagic deaths resulting from this procedure not as yet reported in the medical literature. An aspiration biopsy<sup>3</sup> should always be carried out as part of the same procedure. Clinical details and the results of relevant laboratory tests including the blood count and blood film features must be known before a bone marrow biopsy is performed.

### Indications for a trephine biopsy and areas of controversy

Table 1 shows the accepted indications for performing a trephine biopsy. Indications can be summarised as follows:

- Inadequate or failed aspirate.
- Need for accurate assessment of cellularity, whether increased or decreased.
- Suspected focal lesion (for example, suspected granulomatous disease or lymphoma).
- Suspected bone marrow fibrosis.
- Need to study bone marrow architecture.
- Need to study bone structure or bone marrow blood vessels.

Department of Haematology, St Mary's Hospital Campus of Imperial College School of Medicine, Praed Street, London, W2 1NY, UK  
B J Bain

Correspondence to: Dr Bain  
b.bain@ic.ac.uk

Accepted for publication 26 February 2001

In general, patients who have a hypocellular bone marrow or bone marrow fibrosis are likely to need a trephine biopsy for adequate assessment. In such patients, an aspirate will probably be inadequate or even impossible. Similarly, only a trephine biopsy shows the architecture of the bone marrow and permits the detection of an abnormal distribution of cells, bone marrow granulomas, and focal lymphoid infiltrates. There is also a greater likelihood of detection of infiltration by non-haemopoietic neoplasms. Unexplained pancytopenia and an unexplained leucoerythroblastic blood film are indications for a trephine biopsy because they are likely to indicate bone marrow infiltration or fibrosis.

#### LYMPHOPROLIFERATIVE DISORDERS

Some controversy surrounds the role of the trephine biopsy in lymphoproliferative disorders.

Not all patients with chronic lymphocytic leukaemia require bone marrow examination because the disease can usually be diagnosed without difficulty from peripheral blood cytology and immunophenotyping. Patients with early stage disease do not require active treatment and bone marrow biopsy is not essential for management. However, a bone marrow trephine biopsy is indicated in patients in whom treatment is necessary, either those with more advanced disease or younger patients in whom intensive treatment is planned. A trephine biopsy is essential for follow up of intensive treatment because it may show residual focal disease when a bone marrow aspirate is normal. In fact, a bone marrow aspirate is of little value in chronic lymphocytic leukaemia; it is peripheral blood examination and trephine biopsy that are important.

Hodgkin's disease is sometimes diagnosed from trephine biopsy histology. The biopsy will usually have been done for the investigation of pancytopenia or of systemic symptoms such as fever. Diagnosis by bone marrow biopsy is relatively frequent in human immunodeficiency virus positive patients, who often present with stage IVB disease. Trephine biopsy is not mandatory in the staging of Hodgkin's disease diagnosed at another site.<sup>4</sup> In one series, management was altered by the results of biopsy in less than 1% of patients.<sup>5</sup> Management of the patient will not be altered if it is already known that the patient has stage III or IV disease or if, for any other reason, treatment by chemotherapy is intended. Patients with clinical stage IA disease very rarely have bone marrow infiltration and it has been considered justifiable to avoid trephine biopsy in such patients. Only the rare patients with stage IB disease and some patients with stage II disease require bone marrow examination because the results of the investigation might determine the choice of treatment. If bone marrow examination is required in Hodgkin's disease, a trephine biopsy is essential because, even when the marrow is involved, it is rare for neoplastic cells to be detected in an aspirate.

A trephine biopsy may permit a diagnosis of non-Hodgkin's lymphoma, particularly low

grade lymphoma in which the marrow is often infiltrated. It is also of some use in classification if a lymph node biopsy is not available because paratrabecular infiltration is much more common in follicular lymphoma than in other categories. Although a lymph node biopsy is preferred for precise diagnosis, consideration of the immunophenotype and the pattern of bone marrow infiltration can permit a provisional categorisation, and the addition of cytogenetic or molecular genetic analysis may mean that a precise diagnosis can be made without a lymph node biopsy. Biopsy can also be required for staging purposes. Because of increased reticulin deposition in infiltrated areas, the rate of detection may be higher with a trephine biopsy than with an aspirate. However, the two investigations should be regarded as complementary because either may reveal infiltration when the alternative procedure fails to do so.<sup>6</sup> A trephine biopsy performed for staging sometimes shows discordant histological subtype or discordant grade. When high grade disease is found in a bone marrow biopsy specimen from a patient with low grade disease at another site there are clear therapeutic implications.

A trephine biopsy is unnecessary for the diagnosis of acute lymphoblastic leukaemia as long as a cellular aspirate is obtained.

#### MULTIPLE MYELOMA AND OTHER PLASMA CELL NEOPLASMS

Bone marrow aspiration and trephine biopsy should be regarded as complementary investigations in suspected multiple myeloma. A trephine biopsy is essential for diagnosis in some patients. In others in whom an aspirate permits a definitive diagnosis the trephine biopsy is important as a baseline for comparison with repeat biopsies during follow up. Not all patients with a low concentration of a paraprotein require bone marrow examination, particularly elderly patients in whom the abnormality has been detected almost incidentally. However, if investigation is thought to be indicated this should include a trephine biopsy because this may demonstrate much more extensive infiltration than is suspected from the bone marrow aspirate. Immunocytochemistry and immunohistochemistry can be useful in establishing the presence of a relatively small plasma cell clone but if the patient is known to have a paraprotein these investigations are not essential.

A trephine biopsy is much more useful than an aspirate in assessing suspected light chain associated amyloidosis because the biopsy specimen is far more likely than the aspirate to show amyloid deposition.

#### METASTATIC TUMOUR

A bone marrow trephine biopsy is essential if bone marrow examination is being carried out for suspected metastatic disease because the rate of detection of tumour cells is higher than with an aspirate.<sup>7-10</sup> Nevertheless, trephine biopsy and bone marrow aspiration should be regarded as complementary investigations because either may show tumour cells when the other procedure fails to do so.<sup>7-9 11</sup> A trephine

biopsy has the advantage that, because of the possibility of assessing tissue structure and applying histochemical stains, it may be possible to predict the tissue of origin of metastatic tumour—for example, breast or prostate gland—and this may be of relevance to the choice of treatment.

Whether bone marrow examination is essential for staging patients with malignant disease depends on what treatment is intended, on whether bone marrow infiltration is likely, and on whether any bone marrow infiltration detected will alter the management of the patient. For example, in small cell carcinoma of the lung it has been concluded that bone marrow examination is not necessary<sup>12</sup> or is rarely necessary.<sup>13</sup> The bone marrow is the sole site of detected metastatic disease in less than 2% of patients and those with bone marrow infiltration have a median survival only a little worse than that of other patients with extensive disease.<sup>12</sup> It has been estimated that if an appropriate algorithm is followed and investigation is stopped as soon as the first site of metastatic disease is identified unnecessary investigation can be avoided and a third of initial evaluation costs can be saved.<sup>13</sup>

Bone marrow trephine biopsy is essential in the initial staging of certain paediatric tumours, such as neuroblastoma, rhabdomyosarcoma, primitive neuroectodermal tumour, and Ewing's tumour. The detection of bone marrow infiltration in the initial staging bone marrow biopsy means that trephine biopsy is also required during follow up.

#### GRANULOMATOUS DISEASES

A trephine biopsy is always indicated if bone marrow examination is being performed for suspected granulomatous diseases such as sarcoidosis, tuberculosis, cryptococcosis, or histoplasmosis. The detection rate is much higher because the increased reticulin deposition associated with granuloma formation often means that there is a dry tap or only relatively normal bone marrow is aspirated.<sup>14 15</sup>

#### ACQUIRED IMMUNE DEFICIENCY SYNDROME

A bone marrow trephine biopsy is often indicated in patients with AIDS. Specific indications in this context include pancytopenia, fever, and lymphadenopathy. A bone marrow aspirate is much less useful in such patients and, if bone marrow examination is thought to be indicated, it should always include a trephine biopsy. The biopsy may show granulomas or lymphomatous infiltration when the aspirate is uninformative.

#### Site and technique of biopsy

A trephine biopsy is usually most easily carried out on the posterior superior iliac spine, with the patient in the left or right lateral position and with the knees drawn up. An alternative site is the ilium, just below the anterior superior iliac spine, with the patient supine and the approach being perpendicular to the ilium. It is preferable to use disposable needles to avoid the risks associated with cleaning reusable needles. Various needle designs are satisfactory

including Jamshidi and Islam needles. Appropriate sterile gloves<sup>3</sup> should be worn and an aseptic technique must be used. The procedure has been described in standard textbooks but important points will be discussed.

A trephine biopsy and aspiration biopsy can be carried out through the same skin incision but with the bone being entered at two different points, about 1 cm apart. The aspiration is usually performed first but, if a very large aspirate is taken, this may lead to disruption of the tissues that are subsequently included in the trephine biopsy specimen. However, release of thromboplastic substances is likely to mean that the probability of obtaining an adequate aspirate is less if aspiration is performed after, rather than before, a trephine biopsy. It is also easier to perform the least painful procedure first. On balance, it seems preferable to perform the aspirate first, particularly in those cases where it is likely to be crucial in diagnosis.

Local anaesthesia must be adequate with particular attention being paid to infiltrating an adequate area of the periosteum. The adequacy of anaesthesia must be confirmed before proceeding. In anxious patients, or if technical difficulties are anticipated, sedation is useful. It is not necessary to incise the skin to perform an aspirate but for a trephine biopsy a preliminary skin incision is desirable. This can be done either with a lancet or with the sharp edge of the hollow needle that has been used for injection of local anaesthetic. The biopsy needle should be firmly fixed in the cortex of the bone before the trocar is removed, to avoid including extraneous tissues in the biopsy specimen. Ideally, the biopsy should measure at least 20 mm in length after processing. A large biopsy is particularly important if there is a possibility of a focal lesion—for example, lymphomatous infiltrate, metastatic carcinoma, or granulomas (see below).

Trephine biopsies should be carried out only by appropriately trained personnel, usually consultant haematologists or haematopathologists or trainees in these disciplines.

Trephine biopsies can be carried out safely on patients with severe thrombocytopenia but prolonged pressure is indicated to achieve primary haemostasis and reduce bruising to a minimum. If technical difficulties are anticipated—for example, if a patient with thrombocytopenia is also obese—it is usually possible to delay the procedure until a platelet transfusion has been given to raise the platelet count above  $15 \times 10^9$ /litre. Bleeding problems are more likely in patients with coagulation defects and, if patients with severe liver disease or disseminated intravascular coagulation require a biopsy, the coagulation defect should be corrected, as far as possible, before the procedure is undertaken. If a biopsy appears to be indicated in a patient who is fully anticoagulated, and in whom cessation of anticoagulation is contraindicated, the clinical situation should be re-assessed to determine whether a trephine biopsy is essential for management or

whether a bone marrow aspirate or an alternative diagnostic procedure might yield sufficient diagnostic information.

All patients require an explanation of the purpose of the investigation and how the procedure will be carried out. Written consent should be obtained if the procedure is to be carried out under general anaesthesia or heavy sedation. Oral consent is usually considered sufficient if the patient will be fully conscious during the biopsy but local hospital policy should be followed in this regard.

Trephine biopsies of the posterior superior iliac spine can be carried out successfully in children and adults. A modified technique applicable to the tibia has been described for neonates.<sup>16</sup> A core of bone is cut with a hollow needle and the specimen is then aspirated into a syringe; marrow structure is preserved and although the specimen contains small spicules of bone it can be processed without decalcification.

#### Adequacy of biopsy

It has been suggested that an adequate trephine biopsy specimen should contain at least five to six intertrabecular spaces and, after processing, should be at least 2–3 cm in length.<sup>17</sup> Others have considered that 1.5–2 cm is an acceptable length.<sup>18,19</sup> Any such statement as to “adequate” length is arbitrary because the larger the amount of tissue that is biopsied the greater is the likelihood of focal lesions being detected.<sup>12,20</sup> The amount of assessable haemopoietic marrow included in the biopsy specimen is of more importance than the total length. Evidence as to the relation between length and adequacy was provided by Bishop *et al.*<sup>20</sup> They demonstrated that the likelihood of detecting a metastatic tumour increased as the length of interpretable biopsy specimen increased from 0–0.04 mm to > 2 cm, but there was little further gain above a length of 1.2 cm; biopsy specimens of > 2 cm contained tumour in 30% of cases in comparison with biopsy specimens of 0.8–1.2 cm, 26% of which contained tumour. Measurements given relate to a biopsy specimen that has been paraffin wax embedded and decalcified because biopsy specimens shrink by about 25% during processing. It may reasonably be concluded that a biopsy specimen should measure at least 1.6 cm before processing, but that there will be a higher rate of detection of focal lesions with larger biopsies. In the case of children, biopsies will necessarily be shorter. There are no data on which to base advice as to adequacy. In the case of neuroblastoma, it has been suggested that an adequate biopsy should either contain tumour or have at least 0.5 cm of well preserved bone marrow.<sup>21</sup> Even this standard may not be achieved in as many as 25% of biopsies.<sup>22</sup>

A higher detection rate with bilateral biopsies has been demonstrated for neuroblastoma,<sup>23</sup> small cell carcinoma of the lung,<sup>24</sup> Hodgkin's disease,<sup>25,26</sup> non-Hodgkin's lymphoma,<sup>25,27,28</sup> other malignant disease,<sup>25</sup> and malignant disease in general.<sup>29</sup> The higher detection rate for bilateral biopsies may be merely a reflection of the larger amount of tissue biopsied, although there is some evidence

of a higher detection rate with bilateral biopsies than with two biopsies from the same side.<sup>30</sup> However, the gains from bilateral biopsies may not be very great. In a total of 567 patients with non-Hodgkin's lymphoma reported in three large series of patients,<sup>25,27,28</sup> there was detectable bilateral infiltration in 25% and unilateral in 10% of cases. If one postulates that there is an equal chance of the first of two biopsies being positive or negative in a patient with unilateral detectable disease, then approximately 5% of patients will have disease detected only in the second of two biopsies. The proportion of patients in whom disease management is altered by the second biopsy is likely to be less than 5%.

A reasonable compromise, given that patient discomfort must be considered in addition to the likelihood of gaining extra useful information, would be to measure the size of the biopsy specimen and to perform a second biopsy, preferably from the contralateral iliac crest, whenever the length of the initial unprocessed specimen is less than 1.6 cm and the results of the procedure would be likely to alter the management of the patient.

#### Processing of biopsy specimens and staining of sections

If an adequate aspirate has not been obtained it is useful to make a touch imprint of the biopsy specimen, for cytological examination, before putting it into fixative. This is done by rolling the specimen gently along a slide and permits a differential count similar to that performed on aspirate films.<sup>31</sup> If an imprint is not needed, the biopsy specimen should be placed immediately in fixative and when adequately fixed should be decalcified and processed further.<sup>17</sup>

Plastic embedding of trephine biopsy specimens, which can then be sectioned without decalcifying, gives very good cytological detail. However, excellent results can also be obtained from paraffin wax embedded biopsy specimens if they are processed carefully and if sections are thin (not more than 4 µm and preferably considerably less).

All biopsy specimens should have sections stained with haematoxylin and eosin (H&E) and for reticulin. A Giemsa stain on all specimens is also highly desirable because it yields information not available from an H&E stain. Specifically, a Giemsa stain permits the recognition of mast cells, highlights the presence of increased numbers of plasma cells or eosinophils, and helps in making a distinction between myeloblasts and proerythroblasts. It also aids the detection of abnormal bone structure. The effort needed to achieve consistently good Giemsa stains is worthwhile for the extra information yielded. An iron stain is unreliable on decalcified specimens because some or all of the iron may be removed during decalcification. However, if iron is detected this information can be of use. Either an iron stain can be performed in all cases or this stain can be applied selectively when it appears relevant—for example, if no adequate aspirate was obtained. In either event, it is necessary to remember that absence or apparent reduction of stainable iron in sections

Table 2 Useful immunohistochemical stains

| Specificity of monoclonal antibody (or polyclonal antiserum)   | Condition or circumstance in which immunohistochemical stain is useful   |
|--|--|
| CD3, CD1a, CD5, CD8  | Detection of T cells and T cell subsets (note: CD5 is also expressed on some B cells and B cell neoplasms and CD1a on Langerhan's cells)         |
| CD20, CD79a, CD10, CD23  | Detection of B cells and B cell subsets  |
| Terminal deoxynucleotidyl transferase (TdT)  | Detection of immature cells in most acute lymphoblastic and some acute myeloid leukaemias  |
| CD34   | Detection of immature cells in acute and chronic leukaemias and some cases of myelodysplastic syndrome (also useful for accentuation of vessels) |
| Myeloperoxidase, neutrophil elastase, CD68   | Detection of granulocytic and monocytic differentiation  |
| Glycophorin  | Detection of erythroid cells   |
| CD61 or von Willebrand's factor  | Detection of megakaryocytes  |
| Light chains ( $\kappa$ or $\lambda$ )   | Detection of isotype restriction (and therefore probably monoclonality) in multiple myeloma, MGUS, lymphoplasmacytoid lymphoma, and amyloidosis  |
| CD15   | Confirmation of Reed-Sternberg and mononuclear Hodgkin's cells   |
| CD30   | Detection of infiltration by anaplastic large cell lymphoma and Reed-Sternberg and mononuclear Hodgkin's cells                                   |
| CD31   | Endothelial cells (macrophages, monocytes, megakaryocytes, and plasma cells are also positive)   |
| Cyclin D1  | Detection of mantle cell lymphoma  |
| ALK1   | Detection of anaplastic large cell lymphoma  |
| Epithelial membrane antigen  | Detection of infiltration by carcinoma cells or anaplastic large cell lymphoma   |
| Cytokeratin, prostate specific antigen, prostatic acid phosphatase                                     | Diagnosis of infiltration by carcinoma cells   |
| CD1a   | Diagnosis of Langerhan's cell histiocytosis  |
| Mast cell tryptase   | Detection of mast cells and diagnosis of systemic mastocytosis   |
| HMB45 and melan A (S100 less specific)   | Melanoma   |
| Vimentin, desmin, actin, myoglobin, chromogranin A, protein gene product 9.5, neurone specific enolase | Differential diagnosis of small cell tumours of childhood  |

MGUS, monoclonal gammopathy of undetermined significance.

of a decalcified trephine biopsy specimen is not relevant and the report should therefore be worded carefully. The report should state "the iron stain is negative" rather than "storage iron is absent". The statement that "storage iron is reduced" cannot (and therefore should not) be made in relation to a decalcified biopsy specimen.

Most laboratories cut only the number of sections they intend to stain. A superior approach, but one that is not often followed on grounds of cost, is that of sectioning the entire block and H&E staining 20 representative sections (five/slide) from different levels in the biopsy specimen.<sup>18</sup> If a focal lesion is detected it is then possible to select the section adjacent to the section of interest for immunohistochemistry.

Immunohistochemical stains should be applied selectively using appropriate methods of antigen retrieval when necessary. Table 2<sup>2</sup> summarises the useful immunohistochemical stains and a more extensive and detailed list is provided in the book by Bain *et al.*<sup>17</sup>

### Examining and reporting a trephine biopsy, including areas of controversy

#### WHO SHOULD REPORT A TREPINE BIOPSY?

The question of who should report a trephine biopsy is problematical. Ideally, this should be done by a haematopathologist who has been trained adequately in both laboratory haematology and histopathology and who is also competent to assess blood films and bone marrow aspirates. Problems arise in countries where haematologists and histopathologists are

narrowly trained in their specific disciplines and do not have a broad area of competence. In such circumstances, the haematologist may be more reliable at interpreting the trephine biopsy specimen in patients with acute and chronic leukaemias, myelodysplastic syndromes, and myeloproliferative disorders, whereas the histopathologist may be more competent at diagnosing fungal and mycobacterial infection and interpreting infiltration by non-haemopoietic tumours. In addition, the practical reality of the situation is that, except when there is a very large volume of haematopathological specimens justifying a separate laboratory, biopsy specimens are likely to be processed by a histopathology laboratory even if the stained sections are then presented to a haematologist for assessment. The lack of a single group of pathologists with the training and experience to interpret blood films, bone marrow aspirates, and trephine biopsy specimens is hardly ideal but should be acknowledged and the practical problems created should be confronted. The only solution is close cooperation between disciplines. Ideally, in this circumstance the blood film, bone marrow aspirate, and trephine biopsy specimens should be examined simultaneously by a haematologist and histopathologist working together. If the workload of either does not permit this for all specimens then the minimum acceptable safeguard is that the histopathologist should not release a report on a trephine biopsy without ascertaining the haematologist's opinion on the blood film and bone marrow aspirate. If there is any apparent discrepancy between the findings this should be resolved by a joint examination of the slides of the case or, at the very least, by a telephone conversation between those responsible for reporting the specimens. Reports should only be issued by a qualified haematologist or histopathologist.

#### EVALUATING A TREPINE BIOPSY SPECIMEN

H&E stained sections of the specimen should be examined systematically at low power ( $\times 2.5$ ,  $\times 4$ , or  $\times 10$  objective), at medium power ( $\times 20$ ,  $\times 40$ , or  $\times 50$  objective), and at high power ( $\times 100$ , oil immersion objective).

Low power examination is important for evaluation of the adequacy of the biopsy specimen and for assessment of cellularity and the detection of focal lesions. A short piece of sub-cortical bone marrow is totally inadequate for assessment because it is likely to be of low cellularity. Focal lesions that may be noted at low power include granulomas and focal infiltrates of lymphoma cells or carcinoma cells.

Examination at low to medium power permits assessment of cellularity, bone structure, and megakaryocyte numbers. At medium power the location of cells of erythroid and granulocytic lineages and their relative proportions can be assessed, the nature of any focal lesions can be determined, and blood vessels can be examined.

Examination at high power is important if fine cellular detail is to be appreciated and if fungal and protozoal infections are to be

detected. Although cryptococci are usually readily detected at medium power, histoplasmosis—particularly in patients with a poor immune response as a consequence of AIDS—can easily be missed if high power examination is omitted. The correct diagnosis of acute leukaemia, the myelodysplastic syndromes, megaloblastic anaemia, and lymphoma requires high power examination.

After examination of the H&E stained sections, the Giemsa stain, reticulin stain, and iron stain should be examined. The reticulin should be graded, according to a standardised published system, and any focal increase in reticulin deposition should be noted. The presence of focal reticulin deposition is an indication to re-examine the H&E stained sections in case a focal infiltrate or a granuloma has been missed.

After routine stains have been examined a decision should be made as to whether any specialised cytochemical stains (such as Congo red stain or Ziehl-Neelsen stain) or immunohistochemical stains are needed.

#### REPORTING A TREPINE BIOPSY SPECIMEN

The trephine biopsy report should begin with a macroscopic description and, usually, a statement that the specimen was embedded in its entirety. Usually, the length of the biopsy is the only useful information to be gained, but occasionally the biopsy specimen is macroscopically abnormal.

The report of the microscopical appearances should begin with a statement as to the size of the specimen or its adequacy for diagnosis and should then comment systematically on cellularity, bone structure, haemopoietic cells, and any abnormal infiltrate. Special stains, if any, should be assessed and commented on. The report should finish with a summary of the abnormalities detected and their likely importance.

At this stage, if not earlier, the haematologist or histopathologist should have examined or ascertained the findings on the bone marrow aspirate and, if assessment of cytological and histological features is consistent, should issue a report. If it is necessary to issue a provisional report—for example, because special stains are pending or because a second opinion is being sought—this should be clearly stated.

Laboratories must have procedures in place to ensure that unauthorised provisional reports are not inadvertently released to clinical staff. It is useful for laboratory staff if all provisional reports on laboratory computers are clearly indicated as “UNAUTHORISED REPORT” so that laboratory staff referring to them are instantly aware of their status. At the point when a report has been finalised and authorised the name of the pathologist issuing the report must be added, either as a signature or by computer authorisation using a secure password system.

#### Standard operating procedure

The performance of a trephine biopsy, the processing of the biopsy specimen and the

reporting of the histological sections should be in accordance with standard operating procedures.

I am grateful to colleagues who critically reviewed the manuscript—Dr DM Clark, Dr R Hasserjian, Professor K Gatter, Dr M Reid, Professor I Roberts, and Dr BS Wilkins.

- 1 Ben-Chetrit E, Fusser D, Assaf Y. Severe bleeding complicating percutaneous bone marrow biopsy. *Arch Intern Med* 1984;144:2284.
- 2 Pedersen LM, Jarner D, Winge J. Bone-marrow biopsy of the iliac bone followed by severe retroperitoneal hemorrhage. *Eur J Haematol* 1993;51:52.
- 3 Bain BJ. Bone marrow aspiration. *J Clin Pathol* 2001;54:657–64.
- 4 Ellis ME, Diehl LF, Granger E, et al. Trephine needle biopsy in the initial staging of Hodgkin disease: sensitivity and specificity of the Ann Arbor staging procedure criteria. *Am J Hematol* 1989;30:115–20.
- 5 MacIntyre EA, Vaughan Hudson B, Linch DC, et al. The value of staging bone marrow trephine biopsy in Hodgkin's disease. *Eur J Haematol* 1987;39:66–70.
- 6 Horlyck A, Thorling K. Bone marrow examination in non-Hodgkin's lymphoma: comparison of the diagnostic value of marrow aspirations and trephine biopsy. *Eur J Haematol* 1991;46:54–6.
- 7 Bearden JD, Ratkin GA, Coltman CA. Comparison of the diagnostic value of bone marrow biopsy and bone marrow aspiration in neoplastic disease. *J Clin Pathol* 1974;27:738–40.
- 8 Singh G, Krause JR, Breitfeld V. Bone marrow examination for metastatic solid tumours. *Cancer* 1977;40:2317–21.
- 9 Ingle JN, Tormey DC, Tan HK. The bone marrow examination in breast cancer. *Cancer* 1978;41:670–4.
- 10 Ihde DC, Simms EB, Matthews MJ, et al. Bone marrow metastases in small cell carcinoma of the lung: frequency, description, and influence on chemotherapeutic toxicity and prognosis. *Blood* 1979;53:677–86.
- 11 Anner RM, Drewinko B. Frequency and significance of bone marrow involvement in metastatic solid tumours. *Cancer* 1977;39:1337–44.
- 12 Campling B, Quirt J, deBoer G, et al. Is bone marrow examination in small-cell lung cancer really necessary? *Ann Intern Med* 1986;105:508–12.
- 13 Richardson GE, Venzon DJ, Phelps R, et al. Application of an algorithm for staging small-cell lung cancer can save one third of the initial evaluation costs. *Arch Intern Med* 1993;153:329–37.
- 14 Browne PM, Sharma OP, Salkin D. Bone marrow sarcooidosis. *JAMA* 1978;240:2654–5.
- 15 Kurtin PJ, McKinsey DS, Gupta MR, et al. Histoplasmosis in patients with acquired immunodeficiency syndrome. Hematologic and bone marrow manifestation. *Am J Clin Pathol* 1990;93:367–72.
- 16 Sola CM, Rimsza LM, Christensen RD. A bone marrow biopsy technique suitable for use in neonates. *Br J Haematol* 1999;107:458–60.
- 17 Bain BJ, Clark DC, Lampert IA, et al. *Bone marrow pathology*, 3rd ed. Oxford: Blackwell Science, 2001.
- 18 Brynes RK, McKenna RW, Sundberg RD. Bone marrow aspiration and trephine biopsy: an approach to a thorough study. *Am J Clin Pathol* 1978;70:753–9.
- 19 Islam A. *Manual of bone marrow examination*. Amsterdam: Overseas Publishers Association, 1997.
- 20 Bishop PW, McNally K, Harris M. Audit of bone marrow trephines. *J Clin Pathol* 1992;45:1105–8.
- 21 Reid MM, Roald B. Adequacy of bone marrow biopsy specimens in children. *J Clin Pathol* 1996;49:226–9.
- 22 Reid MM, Roald N, for the European Neuroblastoma Study Group. Deterioration in performance in obtaining bone marrow trephine biopsy cores from children. *J Clin Pathol* 1999;52:851–2.
- 23 Franklin IM, Pritchard J. Detection of bone marrow metastases in neuroblastoma is improved by sampling at two sites with aspirates and trephines. *J Clin Pathol* 1983;36:1215–18.
- 24 Hirsch FR, Hansen HH, Hainau B. Bilateral bone-marrow examinations in small-cell anaplastic carcinoma of the lung. *Acta Pathol Microbiol Scand [A]* 1979;87:59–62.
- 25 Brunning RD, Bloomfield CD, McKenna RW, et al. Bilateral trephine bone marrow biopsies in lymphoma and other neoplastic disease. *Ann Intern Med* 1975;82:365–6.
- 26 Bartl R, Frisch B, Burkhardt R, et al. Assessment of bone marrow histology in Hodgkin's disease: correlation with clinical factors. *Br J Haematol* 1982;51:345–60.
- 27 Ebic N, Loew JM, Gregory SA. Bilateral trephine bone marrow biopsy for staging non-Hodgkin's lymphoma—a second look. *Hematol Pathol* 1989;3:29–33.
- 28 Juneja SK, Wolf M, Cooper IA. Value of bilateral bone marrow biopsy specimens in non-Hodgkin's lymphoma. *J Clin Pathol* 1990;43:630–52.
- 29 Alvarez CJ, Kidd PG, Ettinger LJ. Evaluation of bilateral bone marrow specimens in pediatric disorders. *Am J Clin Pathol* 1999;112:559.
- 30 Haddy TB, Parker RI, Magrath IT. Bone marrow involvement in young patients with non-Hodgkin's lymphoma: the importance of multiple bone marrow samples for accurate staging. *Med Pediatr Oncol* 1989;17:418–23.
- 31 Aboul-Nasr R, Estey EH, Kantarjian HM, et al. Comparison of touch imprints with aspirate smears for evaluating bone marrow specimens. *Am J Clin Pathol* 1999;111:753–8.