CHAPTER 1

Introduction

During the late 1950s, McFarland and Dameshek introduced an acceptable means of obtaining bone marrow core biopsies.[1] This advance made it possible for the histopathologist to diagnose a wide range of haematopathological disorders including the leukaemias, lymphoproliferative and myeloproliferative disease, myelodysplasia, metastases and reactive disorders.

Most biopsies are taken from the posterior superior iliac spine. Ideally in an adult the core of tissue should be at least 1 cm in length. This often raises the question: 'But what is the minimum you need?' There is no standard answer. Although half a crushed marrow space full of carcinoma is diagnostic, in most cases a good rule of thumb is a minimum of five complete marrow spaces for most haematological diagnoses. An aspirate is usually taken from the same site before the biopsy is removed (but from a different needle track, or the biopsy may be a haemorrhagic mess). The haematologist will usually make about 10 smear preparations from the marrow particles that have been aspirated and either discard or send the remainder for histology. We find it useful to have both of these types of specimen since there are occasions when only an aspirate is available, in which case it is then important to have built up experience examining aspirate preparations for which trephines have been available for comparison.

The trephine biopsy has a number of advantages over the aspirate specimen. The most important is to enable examination of the topographical distribution of the cellular constituents of the marrow, their relationships to the bony trabeculae and an assessment of marrow cellularity. Furthermore, in diseases which produce fibrosis, e.g. Hodgkin lymphoma or myeloproliferative disorders, an aspirate often fails to produce an adequate diagnostic sample ('a dry tap').

Close liaison with haematologists is important since it makes the reporting of trephine biopsies easier and ensures that misdiagnoses are kept to a minimum. Many if not most authorities recommend reporting of the trephine biopsy alongside the aspirate, either by yourself or with the haematologist. However, this does not seem to be common practice and, as workloads and specialist referrals rise, will inevitably decline. You can report safely most trephines on their own provided you know your limitations and keep a good dialogue open with your clinicians. Multidisciplinary team meetings are also invaluable, allowing for a review of all malignant haematological diagnoses and discussion of diagnostically difficult cases. The authors appreciate that many trainee histopathologists who see only occasional trephine biopsies find it difficult to observe any order, even in a normal marrow, and often give up on this subspecialty as being 'too difficult'. Our advice is to persist and spend time initially on examining as many normal/reactive marrows as possible.

There has been debate involving the embedding medium for bone marrow biopsies. There are essentially two schools of thought: those who believe that the biopsies should be embedded in plastic and those who believe paraffin embedding with decalcification to be superior. The reason for this divergence is related to the nature of the biopsy itself, which consists of both hard tissue (i.e. bone) and soft tissue (i.e. marrow and fat). In order to cut intact sections one can either make the biopsy material uniformly soft (by decalcification) or uniformly hard (by resin embedding).

Unfortunately, decalcification inevitably produces some tissue distortion and plastic embedding limits the range of immunohistochemical studies. The debate over which is superior continues, with vociferous advocates on either side.[2–6] The advantages and disadvantages of each approach are summarized in Table 1.1.

We believe that, with a little extra care, it is possible to provide sections, from paraffin-embedded trephines, which meet the practical requirements of the diagnostic haematologist.[7]

Just as there has been division among pathologists regarding the best embedding medium, so too has there been debate over the most appropriate general stain. This inevitably involves an element of personal preference. The well-established place of the H&E stain in general diagnostic pathology has assured it of much support as the primary stain in bone marrow histology. We believe that a good Giemsa stain provides more information than its H&E counterpart, e.g. in identifying cell lineage, the detection of fibrosis and the estimation of iron stores. A good Giemsa stain requires fastidious technical preparation (see Chapter 15). The results are worth the initial perseverance required by both the technical staff and the pathologist to

Bone Marrow Diagnosis: An Illustrated Guide, Third Edition. Kevin Gatter and David Brown.

Table 1.1 Comparison of the relative advantages and disadvantages of paraffin and plastic embedding of bone marrow trephine biopsies.

	Paraffin embedding	Resin/plastic embedding
Advantages	1 Widespread antigen preservation allows immunohistochemical studies 2 Pathologists are familiar with sections cut from paraffin-embedded material	1 Superb cytological detail available from the very thin sections obtained by this technique
Disadvantages	1 Loss of some histochemical reactivity within the granules of the granulocyte and mast cell series, e.g. Leder stain. This loss is directly proportional to the strength of the acid used in decalcification 2 Some inevitable tissue distortion is produced by decalcification	1 Loss of some immunoreactivity 2 A separate technique is required solely for bone marrow biopsies 3 Pathologists are unfamiliar with resin-embedded sections and their associated artefacts, e.g. the basophilic hue indicative of erythroid histogenesis is lost in resin-embedded sections

Tables 1.2–1.5 A scheme for assessing the bone marrow trephine with some common pathological conditions as examples.

Table 1.2

Assessment of cellularity	
Hypocellular	Aplastic anaemia Hairy cell leukaemia Acute myeloid leukaemia
Normocellular	Be aware of subtle infiltrates such as myeloma
Hypercellular	
Homogeneous	Non-Hodgkin lymphoma Acute leukaemias
Heterogeneous	Reactive Myeloproliferative neoplasia Myelodysplasias Metastatic cancer Small cell tumours of childhood

Table 1.3

Topography (distribution) of cellular elements
Are all cell types present?
Are any particular cells present in abnormal numbers?
e.g. increased granulocytes in chronic granulocytic leukaemia
Prominent mast cells in Waldenström's macroglobulinaemia
Normal cellular distribution
Granulocytes
Paratrabecular, peri-arterial
Erythroid
Intertrabecular
Megakaryocytes
Intertrabecular and peri-sinusoidal
Common abnormal patterns
Myelodysplasia/myeloproliferation
Paratrabecular erythroid and megakaryocytic colonies
Megakaryocytic clustering
Non-Hodgkin lymphoma
Follicular lymphoma has a paratrabecular pattern
CLL is usually diffuse or nodular

Table 1.4

Assessment of cell morphology
Atypia
Abnormal megakaryocytes in myeloproliferation and myelodysplasia
Maturation abnormalities
Maturation arrest, e.g. drug induced
Asynchronous maturation in myelodysplasia
Abnormal maturation, e.g. megaloblastic anaemia
Imbalance of maturation, e.g. left/right shifted

Table 1.5

Assessment of accessory structures
Vessels
Vasculitis
Amyloid deposition
Sinusoids
Distended in myeloproliferative disorders
Bone
Osteoporosis
Osteomalacia
Paget's disease
Stroma
Iron deposition
Amyloid
Gelatinous transformation
Granulomas
Fibrosis
Metastatic carcinoma
Gaucher's disease
Organisms
Mycobacteria (TB)
Atypical mycobacteria
Leishmania
Histoplasma
Cryptococci

become familiar with it. When indicated, we include a reticulin stain in our bone marrow set.

Reasons for performing bone marrow biopsies

The majority of bone marrow biopsies are performed for the following reasons.[8]

1 Dry tap. The commonest diagnoses are:
   - fibrosis (Hodgkin lymphoma, metastatic cancer, primary myelofibrosis);
   - hairy cell leukaemia;
   - extreme hypercellularity ('packed marrow') such as may be seen in cases of leukaemia and lymphoma.
2 Assessment of cellularity:
   - extent of infiltration by leukaemia, lymphoma and myeloma;
   - amount of residual marrow;
   - assessment of marrow post chemotherapy and after engraftment;
   - investigation of cytopenias.
3 Identification of focal disease:
   - metastatic cancer, lymphomas, granulomas.
4 Lymphoma staging.
5 Assessment of HIV and its opportunistic infections.

Source: De Wolf-Peeters (1991).[8] Reproduced with permission of John Wiley & Sons Ltd.

How to examine a trephine section

It is important to have an organized approach to the examination of bone marrow sections in order not to miss diagnostic features. One possible scheme is based on an assessment of cellularity, topography, morphology and accessory structures, as illustrated in Tables 1.2–1.5 with a selection of some common pathological conditions.

References

1 McFarland W, Dameshek W. Biopsy of bone marrow with the Vim-Silverman needle. JAMA 1958; 166: 1464–1466.
2 Nand NM. Misunderstandings about methyl methacrylate. J Clin Pathol 1993; 46: 285.
3 Jack AS, Roberts BE, Scott CS. Processing of trephine biopsy specimens. J Clin Pathol 1993; 46: 285.
4 Schmid C, Isaacson PG. Bone marrow trephine biopsy in lymphoproliferative disease. J Clin Pathol 1992; 45: 475–450.
5 Schmid C, Isaacson PG. In reply to correspondence. J Clin Pathol 1993; 46: 285–286.
6 Krenacs T, Krenacs L, Bagdi E. Diagnostic immunocytochemistry in Araldite-embedded bone marrow biopsies. Cellular Pathol 1996; 1: 83–88.
7 Gatter KC, Heryet A, Brown DC, Mason DY. Is it necessary to embed bone marrow biopsies in plastic for haematological diagnosis? Histopathology 1987; 11: 1–7.
8 De Wolf-Peeters C. Bone marrow trephine interpretation; diagnostic utility and pitfalls. Invited review. Histopathology 1991; 18: 489–493.