THE PLACE OF THE BONE MARROW TREPHINE BIOPSY IN CURRENT CLINICAL PRACTICE

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The trephine biopsy of the bone marrow is usually a complementary diagnostic technique rather than a primary investigative one. A core of intact marrow is withdrawn, usually 1 to 2 cm in length, which is ideally suited to provide information of an architectural or topographic nature rather than providing the precise cytomorphological detail best seen in aspirated specimens. In the majority of instances the trephine will form part of a collaborative investigative exercise and in many hospitals the pathology department will simply process the specimen for subsequent reporting by the haematologist. In my hospital, partly for historic reasons, histopathologists report the trephines, although there is a close collaboration with the haematologists because it is essential that the maximum information is obtained from the specimen.

In my department, approximately 30 per cent of trephines are sent for staging purposes, i.e. to evaluate marrow involvement by tumour in a patient in whom the primary diagnosis of malignant disease has been made already. The majority of the cases will be carcinomas or lymphomas; sarcomas have a relatively low incidence of marrow metastases in adults. Although double or triple iliac crest sampling may give a better yield of positive results, in most hospitals single crest samples are the norm. Recently, attention has been focused on the detection of micrometastases and its relevance to the management of malignant disease. The techniques usually involve the pooling of aspirates and the application of the polymerase chain reaction rather than the histological evaluation of trephine specimens.

In the majority of the remaining cases the biopsy is taken to provide supportive information about a variety of haematological disorders. This information would include disease extent, progress or response to treatment in neoplastic disease, the degree of marrow fibrosis particularly in the myeloproliferative disorders and occasionally the assessment of iron stores. The bone marrow biopsy may also be examined both histologically, and also cultured, to diagnose specific infections particularly where the index of suspicion is high such as in the immunocompromised host. It should always complement a full clinical assessment of the patient, the appearances of the peripheral blood film and the bone marrow aspirate.

A complete diagnosis may be possible on the trephine biopsy alone but this is rarely necessary or desirable in the majority of cases. In a number of instances however, particularly in cases of idiopathic myelofibrosis and idiopathic aplastic anaemia, the marrow trephine may be the sole source of diagnostic material. It should be remembered that a single trephine biopsy may not be sufficient to reach a diagnosis and that a series of similar investigative examinations may prove necessary as in the diagnosis of some infections, metastatic malignancy or an evolving haematological malignancy. Table 1 illustrates the principal areas where the marrow trephine is useful in current practice. Because of my own area of practice and experience I will limit this discussion largely to adult diseases. The place of bone marrow examination in paediatric practice is the subject of an excellent recent review.
Table 1
Principal uses of the marrow trephine biopsy

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<tr>
<th>Disease Group</th>
<th>Diagnosis</th>
<th>Role of trephine</th>
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<tr>
<td>Neoplastic disease</td>
<td>Metastatic carcinoma</td>
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<td>Lymphomas ('metastatic')</td>
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<td>Myeloma</td>
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<td>Myelodysplasias</td>
<td>Supportive</td>
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<tr>
<td>Myeloproliferative diseases</td>
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<td>Aplastic anaemias</td>
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TREPHINE AS THE STAGING BIOPSY

Metastatic carcinoma
Bone marrow involvement by metastatic carcinoma increases with advancing disease but there are substantial differences in the propensities of different neoplasms to involve the marrow. In childhood, neuroblastoma may involve the marrow in up to 60 per cent of cases at presentation, while in adult practice small cell carcinoma of lung involves the marrow in 20 per cent of cases at presentation. Other tumours which involve the marrow frequently, particularly during the later stages of the disease include non-small cell carcinomas of the lung, and carcinomas of the breast and prostate.

The histological diagnosis of metastatic carcinoma in the marrow is usually straightforward as carcinoma cells are usually much larger than haemopoietic blast cells, frequently they are clustered in small groups and often excite a fibrotic (desmoplasic) tissue reaction in the marrow, and these stand out against the background e.g. metastases from carcinomas of breast, stomach, bronchus and prostate. Sometimes, the desmoplastic reaction may be so florid as to obscure the malignant cells that have initiated it. Metastatic small cell carcinoma of the bronchus may cause diagnostic difficulty partly because the size of these cells more closely approximates that of native marrow blasts. In difficult cases immunohistochemistry may detect the presence or confirm the epithelial nature of these cells. In some tumours (for example prostatic carcinoma) in which relatively specific tumour antibodies are available, it will also give a strong indication of the primary site of origin of the tumour.

Figure 1 shows a focus of metastatic carcinoma of breast in a bone marrow trephine specimen.

Lymphomas
The evaluation of bone marrow trephine specimens for the purposes of staging lymphomas is usually straightforward. Low grade non-Hodgkin’s lymphomas (NHLs), particularly follicle centre cell neoplasms, may involve the marrow in over 50 per cent of cases at presentation, while high grade tumours involve the marrow in 15 - 20 per cent of cases. Follicular NHLs have a characteristic paratrabecular pattern of involvement in contrast to benign lymphoid aggregates and are associated with the localised deposition of reticulin. This latter feature can be helpful in the initial recognition of a subtle infiltrate. Mantle zone lymphomas involve the bone
marrow in up to 65 per cent of cases and their pattern of involvement is much less predictable; the morphology of the infiltrate may not always allow reliable histological separation from metastatic small cell carcinoma of bronchus and follicular non-Hodgkin's lymphoma. High grade NHLs may involve the marrow in a focal, interstitial or diffuse manner with interstitial and diffuse patterns carrying a poorer prognosis.

In some cases of anaplastic large cell lymphoma with marrow involvement, tumour cells may be scarce and look like haemopoietic precursor elements and also may not excite a reticulin reaction. Immunohistochemistry using antibodies to CD30 and EMA antigens has produced a yield of 23 per cent positives in marrows that were originally regarded as negative.

While the infiltrate in marrow would normally be expected to be similar morphologically to the nodal or extra-nodal 'primary', a surprisingly high incidence of morphological non-discordance between these lesions may be observed, with a rate as high as 60 per cent in one series. This should be borne in mind when trying to extrapolate a detailed lymphoma diagnosis from the appearances of a focus of tumour in the marrow.

Hodgkin's disease (HD) involves the marrow in only about 5 per cent of cases at presentation and may be difficult to diagnose because of the problem of reliably identifying the Sternberg-Reed cells. This problem is compounded further because of the relatively frequent occurrence of a non-metastatic hyperplastic eosinophilic marrow reaction in HD that can cause diagnostic confusion. The significance of these reactions is uncertain but they do not signify metastatic involvement elsewhere.
Considerable doubt has recently been cast on the contribution of this procedure to the initial management of patients with Hodgkin’s disease\textsuperscript{18,19} although it may be important in the management of relapsed disease. Immunohistochemistry may be of considerable value in helping to evaluate a focus of possible Hodgkin’s disease in the marrow.

Deposits of lymphoma must always be distinguished from reactive lymphoid aggregates and this may be extremely difficult on occasions.\textsuperscript{20,21}

**MYELOFIBROSIS AND APLASTIC ANAEMIA**

**Mycelofibrosis**
The bone marrow trephine may be the only specimen available for the diagnosis of myelofibrosis and aplastic anaemia. In myelofibrosis, as is often the case in the myeloproliferative disorders, the presence of diffuse fibrosis may prevent the aspiration of marrow tissue and lead to a ‘dry tap’, and so the only material for making a diagnosis may be the trephine. The histological features of myelofibrosis are well described elsewhere\textsuperscript{22} but it is pertinent to note here that caution should be exercised when reporting the trephine from the isolated perspective of the trephine alone; it may be prudent to restrict one’s comments to a description of the findings with a summary giving the more general diagnosis of ‘myeloproliferative disorder’. Myelofibrosis, essential thrombocythaemia and some cases of polycythaemia rubra vera may be indistinguishable histologically and these three labels should be regarded as clinico-pathological descriptors rather than ‘black and white’ diagnoses. Furthermore, during the course of a myeloproliferative disease, the pattern may change, for example from one of essential thrombocythaemia to myelofibrosis. This adds further support to adopting a flexible approach towards the labelling of this group of disorders.

In a recent review of bone marrow trephines from a series of twenty patients with clinical diagnoses of essential thrombocythaemia treated at this hospital almost all showed substantial to marked deposition of reticulin,\textsuperscript{23} a feature previously regarded as indicative of myelofibrosis. The assessment of fibrosis in trephines can be made in a semi-quantitative manner\textsuperscript{24}, and such description may help in monitoring progress of disease and response to treatment.

**Idiopathic aplastic anaemia**
The situation with idiopathic aplastic anaemia contrasts with that of myelofibrosis. Once again the patient may present with anaemia and cytopenias, and the marrow aspirate yields no cells. On this occasion, the marrow trephine is almost completely acellular with marrow elements replaced by fatty tissue. Causes of this type of clinical and peripheral blood presentation would include marrow infiltration (for example by tumours), fibrosis and acute leukaemias, all of which should be distinguishable, at least in broad terms, by the bone marrow trephine.

**MULTIPLE MYELOMA, CHRONIC LYMPHOCYTIC LEUKAEMIA AND HAIRY CELL LEUKAEMIA**
The marrow trephine specimen may provide a firm diagnosis in these three disorders. Although the technique is often an accessory diagnostic aid it will sometimes provide the only material, for example if the aspirate clots or if marrow fibrosis prevents aspiration of marrow tissue.
Multiple myeloma
The diagnosis of multiple myeloma is often based on clinical, radiological and laboratory data; the marrow aspirate will often confirm the diagnosis. Indeed, because of morphological differences between plasma cells seen in aspirate and trephine specimens, it is often easier to confirm the diagnosis with the former technique. The trephine will however give an indication of tumour load and the degree of preservation of native marrow elements, and may provide some baseline data for monitoring subsequent therapy. Histological classifications of plasma cell morphology have been proposed but these provide limited prognostic information. Correlation with a plasma cell labelling index may be helpful also in refining this information further.

Chronic lymphocytic leukaemia
The diagnosis of chronic lymphocytic leukaemia (CLL) is made primarily on the peripheral blood with marrow aspirates and trephines providing only secondary information. The trephine (in contrast to the aspirate) may give an indication about the tumour burden and give information about the residual native marrow, and will also allow assessment of the pattern of infiltration that will give some prognostic information. Nodular infiltrates are associated with a more favourable prognosis, while diffuse infiltration is less favourable prognostically.

Hairy cell leukaemia
The diagnosis of hairy cell leukaemia (HCL) may be suggested by the clinical presentation of splenomegaly and anaemia, and confirmed by the finding of tartrate-resistant acid phosphatase (TRAP)-positive ‘hairy cells’ in the peripheral blood. These cells may be sparse and the diagnosis may rest on bone marrow examination. Because the bone marrow carries a heavy reticulin load in this condition, aspiration is usually unsuccessful and the diagnosis usually relies on examination of the trephine.

The appearances in the typical case are those of a marrow packed with so called ‘hairy’ cells (Fig 2a), that have a characteristic ‘fried egg’ appearance in sections, while the ‘chicken wire’ reticulin pattern is also characteristic (Fig 2b). It should be appreciated that the ‘hairy’ appearance of the cytoplasmic membranes of these cells can only be appreciated in peripheral blood smears and electron-microscopical preparations, and is not a feature of the cells in histological sections. All three cell lines may show dysplastic characteristics, a feature which may resolve after treatment. Immunohistochemistry can be applied to help confirm the diagnosis of this condition which is histogenetically related to CLL.

Myelodysplastic syndromes and myeloid leukaemias
The trephine biopsy plays an important diagnostic role in the myelodysplastic syndromes (MDS), both primary and secondary, and the myeloid leukaemias. It should be stressed that although a diagnosis may be offered from the trephine appearances, the clinical, peripheral blood and marrow aspirate features are the cornerstones of diagnosis with the trephine providing valuable, primarily architectural information in most cases.

Myelodysplastic syndromes
While acknowledging the heterogeneous nature of MDS it is still possible to generalise about some of the key features of this group of diseases. Patients are usually
FIGURE 2a
Hairy cell leukaemia in bone marrow biopsy: H & E x 400

FIGURE 2b
Hairy cell leukaemia in bone marrow biopsy: Reticulin stain showing 'chicken wire' pattern.
middle aged or elderly, and present with tiredness, anaemia and cytopenias. There may only be small numbers of circulating blasts in the peripheral blood. Marrow aspiration will suggest hypercellularity and will demonstrate dysplastic features. In contrast to the marrow aspirate, the trephine will usually demonstrate the hypercellularity convincingly. Architectural disturbances are noted within marrow spaces with abnormal locations of the different precursor elements.

Within erythroid islands and sometimes in myeloid precursor clusters, it may be possible to appreciate a lack of maturation referred to as a ‘left shift’ (the marrow is rather graphically depicted as maturing from left to right). The trephine is less suited to the demonstration of cytological features of dysplasia which should be more apparent in an accompanying aspirate. The size and location of blastic aggregates and possibly their CD34 immunostaining characteristics, may act as pointers indicating a higher risk of leukaemic transformation.

**Myeloid leukaemias**

It is beyond the scope of this review to describe the trephine biopsy features of all the subtypes of acute and chronic myeloid leukaemia. The diagnosis of these disorders is made primarily on the peripheral blood and marrow aspirate specimens. The trephine biopsy may occasionally be the only diagnostic material available but usually the procedure is only performed to try and gauge tumour load, fibrosis and response to treatment. It should be appreciated that it is often difficult to be certain about the precise degree of maturation of a precursor or even its lineage and therefore giving percentages of blasts required to make a particular diagnosis may be impossible. In chronic myeloid leukaemia the trephine may be helpful to confirm the transformation to an acute phase of the disease.

**The Future**

With advancing sophistication of diagnostic techniques and therapy, it is unlikely that the role of the trephine biopsy will diminish in the early years of the next century. Despite the growth of molecular techniques in the past decade no reduction has been noted in the requirement for the histological information in trephine biopsies which, if anything, has grown. We can look forward to developing further immunohistochemical techniques to allow us to obtain the maximum information from trephines, and also pursue more detailed investigative approaches offered by newer techniques, such as *in situ* hybridisation and the polymerase chain reaction. The significance of detection of minimal residual disease in cancer, leukaemia and lymphoma has yet to be evaluated fully and these techniques have the potential not only for defining the extent of this problem but also for providing a rational scientific basis for launching an appropriate therapeutic response.

It is likely that in future the simple diagnosis of malignancy using late 20th century labels will seem as imprecise as was the calling of a lymphoma a ‘qualitative derangement of the lymphatic system’ in the 19th century. Even in an overtly straightforward malignancy it is likely that the outcome of the disease is at least partly determined by the relationship of neoplastic cells to their non-neoplastic neighbours. The trephine biopsy will provide diagnostic material that will allow not only precise definition of these disease processes but also an appreciation of the complex interrelationships of the various cell types present.
ACKNOWLEDGEMENTS

I would like to thank the Trustees of the Myre Sim Bequest and also the Western General Hospitals NHS Trust who gave financial assistance for me to attend a course on haematopathology in the United States at the end of 1994. This has contributed greatly to my understanding of this subject.

REFERENCES


23 Thomas J and Shepherd P. Unpublished observations.