ICSH guidelines for the standardization of bone marrow specimens and reports

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SUMMARY

The bone marrow examination is an essential investigation for the diagnosis and management of many disorders of the blood and bone marrow. The aspirate and trephine biopsy specimens are complementary and when both are obtained, they provide a comprehensive evaluation of the bone marrow. The final interpretation requires the integration of peripheral blood, bone marrow aspirate and trephine biopsy findings, together with the results of supplementary tests such as immunophenotyping, cytogenetic analysis and molecular genetic studies as appropriate, in the context of clinical and other diagnostic findings. Methods for the preparation, processing and reporting of bone marrow aspirates and trephine biopsy specimens can vary considerably. These differences may result in inconsistencies in disease diagnosis or classification that may affect treatment and clinical outcomes. In recognition of the need for standardization in this area, an international Working Party for the Standardization of Bone Marrow Specimens and Reports was formed by the International Council for Standardization in Hematology (ICSH) to prepare a set of guidelines based on preferred best practices. The guidelines were discussed at the ICSH General Assemblies and reviewed by an international panel of experts to achieve further consensus.

CONTENTS

1. INTRODUCTION

Examination of the bone marrow (BM) aspirate and trephine biopsy is essential for the diagnosis of BM disorders. A comprehensive diagnosis of a BM disorder often requires the integration of various diagnostic approaches. These include peripheral blood (PB) counts and smear evaluation, BM aspirate smear, particle clot section, BM trephine biopsy and imprint morphology, together with results of other relevant investigations such as cytochemistry, immunophenotypic analysis, cytogenetic and molecular genetic techniques, as well as biochemical and microbiological test results, as appropriate. The final interpretation should be in the context of clinical and preliminary diagnostic findings. Current methods for the preparation, processing and reporting of BM specimens can be highly variable. The lack of uniformity can lead to inconsistencies in disease diagnosis and classification, and thereby affect treatment and clinical outcomes. In an attempt to standardize the indications for BM examination, the specimens required and report format, an international Working Party for the Standardization of Bone Marrow Specimens and Reports was formed by the re-registered and independent foundation of the new International Council for Standardization in Hematology (ICSH) (McFadden *et al.*, 2008) in Miami, FL, USA, in April 2007. By e-conferencing, the Working Party drafted a set of consensus guidelines that were presented to the ICSH General Assemblies in London, UK, in October 2007, and in Sydney, Australia in May 2008. After revisions, the guidelines were presented to an international panel of experts for further comment prior to publication.

An extensive discussion of the technical aspects of the procedures for obtaining BM aspirates and trephine biopsies is beyond the scope of the guidelines and the reader is referred to several monographs for these details (Brynes, McKenna & Sundberg, 1978; Frisch & Bartl, 1999; Perkins, 1999; Bain, 2001a,b; Foucar, 2001; Fleming, Kutok & Skarin, 2002; Lewis, Bain & Bates, 2006). Where pertinent, some brief comments are provided.

Although the Working Party makes the following recommendations based upon preferred best practices, it recognizes that there may be variations in practice because of costs or practical constraints, and that these variations may be acceptable in the local context.

1.1 Indications for BM examination

The indications for BM examination are listed in Table 1. The aspirate and trephine biopsy provide complementary and useful information. It is recommended that both BM aspirate and biopsy be routinely performed so that respective findings can be correlated. However, in some clinical situations, a BM aspirate alone may suffice. If a trephine biopsy is

Table 1. Indications for bone marrow examination

Investigation of unexplained anaemia, abnormal red cell indices, cytopenias or cytoses
Investigation of abnormal peripheral blood smear morphology suggestive of bone marrow pathology
Diagnosis, staging and follow-up of malignant haematological disorders (e.g. acute and chronic leukaemias, myelodysplastic syndromes, chronic myeloproliferative disorders, lymphomas, plasma cell myeloma, amyloidosis, mastocytosis)
Investigation of suspected bone marrow metastases
Unexplained focal bony lesions on radiological imaging
Unexplained organomegaly or presence of mass lesions inaccessible for biopsy
Microbiological culture for investigations of pyrexia of unknown origin or specific infections, e.g. military tuberculosis, leishmaniasis, malaria
Evaluation of iron stores
Investigation of lipid/glycogen storage disorders
Exclusion of haematological disease in potential allogeneic stem cell transplant donors

indicated and not performed, or is not obtainable, a particle clot preparation is recommended. If an aspirate is unobtainable (e.g. in a 'dry tap'), trephine imprints should be prepared.

1.2 Preliminary procedures

It is important that the operator is aware of the clinical indications for the marrow examination and the specimens required to be taken. The operator should also be aware of the need for adequate sedation and analgesia, and of safety issues with regard to thrombocytopenia or coagulopathic risks, which may require platelet transfusion support or the reversal of anticoagulation. Special considerations of the site for BM examination and positioning of the patient should be given to immobile patients, obese patients, paediatric patients or infants, patients with lytic bone lesions or BM necrosis, or who have had prior radiotherapy. Before the BM examination, the following should be performed:

- The procedure should be explained in detail to the patient. The past clinical history of the patient should be obtained, any allergies and co-morbidities documented and any premedications explained.
- Informed consent should be obtained from the patient. Consent should also be obtained, according to local guidelines, for the use of any specimens for non-diagnostic purposes, e.g. research, tissue banking, education or quality assurance.
- A blood count and smear should be obtained if these have not been collected in the previous 2 days.

1.3 Anatomic site

The preferred anatomic site for BM aspiration and trephine biopsy is the posterior iliac crest. The anterior iliac crest can be used if the patient is immobile. The medial surface of the tibia can also be used in infants. A sternal aspirate may be appropriate in certain circumstances, e.g. if the patient is immobile, has received radiotherapy to the pelvis or other sites have yielded a 'dry tap' or if a trephine biopsy is not required. Sternal aspiration should only be performed by an experienced operator who is aware of the risk of cardiac tamponade. It should be noted that sternal aspiration should not be attempted in patients with suspected plasma cell myeloma or other disorders associated with bone resorption. If there is a known focal bone lesion (from radiological imaging), diagnostic information may be obtained if a needle aspirate and bone biopsy is also performed at the site.

Either the aspirate or the trephine biopsy may be performed first. If the aspirate is performed first, the trephine biopsy should be performed through the same incision, approximately 0.5–1 cm away from the site of aspiration to avoid obtaining a damaged or haemorrhagic trephine biopsy. If the trephine biopsy is performed first, the aspirate needle should be positioned on the bone surface approximately 0.5–1 cm away from the biopsy site, to avoid clotting of the aspirate. It is recommended that the aspirate and trephine biopsy be obtained using the respective needles separately, and not through a trephine needle. If the aspirate is performed with a trephine needle, the aspirate sample may be haemodiluted. If the biopsy is then performed using the same needle, the trephine core may be damaged or haemorrhagic.

2. THE BM ASPIRATE

2.1 Collection of aspirate specimens

The collection and processing methods for BM aspirates and trephine biopsy specimens are outlined in Table 2. It is recommended that the aspirate should be drawn with a 10- or 20-ml plastic syringe, to provide adequate negative pressure, attached to the aspiration needle. To preserve morphology, the syringe should not contain anticoagulant. Approximately 0.5 ml of the first draw of the aspirate should be collected to make BM smears by the bedside. With increasing volumes of BM aspirate drawn, there is progressive dilution of the aspirate with PB. Additional aspirate should be placed into a tube containing ethylene diamine tetra-acetic acid (EDTA) to make smears, in case the sample clots rapidly. The amount of aspirate should be proportional to the amount of EDTA in the tube to minimize EDTA-induced artefacts. For peripheral blood samples, the ICSH recommends the dipotassium EDTA salt at a concentration of 1.50 ± 0.25 mg/ml of blood (ICSH,

Table 2. Collection and processing of bone marrow aspirate and core biopsy specimens

Specimen	Test	Anticoagulant or media	Fixative	Further processing	Staining (no. of slides or sections)*
Aspirate	Smear (6 slides)	None/EDTA	Air dry, methanol-fix	–	MGG or Wright stain (2 slides), Prussian Blue (1 slide), cytochemistry
Aspirate	Squash (≥2 slides)	None/EDTA	Air dry, methanol-fix	–	MGG or Wright stain (1 slide), Prussian Blue (1 slide)
Aspirate	Particle clot	–	NBF, AZF, B5, Bouin's etc	Paraffin embed, cut sections	H&E (3 sections), Giemsa, IHC, histochemistry, FISH
Aspirate	Flow cytometry	Heparin	Further processing according to specific protocols		
Aspirate	Molecular genetics	EDTA			
Aspirate	Cytogenetics, FISH	Sterile tissue culture media e.g. RPMI with 10% bovine fetal serum			
Aspirate	Microbiology	Sterile plain or heparinized tubes, lysis centrifugation tubes or culture media			
Core biopsy	Histology	–	NBF, AZF, B5, Bouin's, etc.	Decalcify, paraffin embed, cut sections	H&E (2–4 sections), reticulin (1 section), Giemsa, IHC, histochemistry, FISH
Core biopsy	Touch imprint (≥2 slides)	–	Air dry, methanol-fix	–	MGG or Wright stain (1 slide), cytochemistry

*Several smears and imprints should be left unstained for possible immunostains, cytochemical stains, FISH or DNA extraction. Additional sections of particle clots and BM biopsy specimens should be cut as required.
AZF, acetic acid–zinc–formalin; B5, mercuric chloride, sodium acetate and formalin; EDTA, ethylenediamine tetra-acetic acid; FISH, fluorescent *in-situ* hybridization; H&E, haematoxylin and eosin; IHC, immunohistochemistry; MGG, May–Grünwald Giemsa; NBF, neutral buffered formalin.

1993). An appropriate volume of aspirate may be added to a tube containing isotonic EDTA solution to achieve the desired concentration of EDTA. Alternatively, an appropriate volume of aspirate may be added to a paediatric EDTA tube.

A second syringe should be attached to the aspiration needle to draw additional samples for supplementary tests, such as flow cytometry, cytogenetic analysis and molecular genetic studies, microbiology, electron microscopy or BM culture. In the event of a 'dry tap', or if no particles ('fragments') have been obtained, the BM aspirate can be repeated at a slightly different angle or at another site. It is suggested that samples for flow cytometry, cytogenetics and molecular genetic studies be collected with all BM aspirates. The samples may be discarded if the investigations are considered to be unnecessary after the BM slides have been reviewed. The volumes required will be specified by the respective laboratories.

Bone marrow aspirate samples for supplementary tests should be collected as recommended by the local laboratory. Guidelines for the processing of BM aspirates for flow cytometry have been published by the Clinical Laboratory Standards Institute (CLSI, 2007). BM aspirate for flow cytometry should be placed into a sodium heparin tube. Dipotassium EDTA can be used, however, specimens must be analysed within 12–24 h (CLSI, 2007). In the event of a 'dry tap', a trephine biopsy core of 2–3 cm in aggregate length can be submitted for flow cytometric analysis in tissue culture media, such as RPMI in a sterile container. However, the quality of the cell suspension obtained by resuspending a biopsy is usually inferior to that of an aspirate, due to an increase in dead cells and debris. BM aspirates for cytogenetic analysis or fluorescent in-situ hybridization (FISH) should be collected as recommended by the cytogenetics laboratory, preferably into sterile tubes with tissue culture media such as RPMI with 10% bovine fetal serum for good cell viability, or into tubes with sodium heparin for subsequent transfer into tissue culture media in the laboratory. If the BM cannot be aspirated, a trephine biopsy specimen may be placed in sterile tissue culture medium or saline for cytogenetic analysis (Novotny *et al.*, 2005). Aspirate samples for molecular studies such as PCR or RT-PCR should also be collected as recommended by the molecular laboratory, usually into EDTA tubes. Specimens for microbiology should be placed in sterile plain or heparinized tubes, lysis centrifugation tubes or inoculated into appropriate culture media as specified by the microbiology laboratory. If specimens for BM culture or electron microscopy are required, they should be collected into appropriate media as specified by the respective laboratories. Excess aspirate can be used to make particle clot preparations or placed in an EDTA tube for a number of purposes, including making additional smears, flow cytometry, molecular genetic studies, or cryopreservation and tissue banking.

2.2 Preparation of aspirate slides

Bone marrow smears should be prepared immediately following aspiration. Smears prepared from EDTA samples should be made as soon as possible to reduce storage artefact. To prepare smears, the aspirate should be expelled into a small plastic or siliconized glass dish, and a Pasteur pipette used to draw up particles, which are placed on glass slides and then smeared. Alternatively, a drop of aspirate can be placed on each glass slide and the excess blood drained off the slide by tipping the slide, or aspirated with a Pasteur pipette or plastic syringe, before making the smear. Smears are made with a glass spreader with bevelled edges so that the width of the spreader is narrower than the width of the specimen slide. The spreader is placed in front of the drop of aspirate at an angle of approximately 30° and pulled back to make contact with the drop, to enable the drop to spread along the line of contact with the slide. The spreader is then pushed forward in a smooth action, in contact with the slide. A minimum of six smears and two particle squash ('crush') slide preparations should be made. To make a squash slide, a drop of BM containing particles is placed in the middle of one slide, and a second slide is placed on top of the first. The weight of the second slide on the first is sufficient to squash the marrow particles; no downward force should be applied. The slides are drawn apart away from each other, in the direction of the long axis of the slide. BM smears and squash preparations must be labelled at the bedside with the surname and first name or initial, unique patient identifier and date. The glass slides should be frosted glass at one end so that details can be written in pencil.

2.3 Preparation of particle clots

Particle clot preparations may provide additional information and can be made if a trephine biopsy is not taken or if one suspects that the biopsy was not adequate. To make a clot preparation, the aspirate is spread on a clock glass and a pipette or a 21-gauge needle is used to collect the BM particles into a small clot which is placed onto a piece of filter paper. Bovine thrombin powder (e.g. Thrombostat®, Parke-Davis, Rochester, MI, USA; Thrombin, tropical) may be added to facilitate clotting. The clot is dropped into a tube containing appropriate fixative as with trephine biopsy specimens and processed using the same method as biopsy specimens, except that decalcification is not required. Particle clot sections can be used for the assessment of BM cellularity, megakaryocyte morphology or tumour infiltrates complementary to a biopsy specimen, and also for immunohistochemistry (IHC) or FISH. An important advantage of particle clot preparations is the lack of decalcification-associated nucleic acid or protein damage. The clot sections should be reported as for the BM trephine biopsy specimen.

2.4 Staining of BM aspirate slides

Two air-dried smears and one squash slide should be fixed with fresh acetone-free absolute methanol and stained with a Romanowsky stain, such as May–Grünwald Giemsa (MGG) (Lewis, Bain & Bates, 2006) or Wright Giemsa stain (Peterson & Brunning, 2001; Woronzoff-Dashkoff, 2003). A methanol-fixed smear and a squash slide should be stained with Prussian Blue (Perls' reaction) and counterstained with Safranin-O or Kernecht Red (nuclear fast red). All BM smears should be coverslipped using a mounting medium that hardens and dries rapidly. Mounting media may contain toxic organic compounds such as toluene or xylene and should be handled with appropriate safety precautions. It is recommended that this should be performed in a chemical fume hood. After staining, a paper label should be affixed to the slide with the patient's identity details and date. Additional slides may be used for cytochemistry (e.g. myeloperoxidase or nonspecific esterases), IHC, FISH, or archived as unfixed, unstained smears, as required.

Spare BM aspirate slides may be wrapped tightly in aluminium foil for storage at −20° Celsius to preserve cellular antigens. In any subsequent processing, they should not be unwrapped until they have been warmed to room temperature, to prevent condensation. Unfixed and unstained aspirate smears stored at room temperature for long periods may give variable results on retrospective Giemsa staining. Aspirate slides fixed in absolute methanol preserve DNA (and possibly many antigens) for future FISH, or DNA extraction and subsequent PCR amplification.

2.5 Microscopy

The BM smear or squash preparation should first be viewed under low power magnification (×100) to determine the number and cellularity of particles, the number of megakaryocytes, and to scan for clumps of abnormal cells and for abnormal cells of low incidence. Areas of well-spread marrow cells in the cellular trails of the BM smear behind the particles are selected for assessment at higher magnification (i.e. ×200, ×400, ×600, ×1000) for morphological assessment of cells, including cytological detail, parasites or cell inclusions. BM smears are particularly useful for cellular detail and differential cell counts. The squash preparation is useful for the assessment of cellularity, megakaryocyte numbers, focal disease (e.g. lymphoma, plasma cell myeloma, mast cells, metastatic carcinoma, storage histiocytes, granulomas), fibrotic marrows, and the detection of abnormal cells of low incidence. In the absence of particles, megakaryocytes or other haemopoietic precursors, the sample should be reported as a 'blood tap' or peripheral blood. In the absence of particles, but in the presence of megakaryocytes or other precursor cells, the sample should be reported as a dilute BM sample and a qualitative evaluation can be performed. In the presence of particles with absent or very reduced cellularity, only a qualitative description should be provided. The patient's blood count and a PB smear stained with a Romanowsky stain should always be reviewed in conjunction with the aspirate slides.

2.6 The nucleated differential count

A BM nucleated differential cell count (NDC) should be performed to assess haemopoietic activity and to

compare the proportions of the different cell lineages with known reference ranges, and also to quantify abnormal cells, if present. The NDC should be performed in the cellular trails of the BM smear behind the particles, which is minimally diluted with PB. BM cells should be counted in an area where the cells are well dispersed with good cytological detail, and where there is the least number of smudged (lysed) cells.

The NDC should comprise blast cells, promyelocytes, myelocytes, metamyelocytes, band forms, segmented neutrophils, eosinophils, basophils, mast cells, promonocytes and monocytes, lymphocytes, plasma cells and erythroblasts. The NDC should not include megakaryocytes, macrophages, osteoblasts, osteoclasts, stromal cells, smudged cells or non-haemopoietic cells such as metastatic tumour cells. Lymphoid aggregates, if present, should not be included in the NDC, but their presence should be commented upon.

At least 500 cells should be counted in at least two smears when a precise percentage of an abnormal cell type is required for diagnosis and disease. At least 300 cells should be counted if the NDC is not essential to the diagnosis. To reduce imprecision from sampling error, the total number of cells counted in the NDC should be increased, by counting in another smear, or counted by a second observer, if the abnormal cell count is very close to a critical threshold for disease stratification or to a low threshold (e.g. 5%) or when the appearance suggests a patchy involvement of the BM with abnormal cells. The total number of cells counted in the NDC should be stated in the report. The counts obtained should be compared with published normal ranges for the NDC in adults (Brunning & McKenna, 1994; Lewis, Bain & Bates, 2006) or in infants and children (Glaser, Limarzi & Poncher, 1950; Rosse *et al.*, 1977).

The myeloid:erythroid (M:E) ratio should be calculated by expressing the ratio of all granulocytes and monocytes and their precursors (i.e. myeloblasts, promyelocytes, myelocytes, metamyelocytes, band forms, segmented neutrophils, eosinophils, basophils, promonocytes and monocytes) to erythroblasts (at all stages of differentiation). Flow cytometric differential counts should not be used as surrogates for the NDC obtained from the smear. Flow cytometry and morphology are complementary methods that give different and valuable information, but the degree of correlation varies greatly.

2.7 Storage iron

A Prussian Blue stain should be performed on a BM smear for the evaluation of storage iron and sideroblasts. A BM smear with increased iron stores should be included as a positive control. An iron stain should be performed on all initial BM aspirates, but may not be necessary on follow-up BM aspirates, e.g. for leukaemia. Squash preparations and particle clot sections can also be stained for iron. If there is a 'dry tap', a core biopsy section and imprint can be stained for iron. Core biopsy sections are less reliable than the aspirate for the assessment of storage iron, since decalcification removes storage iron. They should not be used for the assessment of sideroblast iron, which can be identified in core biopsy imprints.

The presence or absence of iron stores should be evaluated by examining BM macrophages in several particles in the BM smear. Iron stores in smears may be graded subjectively as absent, reduced, normal, increased, or markedly increased. It should be noted that quantitative estimates of iron stores are subject to observer variation and may not be reproducible.

The total number of sideroblasts (normal, reduced or increased) should be reported and the frequency and location (cytoplasmic or perinuclear) of siderotic granules (Bowman, 1961; Cartwright & Deiss, 1975) should be noted, if relevant to the diagnostic query. Ring sideroblasts are defined by the presence of five or more siderotic granules encircling one third or more of the nucleus in an iron-stained smear. At least 100 erythroblasts should be evaluated for the percentage of ring sideroblasts, if present.

2.8 Supplementary investigations

Additional investigations including flow cytometric immunophenotyping, cytochemistry, FISH, and molecular genetics can be performed on the BM aspirate. The reader is referred to recent guidelines (CLSI, 2007) for details of techniques and antibody panels for flow cytometric analysis of neoplastic haematolymphoid cells. Results of flow cytometry should be correlated with morphology and also with IHC performed on the corresponding histologic material, if obtained. For cases with suspected acute leukemia, cytochemical stains for myeloperoxidase and nonspecific esterases (e.g. α-naphthyl butyrate esterase) are recommended.

These are particularly valuable in cases in which flow cytometry is inconclusive or unavailable.

2.9 The aspirate report

A BM aspirate report should contain the information shown in Table 3. The appearances of the aspirate and the trephine biopsy specimen should always be correlated and if there are major discrepancies, the reason needs to be explained. It is desirable to combine the aspirate and trephine biopsy into one report (see 4. Combined BM aspirate and trephine biopsy report).

The aspirate report should include the results of the blood count, i.e. the haemoglobin concentration, total and differential white cell count (neutrophils, eosinophils, basophils, monocytes, lymphocytes and other circulating cells if present) and platelet count, and a PB smear comment. The adequacy of the aspirate should be mentioned in the report. Whether particles are present in the BM smear (particulate or aparticulate) or if the aspiration was a 'dry' or haemodilute tap should be stated.

The cellularity of BM particles should be evaluated by assessing several particles in smears or squash preparations. Cellularity is better assessed in squash preparations than smears. Cellularity can be described as acellular, reduced, normal, increased or markedly increased. It should be noted that overall BM cellularity is generally better assessed in trephine biopsy sections than the aspirate. Cellularity of particles and trails can differ, e.g. in fibrotic marrows, and if so, these should be described separately. Particles with an abnormal appearance should be commented upon; for example, necrotic, gelatinous and oedematous. The presence of background staining or intercellular proteinaceous material, rouleaux or crystals should be reported.

Quantitative and qualitative comments should be made for all cell lineages and any abnormal cells detected. The numbers (decreased, normal or increased), whether maturation is normal or abnormal and the morphology of the erythroid and myeloid lineages should be commented upon and described if abnormal. The numbers of blast cells should be reported. The numbers of lymphocytes and plasma cells and whether their morphology is normal or abnormal should also be noted. Megakaryocyte numbers and morphology should be documented; their numbers are best assessed in the trephine biopsy specimen. When macrophage numbers are increased, this should be documented and abnormalities of morphology (e.g. haemo- or erythro-phagocytosis, presence of inclusions such as micro-organisms or crystals, vacuoles or sea-blue histiocytes) recorded. An increase in the numbers of mast cells and any atypical morphological features or aggregates should be noted. Any abnormal cells or metastatic tumour cell aggregates should be described and the presence of significant numbers of smudged cells should also be documented. The results of the iron stain and other cytochemical investigations should be reported. The reader is referred to several monographs for images and descriptions of BM cell morphology (Brunning & McKenna, 1994; D'Onofrio & Zini, 1998; Glassy, 1998; Bain *et al.*, 2001; Foucar, 2001; Jaffe *et al.*, 2001; Orazi, O'Malley & Arber, 2006).

Relevant flow cytometric findings, if available, should be summarized in the aspirate report. The aspirate report should not be delayed whilst awaiting results of supplementary investigations and results that are pending should be noted in the initial report. When the results of molecular genetic studies are known and they impact upon diagnosis, they should be commented upon in a Supplementary Report, to be issued when the results of these or other supplementary investigations are available. An Amended Report may also be required if the final conclusion and diagnosis is altered as a consequence of these additional test results. If the slides were reviewed with another individual, this should be stated in the report with the name of the individual consulted, or the report should be signed out by both individuals.

The conclusion of the BM aspirate report should document the diagnosis or differential diagnosis, with reference to international consensus guidelines (Jaffe *et al.*, 2001) where applicable. Major findings may be summarized and other investigations to be undertaken mentioned. The findings should be compared with previous BM reports if the aspirate was performed for disease monitoring. If the aspirate was performed to confirm a clinical diagnosis and the result was negative, this should be stated. An appropriate disease code according to the SNOMED CT® (Systematized Nomenclature of Medicine – Clinical Terms®; http://www.snomed.org/) or ICD (International Classification of Diseases; http://www.who.int/classifications/

Table 3. The bone marrow aspirate report

Name of institution
Unique specimen identifier (laboratory accession number)
Details of patient: surname, first name(s), identification number, age or date of birth, gender, contact details (e.g. address, hospital location)
Name of responsible physician
Name of requesting doctor
Date of procedure
Significant clinical history including physical findings, recent chemo/radiotherapy, cytokine therapy and pertinent lab results.
Indication for bone marrow examination
Procedure performed (aspirate/trephine biopsy)
Anatomic site of aspirate/biopsy
Ease/difficulty of aspiration
Blood count: Haemoglobin concentration, total and differential white cell count (neutrophils, eosinophils, basophils, monocytes, lymphocytes) and platelet count
Blood smear description and diagnostic conclusion
Cellularity of particles and cell trails
Nucleated differential cell count
Total number of cells counted
Myeloid:erythroid ratio
Erythropoiesis
Myelopoiesis
Megakaryocytes
Lymphocytes
Plasma cells
Other haemopoietic cells
Abnormal cells (e.g. blast cells, metastatic infiltrates)
Iron stain
Cytochemistry
Other investigations (e.g. cytogenetics, PCR, FISH, microbiology)
Summary of flow cytometry findings, if available
Conclusion
WHO classification (if relevant)
Disease code
Signature and date of report

icd/en) terminology may be entered as required by national regulatory guidelines. The report should be signed manually or electronically and dated.

3. BM TREPHINE BIOPSY SPECIMENS

3.1 Collection of BM trephine biopsy specimens

The BM trephine biopsy may be performed either before or after the aspirate. The length of the core from an adult should be at least 2 cm. A shorter core (e.g. 1 cm) may sometimes contain sufficient diagnostic information. The biopsy specimen shrinks by approximately 20% after processing. However, the larger the amount of tissue that is biopsied, the greater is the likelihood of a focal lesion (e.g. lymphoma, metastatic tumour, granulomas) being detected. Bilateral trephine biopsies may be performed to increase the yield of detecting focal lesions.

When the core specimen has been obtained, a blunt stylet is inserted through the distal end of the biopsy needle to expel the core from the proximal end of the biopsy needle onto a glass slide. The specimen should be handled gently to avoid crush artefact and distortion. Touch imprints should be made from the trephine biopsy prior to placing in fixative. Imprints are especially important if there is a 'dry tap' on BM aspiration. Imprints are made by gently touching the fresh unfixed core on the slide, or the slide on the core. The imprints are fixed and stained using the same method as for aspirate smear and squash preparations. If no aspirate is available, the imprint may be the only specimen available on which to examine cell composition and cytologic detail and a NDC can be performed on a representative field of the imprint.

After the imprints have been made, the core specimen should be placed into a container with appropriate fixative. The container must be appropriately labelled at the bedside with the patient surname, first name, unique patient identifier, and date and time of collection, so that the time when the biopsy specimen should be removed from the fixative can be calculated.

3.2 Fixation

Trephine core specimens may be fixed by a number of different methods. Fixation methods can significantly affect morphology, cytological detail and immunoreactivity. A standard fixative is neutral buffered formalin for 6 h. Other fixatives commonly used include zinc formaldehyde, B5 (mercuric chloride, sodium acetate and formalin), AZF (acetic acid-zinc-formalin), IBF (isotonic buffered formalin), Bouin's fixative (picric acid, acetic acid and formaldehyde), or formaldehyde and glutaraldehyde. Fixation time varies depending on the fixative used, from a minimum of 1 h to maximum of >24 h. Although B5 gives good morphology with a short turnaround time (TAT), it contains mercuric chloride that has led to its prohibition in several countries, due to safety and environmental concerns. Picric acid, used in Bouin's fixative, is an explosive that has led to its prohibition in the United States. Neutral buffered formalin with EDTA decalcification gives adequate morphology, preserves antigens for IHC and nucleic acids for molecular studies.

3.3 Decalcification

Many different decalcification methods are available. Commonly used solutions are EDTA, formic acid, acetic acid, picric acid, nitric acid or commercial decalcifying agents (e.g. Shandon TBD.1®, Basingstoke, Hampshire, UK; Surgipath Decalcifier II®, Richmond, IL, USA). Decalcification time varies from 15 min to 72 h, depending both on the type of decalcifying agent as well as on the size of the biopsy specimen. Decalcification chelates storage iron, affects morphology and cytological detail, the ability to perform histochemistry and IHC, and to retrieve material suitable for molecular analysis. Decalcification with EDTA results in better preservation of nucleic acids, but is slower than with other acid reagents.

3.4 Processing of the trephine biopsy specimen

After decalcification, the biopsy specimen is embedded in paraffin wax and sections cut on a microtome. The recommended thickness of sections is two to three microns. At least six sections should be cut at three levels: 25%, 50%, and 75% into the cross-sectional diameter of the core, and serial sections mounted stepwise on glass slides. Additional sections need to be cut if IHC or histochemical stains are required.

Bone marrow core biopsy specimens can also be embedded in plastic. This gives good cytological detail, but is technically more difficult and limits the range of immunohistochemical studies (Brown *et al.*, 2006), and probably FISH. Plastic embedding does not require decalcification and may be useful for the evaluation of metabolic bone diseases and histochemical reactions that are ablated by the decalcification process (Brunning & McKenna, 1994).

3.5 Staining of sections

Trephine biopsy sections should be stained with haematoxylin and eosin (H&E) (Bancroft & Cook, 1994). Giemsa staining (Peterson & Brunning, 2001) may be carried out in addition to H&E stains. The Giemsa stain may be helpful for identifying plasma cells, mast cells, lymphoid cells, eosinophils, and for distinguishing between myeloblasts and proerythroblasts. One section may be stained for reticulin by the silver impregnation method (Gordon & Sweets, 1936; Gomori, 1937; Thiele *et al.*, 2005). A BM section previously shown to have increased reticulin should be included as a positive control since silver impregnation staining methods are fixation dependent. Automated reticulin staining methods should be correlated with manually stained slides in the laboratory before implementation. A trichrome stain may be used to identify collagen fibrosis, which is readily recognized in well-stained H&E specimens.

3.6 Microscopy

Two to four sections should be reviewed routinely. In lymphoma staging and in cases of suspected metastatic infiltrate, the chances of detecting a focal lesion are increased if more sections are reviewed (Jatoi, Dallal & Nguyen, 1999). If IHC is performed, additional sections will be reviewed.

Trephine sections are particularly useful for the assessment of overall marrow architecture and cellularity and provide greater sensitivity for the assessment of focal lesions and patchy infiltrates. The percentage cellularity may be obtained by estimating the proportion of cells occupying the total marrow cavity. BM cellularity varies with age and should be assessed with reference to the age of the patient. In healthy paediatric samples, cellularity is highest in patients younger than 2 years (approximately 80%), and declines in patients 2 to 4 years old (approximately 70%) and 5 to 9 years old (approximately 60%; Friebert *et al.*, 1998). Generally, iliac crest specimens gradually decrease from 60% cellularity after puberty to 30% by the 8th decade. Wide normal ranges often obscure this downward trend, which is in part related to loss of trabecular bone mass in the elderly (Hartsock, Smith & Petty, 1965; Frisch & Bartl, 1999). Intertrabecular spaces adjacent to the cortex are frequently hypocellular, particularly in the elderly, and should not be included in the assessment of cellularity.

Trephine sections should be viewed initially at low power (×40–×100) for adequacy, pattern, cellularity, presence of focal lesions, megakaryocyte number, abnormal cell clusters and location, bone structure (trabecular number and thickness), and osteoclastic and osteoblastic activity. The sections are next viewed under higher magnification (×200–×400) to assess haemopoietic activity (e.g. erythroid, myeloid, megakaryocytic lineages, lymphoid cells, plasma cells and macrophages) and cytological detail. Higher magnifications of ×600–×1000 may be useful for fine cytological details, e.g. intracellular granules, Auer rods.

Reticulin staining can be quantified by grading from 0 to 3 by a European consensus scoring system (Thiele *et al.*, 2005) or from 0 to 4 by alternative scoring systems (Bauermeister, 1971; Manoharan, Horsley & Pitney, 1979). The scoring system used must be stated. Reticulin may also be graded as normal, slightly increased, moderately increased, markedly increased or absent. Focal increase in reticulin (e.g. seen after therapy) should be commented on if necessary for diagnosis.

3.7 Supplementary investigations

Immunohistochemistry may be required for the determination of the lineage and differentiation stage of normal or abnormal cells or cellular infiltrates, detection of low level or minimal residual disease, monitoring a specific cell phenotype, assessment of extent of disease and focal disease, disease classification, detecting markers of prognosis, determination of clonality and disease monitoring. However, if the phenotype of an abnormal cell population is already known from the aspirate (e.g. by flow cytometry), IHC may not be necessary on the BM trephine specimen. Some phe-

notypic markers can be assessed by IHC on BM trephine specimens, but not by flow cytometry, e.g. nucleophosmin in acute myeloid leukaemia. IHC can be performed by immunoperoxidase or immunoalkaline phosphatase methods manually or using automation. It is not recommended that IHC be routinely performed on all trephine biopsy specimens.

A large range of monoclonal antibodies can be used on fixed decalcified trephine sections. It is beyond the scope of this guideline to give a complete list of all antibodies that can be utilized or all applications and the reader is referred to several monographs for further details of IHC on BM biopsy specimens (Bain *et al.*, 2001; Fleming, Kutok & Skarin, 2002; Gudgin & Erber, 2005; Brown *et al.*, 2006). The panel of antibodies required will depend upon the diagnostic query. The same principle applies to *in-situ* hybridization investigations which can be performed on BM biopsy specimens, although some fixatives and decalcifiers may render *in-situ* hybridization impossible.

Useful histochemical stains include Congo Red for amyloid, Ziehl-Nielsen stain for acid-fast bacilli, Gomori's methenamine silver (GMS) stain for fungi, and the periodic acid Schiff (PAS) reaction for carbohydrates. If the aspirate is a dry or aparticulate tap, sections may be stained with Prussian Blue to assess storage iron, however decalcification removes storage and sideroblast iron.

3.8 The trephine biopsy report

The trephine biopsy report should contain the information shown in Table 4. In the report, the aggregate length of the biopsy core, the macroscopic appearance and adequacy, integrity and quality of the specimen should be recorded. The percentage cellularity, pattern of cellularity and any necrotic, fibrotic or haemorrhagic areas should be noted. Bone architecture should be commented on if abnormal. The cellular composition should be described in terms of location, relative proportions, morphology and pattern of differentiation for erythroid, myeloid, megakaryocytic lineages, lymphoid cells, plasma cells and macrophages. Any abnormal cells if present should be described. The reader is referred to several monographs for images and descriptions of trephine biopsy sections (Wilkins, 1992; Brunning & McKenna, 1994; Bain *et al.*, 2001; Foucar, 2001; Jaffe *et al.*, 2001; Brown *et al.*, 2006; Orazi, O'Malley & Arber, 2006). The reticulin grade and the results of IHC and histochemical stains, whether positive or negative, should be reported, if performed. The results of FISH should also be included if available. If the procedure was performed for lymphoma staging, involved marrows should be compared with the original diagnostic marrow specimen, and similarities or discordant changes in histological grade noted.

The conclusion should summarize the findings and diagnosis or differential diagnosis, with reference to international consensus guidelines (Jaffe *et al.*, 2001) where applicable. An appropriate disease code may be entered as required by national regulatory guidelines. The trephine biopsy report should be correlated with the aspirate findings, and any discrepancies accounted for. The findings should be compared to previous results in the same patient when relevant. Any further investigations that are pending should be mentioned and results may be appended in a Supplementary Report at a later date. The report should be signed manually or electronically and dated.

4. COMBINED BM ASPIRATE AND TREPHINE BIOPSY REPORT

It is recommended that a combined BM aspirate and trephine biopsy report be issued. A protocol has been proposed (Peterson, Agosti & Hoyer, 2002). A combined report would merge all the information in Tables 3 and 4, and thus provide a comprehensive BM evaluation. An overall conclusion that integrates the findings of both aspirate and trephine biopsy specimens is recommended. This is best achieved if the aspirate and trephine biopsy are read by the same individual. If the specimens are reported by different individuals, a combined reporting session is recommended to reach the final diagnosis or differential diagnosis. If the slides are reviewed by more than one individual, the names of the individuals should be included in the report.

A final integrated consensus conclusion that includes all test results (e.g. flow cytometry, cytogenetics and molecular genetics) is also desirable, although this may be logistically difficult to achieve. Weekly clinical conferences in which BM findings are discussed by clinicians and pathologists are recommended to facilitate communications and to ensure a

Table 4. The bone marrow trephine report

Name of institution
Unique specimen identifier (laboratory accession number)
Details of patient: surname, first name(s), identification number, age or date of birth, gender, contact details (e.g. address, hospital location)
Name of responsible physician
Name of requesting doctor
Date of procedure
Significant clinical history including physical findings, recent chemo/radiotherapy, cytokine therapy and pertinent lab results
Indication for bone marrow examination
Procedure performed (aspirate/trephine biopsy)
Anatomic site of aspirate/trephine biopsy
Aggregate length of biopsy core
Adequacy and macroscopic appearance of core
Percentage and pattern of cellularity
Bone architecture
Location, number, morphology and pattern of differentiation for erythroid, myeloid, megakaryocytic lineages, lymphoid cells, plasma cells and macrophages
Abnormal cells and/or infiltrates
Reticulin stain
Immunohistochemistry
Histochemistry
Other investigations (e.g. FISH, PCR)
Conclusion
Disease code
Signature and date of report

consistent approach. Because not all data may be available, any pending data should be correlated when it becomes available. Digital images that demonstrate the major abnormal findings in specimens may be appended to the electronic report, if this function is supported by the laboratory information system.

5. VERBAL REPORTS

When a verbal report is given to a clinician, a comment should be added to indicate the name of the pathologist providing the information, to whom the report was given, and the time and date.

6. TURNAROUND TIMES

6.1 Aspirates

The processing TAT for the BM aspirate, or the time from collection of the aspirate to the time when slides are available for microscopy should be about two to six working hours. However, this may not be achievable with remote laboratories. If results are required urgently by the requesting clinician, the Reporting TAT, or the time from when the slides are available for microscopy to the time when a verbal or written report is issued, should be about three working hours

for a verbal report or up to 24 h for a written report. In less urgent cases, a written report on the aspirate alone should be available in 48 h, or in less than five working days if a combined aspirate and trephine report is issued.

6.2 Trephine biopsy specimens

The Processing TAT for BM biopsy specimens, or the time from collection of the specimen to the time when slides are available for microscopy will depend on fixation and decalcification regimens as well as individual laboratory procedures and should be as short as possible, usually 24–72 h. If the results are required urgently by the requesting clinician, the Reporting TAT, or the time from when the slides are available for microscopy to the time when a verbal or written report is issued, should be about three working hours for a verbal report or up to 24 h for a written report. In less urgent cases, a written report should be available in less than five working days. An additional 24–48 h may be required if IHC or other stains are performed.

7. STORAGE

The duration of storage of BM specimens and reports should comply with national regulatory guidelines. Where these are not available, BM slides should be stored for at least 20 years, or indefinitely, if possible. Digital images and electronic reports may be stored indefinitely.

8. EXTERNAL QUALITY ASSURANCE

Participation in external quality assurance (EQA) schemes for both technical and interpretative elements of BM examination are encouraged and recommended to ensure accuracy, reproducibility and standardization.

DISCLAIMER

While the advice and information in these guidelines is believed to be true and accurate at the time of going to press, neither the authors, ICSH nor publishers can accept any legal responsibility for the content of these guidelines.

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