Pitfalls in bone marrow pathology: avoiding errors in bone marrow trephine biopsy diagnosis

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ABSTRACT

Avoiding errors in the histological interpretation of bone marrow trephine biopsy specimens requires an unprecedented degree of collaboration between histopathologists, haematologists, specimen requesters, specimen takers, laboratory technical staff and other scientific staff. A specimen of good quality, with full, relevant clinical information is the essential starting point. This must then be processed optimally and investigated appropriately, involving immunophenotyping and molecular testing when needed. A wide range of pathologies may involve bone marrow haemopoietic and stromal components, and a systematic approach to analysing each of the components in turn is required to avoid overlooking abnormalities; correlation with bone marrow cells aspirated in parallel is particularly important. Final interpretation should be a synthesis of the histological findings with information from such haematological and other investigations, interpreted with due regard to clinical context.

The technical challenges and diagnostic complexity of bone marrow trephine biopsy specimens (BMT) are insufficiently appreciated. Problems arise commonly as a result of poor information accompanying the specimen, poor specimen quality, insufficient sections and stains and inexperience in interpreting BMT histology. No other specimen type is regularly encountered in the histology laboratory that represents only half of the sample for interpretation, with the other half (aspirated bone marrow) being handled in another laboratory and often reported through a separate pathway. Arguably, there is also no other field of diagnostic histopathology in which the pathologist routinely needs so much additional information from diverse investigations to make a full interpretation. Sources of potential error are therefore numerous (box 1).

There has been a long controversy, reflected in ongoing differences in local practice, as to whether haematologists or histopathologists are best placed to report BMT. Clearly, local expertise varies but, with the strengthening position of haematopathology as a subspecialisation in histopathology and a continuing decline in the direct involvement of haematologists in laboratory work, the case for BMT to be reported primarily by haematopathologists becomes increasingly persuasive. However, the fundamental caveat in this regard is that haematopathologists cannot work in isolation from their haematology colleagues in this task; clinical information, blood and marrow aspirate findings, genetic and imaging data are all crucial elements in the dialogue required for BMT interpretation (box 2). There is no place in current pathology services for BMT reporting by histopathologists who do not have specialist knowledge of this particular field and the ability to work closely with colleagues in haematology and genetics. There has been no audit of BMT reporting practice (haematologist vs histopathologist or specialist vs non-specialist histopathologist) equivalent to those undertaken regarding expert versus non-expert lymphoma diagnosis. It has to be suspected that a high frequency of discrepancies would be revealed by such comparisons.

GOOD INFORMATION

It hardly needs saying that the production of an accurate BMT report requires provision with the specimen of accurate clinical information, stating clearly the context and questions that are being investigated by the procedure. It is particularly important in any investigation of neoplastic disease that it is clear whether the specimen represents a primary diagnostic investigation, a staging procedure or a re-examination to assess response to treatment (including transplantation). The type and timing of previous bone marrow transplantation are also important factors, because kinetics of engraftment differ between conditioning regimes and graft types. Knowledge of the recent therapeutic use of growth factors such as granulocyte colony-stimulating factor is a further matter of great relevance, because these may transiently have major modifying effects on haemopoiesis that can mask or mimic genuine pathology.

A GOOD SPECIMEN: COLLECTION

There is no single definition of the perfect BMT, since requirements in different clinical contexts vary and there is always a need to balance specimen size and quality against discomfort or potentially major risks such as haemorrhage for each individual patient. In general, the amount of extramedullary, cortical and immediately subcortical tissue included in the sample should be minimised, because their presence compromises the successful capture of deeper, more informative, elements and may be misleading (eg, physiological but unrepresentative hypocellular subcortical marrow mimicking hypoplasia, or extramedullary tissue inclusion mimicking metastatic non-haemopoietic malignancy). Premature trocar removal from the trephine needle is the usual cause of the excessive collection of these unwanted elements in BMT. It is also important to avoid introducing the BMT needle into the track created by immediately preceding aspiration (figure 1), and to try to avoid previous BMT tracks when repeat sampling is
undertaken after a relatively short interval (within 2–3 months of preceding BMT). Haemorrhage, microfracturing of trabecular bone and focal tissue scarring, all of which may be misinterpreted as genuine pathology, take this amount of time to resolve.

There is only limited guidance about optimum specimen size1–3 but, for diffuse pathologies, examination of a minimum of three intact intertrabecular spaces is the accepted dogma. This is much easier to achieve, and requires a shorter BMT core, using a wider bore trephine needle than a narrower one. Operators who use wide bore needles regularly will appreciate the greater stability of the process; many become highly proficient in obtaining cores that are not only of excellent integrity because of their substantial width but also of exceptional length. Using an 8-gauge needle (~4 mm diameter core) makes it relatively easy to obtain cores of 3–4 cm length with minimal traumatic artefacts from rotation, rocking or compression—decompression. The histological consequences of these artefacts are illustrated in figure 2. There is no evidence that wider needles cause greater morbidity.45 Modern disposable needles should avoid all risk of a poor specimen due to bluntness, previously encountered on occasion with re-sharpened, re-usable needles. Many modern trephine needles also have ingenious capture devices to assist specimen retrieval.

**A GOOD SPECIMEN: LABORATORY ASPECTS**

Bone marrow trephine cores generally fix well, as they are usually expelled directly from the needle into fixative and they are relatively small specimens, readily permeable by the fixative solution. Standard neutral-buffered formalin or aceto-zinc formalin are both very suitable for use with BMT specimens regardless of subsequent processing. It is, however, critical to ensure an adequate period of fixation before decalcification and processing for wax embedding, or before exposure to heat generated during resin embedding. Resin embedding is used relatively little now although it affords superb thin sections in laboratories with appropriate expertise. However, the potential for immunostaining and PCR is more restricted with resin-embedded specimens, whereas EDTA-decalcified, wax-embedded sections allow a superb range of immunostains with current antigen retrieval techniques. EDTA decalcification also generally preserves good quality DNA for PCR studies, and fluorescence in-situ hybridisation for lymphoma-associated chromosomal translocations is entirely feasible using intact EDTA-decalcified BMT sections. Premature exposure to EDTA before adequate fixation, or exposure to poorly buffered EDTA (excessively alkaline), causes severe loss of morphological definition; it also destroys many antigens and degrades nucleic acids.

**Box 1 Sources of error in interpreting BMT histology**

- Inadequate clinical, haematological (blood and aspirate findings), genetic and radiological information
- Inadequate specimen
  - Too small
  - Too crushed/distorted
  - Both
  - Poorly decalcified/processed
- Inadequate sections (thickness, number of levels…)
- Inadequate stains (poor technical quality, range too limited…)
- Insufficient experience to avoid common pitfalls (eg, differential diagnosis of granulomas or fibrosis)
- Insufficient confidence to avoid concluding ‘consistent with…’
- ‘Invisible’ pathology
- Forgetting to look at the bone trabeculae and stroma

**Box 2 Summary: avoiding errors in BMT interpretation requires**

- Dialogue between pathologists, haematologists, other clinicians and scientists
- Time
- Knowledge
- An adequate specimen and facilities for full analysis
- Excellent technical and clerical staff support
- More dialogue with other clinicians, including other haematopathologists, if you are a haematopathologist

**Points of particular note regarding immunostaining and in-situ hybridisation of BMT sections**

Relevant general points about use of immunohistochemistry in haematopathology have been included in the companion article on avoiding errors in lymph node diagnosis.6 It is important to keep in mind that not all antibody reactivities are directly equivalent in bone marrow and lymphoid tissues. This largely reflects the additional expression of some lymphoid cell-associated antigens by myelomonocytic cells. Of particular note is the need for caution when using CD43 as a potential marker of T cells or abnormal B cells in bone marrow.

Many other T-cell-associated antigens are also expressed by myelomonocytic cells at early stages of differentiation, which can complicate the interpretation of lymphoid infiltrates and lineage assignment in acute leukaeemias. Conversely, as in lymphoid tissues, it should be remembered that the variable loss of such antigens is common in many T-cell lymphomas. Antibody clone selection can improve the utility of immunohistochemistry for some T-cell-associated antigens in bone marrow. Clones UCHL1 and OPD4, reactive with different epitopes of the CD45RO antigen, perform similarly when used for immunostaining of lymphoid tissues. However, OPD4 has much less myeloid reactivity and so is preferable for use as a T-cell marker in bone marrow specimens.

**Figure 1** Trephine needle introduced along a track coinciding with that created by the immediately preceding aspiration. A linear zone of tissue loss and haemorrhage is seen. With time, granulation and either regeneration or fibrous scarring will progressively repair this deficit. H&E-stained bone marrow trephine biopsy section.
Such variations between the performance of antibodies in lymphoid tissue and bone marrow highlight the need, whenever possible, to use BMT sections (normal or containing relevant pathology) as positive controls for bone marrow immunohistochemistry. It may be misleading to rely on sections of tonsil or other readily available surplus lymphoid tissue.

Haematogones, which are physiological B-cell precursors in the marrow that express CD10 and (variably) terminal deoxynucleotidyl transferase (TdT), should not be misinterpreted as subtle interstitial infiltrates of follicular lymphoma (FL) or B-acute lymphoblastic leukaemia (B-ALL). Most of these cells lack CD20 expression, despite CD79a positivity, and they are abundant in early childhood, so that they are particularly likely to raise concern with regard to early, or to minimal residual, B-ALL if their true nature is not recognised (see below). They also occur in adult bone marrow, particularly during regeneration following chemotherapy, but not always with an identifiable cause (figure 3).

Immunostaining of immunoglobulin heavy and light chains is technically challenging in all tissues but presents particular problems in bone marrow, in which the interstitium is rich in tissue fluid containing abundant immunoglobulins. High background staining is therefore almost always encountered, making specific staining of lymphoid cell membranes and plasma cell cytoplasm difficult to assess. For plasma cell infiltrates, cleaner and more readily interpretable results for light chain determination can be achieved by the use of in-situ hybridisation to demonstrate messenger RNA rather than protein (figure 4). Unfortunately, this technique remains insufficiently sensitive to demonstrate the low level of mRNA in other lymphoid cells. In-situ hybridisation can conveniently be incorporated alongside immunohistochemistry on automated platforms or performed on the bench using reagents supplied in kit form.

**MAKING THE MOST OF THE PATHOLOGY YOU CAN SEE**

It is beyond the scope of this article to summarise the entire field of histological assessment of BMT specimens. It is important for haematopathologists and haematologists reporting these specimens to establish a sound basic knowledge from current textbooks, updated with topical review articles and research papers, plus attendance at relevant educational events. It is wise practice, and not an admission of failure, to seek specialist support in difficult or rare cases, or simply for increased confidence in areas of uncertainty; the feedback received is generally educational in its own right.

Key differential diagnostic scenarios requiring a good conceptual understanding and systematic approach to analysis are summarised in box 3. Some of these topics are discussed briefly later in this article.
It is important to appreciate the value of immunostains and, increasingly, molecular investigations in assessing BMT specimens; there is a tendency among haematologists, as most work is at a remove from the histopathology laboratory, to under-use immunostains on trephine sections, and to place too great a reliance on morphological interpretation alone. A simple example is the underestimation of the full extent of increased megakaryocytes and the spectrum of their variation in myeloproliferative or myelodysplastic conditions because our familiarity is with readily visible, large, normal cells of moderate to high ploidy. Only with immunostaining for CD42b or CD61 can true micromegakaryocytes and many other small atypical variants be identified (figure 5). The reciprocal erroneous tendency of haematopathologists is to pay insufficient attention to cytological features in aspirated bone marrow and the results of flow cytometry.

DEALING WITH DISCORDANCE BETWEEN ASPIRATED MARROW AND BMT
Discrepancies between the findings in aspirated bone marrow and the paired trephine specimens from individual patients are inevitable. Focal or ‘sticky’ pathologies, such as compact lymphoid or plasmacytic aggregates, will be underrepresented in aspirates. Criteria for recognition of leukaemic blast cells are much better defined in aspirates than in trephine specimens and histological criteria tend to be conservative. Genuine sampling differences will also arise sometimes, particularly when good practice is followed to minimise specimen trauma, with aspirate and trephine needles being directed deliberately to different parts of the iliac crest. It is unrealistic to expect information from these two types of specimen always to be equivalent, and it is important to recognise the circumstances in which one is more likely than the other to represent the marrow as a whole.

A valuable rule of thumb, which can save great embarrassment in reporting trephine specimens, can be encapsulated as follows: if something is seen in trephine sections, it may be absent from the aspirate but, if something is found in the aspirate, it should also be in the trephine specimen.

RECOGNISING ‘INVISIBLE’ PATHOLOGY
Pathology is sometimes represented by the absence of features that should normally be present. This is particularly the case in hypoplastic states. In these circumstances, the aspirated marrow and trephine specimen will generally match, although there are more artefactual reasons for a dry/blood aspirate tap mimicking aplasia than for a falsely empty-appearing trephine core. The major differential diagnosis in severely hypoplastic or aplastic bone marrow lies between true aplasia, a hypoplastic myelodysplastic syndrome or hypoplastic acute leukaemia.

Normal haemopoietic components may be invisible because of maturation arrest or altered maturation, as in megaloblastic anaemia or the myelodysplastic syndromes (MDS). In these cases, aspirate cytology is usually the most sensitive investigation, with histology being supplementary. It is crucial not to be misled by sometimes alarming histology in these circumstances; megaloblastic anaemia can mimic acute myeloid leukaemia if blood and aspirate findings are not considered. In hypercellular MDS, simple histological assessment in H&E or Giemsa-stained sections will tend to overestimate the number of immature haemopoietic cells. Despite studies attempting to equate or substitute CD34-positive cell counting in trephine sections from MDS patients7–9 with blast cell or CD34-positive cell counting...

Box 3 A systematic approach to diagnosis is particularly important for

- Assessing patterns of lymphoid infiltration associated with various lymphomas, especially small B-cell lymphomas
- Differential diagnosis of granulomatous pathologies
- Assessing key histological features of myelodysplastic and myeloproliferative haemopoiesis
- Differential diagnosis of bone marrow fibrosis
- Differential diagnosis of hypoplasia/aplasia
in aspirates, correlation is not precise. Aspirate-based methods probably remain the more reliable modalities at present and are of increasing importance as immunophenotypic and gene expression subset analyses of haemopoietic cells gain importance in prognostication.10–13 It is important not to over-call the aggressiveness of MDS on the basis of histological features. On the other hand, it is important to appreciate that features such as micromegakaryocytes, megakaryocyte clustering and abnormal localisation of immature precursors can only be assessed adequately by histology supplemented by immunohistochemistry. Histology and immunohistology are also critical in patients with hypoplastic bone marrow suspected of showing myelodysplasia.

While dense interstitial and solid lymphoid or plasma cell infiltrates in trephine sections are usually readily visible, dispersed interstitial infiltrates may be almost completely invisible without immunostaining (figure 6). These infiltrates are a paradigm for the situation in which an aspirate with evident infiltration appears discordant with unremarkable trephine histology. Close examination of histological features and an immunohistochemical screen for lymphoid or plasma cells are essential.

In undertaking more immunohistochemical screening for such lymphoid cells, however, one normally invisible population may cause unnecessary concern if not recognised. These are the haematogones, described above. They are CD79a and CD10 positive but most lack CD20. Most are also TdT positive but, as they represent a developmental spectrum, heterogeneity is found; TdT is lost and CD20 gained as the cells mature. Leukaemic blasts in B-ALL, with which haematogones may be confused, have a more uniform immunophenotype and, at the minimal detectable level by histology, form small clusters in the interstitium rather than being truly dispersed, as haematogones are. Occasionally, it can be extremely difficult to make this distinction with certainty; however, B-ALL will progress rapidly from such subtle appearances while haematogones will remain stable or decline over time. Consequently, re-examination of the bone marrow after a short interval will usually resolve any dilemma. Re-aspiration should involve, as a priority, providing a sample for flow cytometry, because the differing antigen expression patterns of haematogone subpopulations are readily resolved by multicolour immunofluorescence.

Lymphomas having a tendency for intravascular infiltration within bone marrow, unless very extensive or forming additional solid aggregates, and may be missed without immunostaining. Of particular note in this regard are splenic marginal zone B-cell lymphoma (SMZL), intravascular large B-cell lymphoma, anaplastic large cell lymphoma and, although rare, hepatosplenic T-cell lymphoma.

In the case of plasma cell infiltrates, whether obvious or inapparent, there may be accompanying invisible pathology in the form of vascular or interstitial amyloid deposition. When sought systematically, amyloid can be demonstrated in trephine specimens in a small but significant proportion of patients with plasma cell neoplasia (monoclonal gammopathy of unknown significance - MGUS - or myeloma).14 It may emerge late in the course of disease. Consequently, not only initial diagnostic specimens but also those obtained during follow-up should be screened by Congo red or Sirius red staining and visualisation under polarised illumination.

Some ‘foreign’ infiltrates in bone marrow are also notorious for invisibility and these are frequently not represented or are not easily recognisable in aspirate films. The subtle infiltration of bone marrow by metastatic breast carcinoma is a well-known occurrence, sometimes after many years of remission and without other evidence of disease recurrence. In contrast with overt involvement, in which there is usually a marked fibrotic stromal reaction to tumour cells, minimal infiltrates may provoke no response and be virtually invisible unless revealed by immunohistochemistry (figure 7). In any patient with a history of breast cancer, immunostaining with broad-spectrum anticytokeratin antibodies is indicated as a screen for this form of otherwise invisible pathology.

**Figure 5** Greater megakaryocyte variation can be appreciated with immunostaining, in this case with CD61 (A), when compared with the impression gained from H&E staining alone (B).

**Figure 6** Interstitial infiltration by T-cell large granular lymphocytic leukaemia. Peripheral blood and marrow aspirate indicated CD8-positive T-cell lymphocytosis with cytology suggesting large granular lymphocytic leukaemia. The infiltrate is virtually invisible in an H&E-stained bone marrow trephine biopsy specimen section (A) plus high magnification insert, but is readily revealed by immunohistochemistry for T-cell-associated antigens; (B) immunostained for CD8.
BE FAMILIAR WITH BONE MARROW STROMA

It is important to assess the bone marrow stroma systematically in all cases. Trabecular architecture provides clues to abnormal calcium metabolism (as in renal or hyperparathyroid osteodystrophy). Sensitive assessment of osteomalacia requires the use of non-decalcified sections but the increased non-mineralised osteoid can be seen in standard sections if severe; Giemsa staining is helpful for visualisation; Paget disease, osteosclerosis and osteopenia or osteoporosis can be appreciated in standard sections. Osteonecrosis secondary to tumour infiltration or fibrosis and osteopenia or osteoporosis can be appreciated in standard sections. Osteonecrosis secondary to tumour infiltration or treatment indicates particularly severe stromal injury that may impair subsequent haemopoietic recovery. Recognition of abnormal new bone formation is critical in assessing primary myelofibrosis and myelofibrotic progression from other myeloproliferative neoplasms.

Fibrosis and necrosis affecting non-bony stromal components have very diverse causes; because such changes are always secondary to other events, attempts should be made to identify the underlying cause. For fibrosis, artefact, scarring from previous sampling or a reaction to metastatic tumour, plasma cell myeloma or myeloproliferative neoplasm should always be considered. In the differential diagnosis of the myelodysplastic syndromes and chronic myeloproliferative neoplasms, fibrosis ranging from minor to severe, accompanied by other inflammatory changes such as oedema and macrophage activation, should prompt consideration of an alternative, inflammatory or toxic cause (figure 8) for inflammatory myelopathy. For necrosis, context is essential (diagnostic specimen or patient undergoing myelotoxic treatment for known disease). In the absence of an established diagnosis, limited immunostaining for antigens that survive cell death should be considered; CD20 and some cytokeratins (the latter detectable with broad-spectrum anti-cytokeratin antibodies) may still be shown reliably in necrotic neoplastic infiltrates. Examination of sections from additional deeper levels may reveal viable or semiviable tissue to assist interpretation.

Macrophage infiltrates and other pathologies that resemble granulomatous infiltration are a frequent source of diagnostic confusion. In particular, the possibility of abnormal mast cell infiltrates mimicking granulomas should always be considered. As mast cells express CD68 and CD68R, their true nature requires confirmation by immunostaining for tryptase or CD117. Small granulomas containing epithelioid macrophages are relatively common in patients with Hodgkin lymphoma and do not indicate direct bone marrow involvement by the neoplasm. Such granulomas may also accompany infiltrates of small B-cell lymphomas and, in that context, should prompt investigations to exclude reactivation of tuberculosis secondary to immunosuppression caused by the disease or its treatment. Dispersed populations of unusual storage macrophages (Gaucher type and Pick type) may also be present. If not accompanying pathology in which high cell turnover can be inferred, investigations for underlying storage disorders may be required.

LYMPHOID INFILTRATES

Detailed discussion of the differential diagnosis between reactive and neoplastic lymphoid aggregates in bone marrow histology is beyond the scope of this article. It is worth noting, however, that immunostaining is often of limited value unless a distinctive phenotype of small lymphocytic lymphoma/chronic lymphocytic lymphoma (SLL/CLL) or mantle cell lymphoma (MCL) is demonstrated. Helpful tips to remember are that reactive lymphoid aggregates are rarely numerous, except in very elderly individuals, and that reactive lymphoid cells do not form paratrabeclar infiltrates (unless other pathology is present to distort the stroma). If BMT specimens have been appropriately decalcified, clonality analysis by PCR to detect immunoglobulin heavy chain (IGH) and T-cell receptor (TCR) gene rearrangements can be helpful, but it is important to remain aware that the detection of monoclonality does not equate with a diagnosis of lymphoma. The PCR results themselves will require careful interpretation in the particular context of each case.

In the differential diagnosis of small lymphoid cell aggregates strongly suspected of, or clearly representing, lymphoma it is worth remembering that paratrabeclar infiltration should not be present in SLL/CLL and that follicles are only rarely formed in
bone marrow by FL, which typically forms paratrabecular infiltrates. Follicle formation may be mimicked in SLL/CLL by proliferation centres, as reflected in their alternative designation as ‘pseudofollicles’. Dispersed interstitial infiltration is uncommon in FL and usual in all of the other common small B-cell lymphomas (SLL/CLL, lymphoplasmacytic lymphoma, MCL and SMZL). Intrasinusoidal infiltration is associated particularly, although not specifically, with SMZL and MCL. Patterns are generally mixed in any individual specimen, apart from pure paratrabecular involvement in some cases of FL and pure nodular infiltration in some examples of SLL/CLL. Pure or almost exclusively intrasinusoidal small B-cell infiltration should raise the possibility of persistent polyclonal B lymphocytosis, a rare disorder found typically in female patients who are heavy smokers. The cellular immunophenotype in persistent polyclonal B lymphocytosis resembles that of SMZL apart from the absence of light chain restriction. Examined closely, however, the cells have distinctive, bi-lobed nuclei that can be appreciated in histology as well as cytology preparations.

In patients undergoing bone marrow re-staging after lymphoma treatment with regimes that have included anti-CD20, two potential pitfalls should be remembered. First, CD20 is down-regulated by residual B cells after such treatment, sometimes for weeks or months, so that alternative immunohistochemical markers should be used to assess residual B-cell populations. Second, T-cell-rich lymphoid nodules are common after such treatment and do not seem to indicate persistent disease.

PROVIDING A GOOD REPORT

A good report is systematic, concise, informative, accurate, contextual and timely. Context includes not only the patient’s current clinical circumstances and parallel investigations (some undertaken in separate laboratories, such as haematology, cytogentic and molecular genetic laboratories) but also their previous history and histology. Considerable review and cross-referencing of other specimens may be required and these actions should not be skimped even though they can be time-consuming. It is to be hoped that the ongoing development of regionally centralised haematological malignancy diagnostic services will support the wider availability of computing tools to make compiling of such diverse data a lesser challenge. The diagnostician’s time and skills can then be more valuably devoted to integration and synthesis.

Other aspects of creating a good bone marrow trephine biopsy report are fundamentally no different to those for other histology specimens. It is important to be aware of the strengths and weaknesses arising from one’s professional background. Haematologists reporting trephine specimens are likely to know the patient as their clinician but most are not experts in tissue structure and pathology. Histopathologists reporting these specimens will have a wider appreciation of tissue organisation and pathological responses but will be working at arms length from clinical care of the patient, often supplied with synoptic or frankly inadequate background information. From both perspectives, dialogue is the best, if not the only, solution to providing an appropriate report.

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