

# Leaders

## Bone marrow aspiration

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### Abstract

**Bone marrow aspiration biopsies are carried out principally to permit cytological assessment but also for immunophenotypic, cytogenetic, molecular genetic, and other specialised investigations. Often, a trephine biopsy is carried out as part of the same procedure. Bone marrow aspirations should be carried out by trained individuals who are aware of the indications, contraindications, and hazards of the procedure. They should follow a standard operating procedure. The operator should have made an adequate assessment of clinical and haematological features to ensure both that appropriate indications exist and that all relevant tests are performed. For the patient's comfort and safety, the posterior iliac crest is generally the preferred site of aspiration. Films of aspirated marrow and, when appropriate, films of crushed particles should be made and labelled. Once thoroughly dry, films should be fixed and stained. As a minimum, a Romanowsky stain and a Perls' stain are required. A cover slip should be applied. The bone marrow films should be assessed and reported in a systematic manner so that nothing of importance is overlooked, using a low power, then intermediate, then high power objective. A differential count should be performed. An interpretation of the findings, in the light of the clinical and haematological features, should be given. The report should be signed or computer authorised, using a secure password, and issued in a timely manner.**

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Bone marrow aspiration biopsies are carried out principally to permit cytological assessment of bone marrow cells. However, in some clinical circumstances other tests—for example, cytogenetic and immunophenotypic analysis—are equally important (table 1). In many patients, a trephine biopsy will be carried out as part of the same procedure.<sup>1</sup> Bone marrow aspiration should be preceded by evaluation of the medical history and clinical features, examination of a blood film and assessment of

results of a full blood count, other laboratory tests, and radiological investigations. This is essential to ensure that all appropriate tests are performed on the material obtained and to permit an adequate evaluation. For example, it is necessary to know whether the patient is receiving, or has recently been receiving, any medication that may influence the blood count or bone marrow cytology. This includes drugs that may have an adverse effect on the bone marrow and cytokines that have been given to stimulate haematopoiesis. Even remote history may be relevant—for example, travel to an area where leishmaniasis or histoplasmosis is prevalent or previous irradiation of a site where a biopsy might otherwise have been performed. A request for bone marrow aspiration should be regarded as a request for a consultation on the patient in question and not simply as a request to carry out a technical procedure.

### Indications for bone marrow aspiration including areas of controversy

Table 2 shows the accepted indications for bone marrow aspiration. The list is not exhaustive. Some indications are controversial.

This table also summarises the role of supplementary investigations that can be applied to a bone marrow aspirate.

#### MEGALOBLASTIC ANAEMIA

It is now uncommon for a bone marrow aspirate to be performed in suspected megaloblastic anaemia. It is acceptable to omit bone marrow examination if the peripheral blood features are totally typical and if assays of vitamin B<sub>12</sub> and folic acid are suggestive of a deficiency state. Otherwise, bone marrow aspiration is still indicated. If the diagnosis does not appear straightforward, or if the patient requires urgent treatment and haematinic assays are not available, bone marrow aspiration is indicated.

Table 1 Tests that might be required on a bone marrow aspirate

<i>Tests applied routinely</i>
Cytology on a Romanowsky stained film
A Perls' stain for haemosiderin
<i>Tests applied selectively</i>
Other cytochemical tests
Immunophenotyping
Cytogenetic analysis
Molecular genetic analysis
Culture for mycobacteria, leishmania, histoplasma, or other microorganisms
Deoxyuridine suppression test
Culture of colony forming units
Ultrastructural examination

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Table 2 Indications for a bone marrow aspiration with or without a trephine biopsy and relevance of other techniques applicable to the aspirate

Indication	Need for a trephine biopsy	Notes on other useful investigations
Investigation of unexplained microcytosis	Only if MDS is suspected	
Investigation of unexplained macrocytosis	Only if MDS is suspected	Deoxyuridine suppression test may be useful but is mainly a research technique
Investigation of unexplained anaemia	Usually	Cytogenetic analysis if MDS is suspected; ultrastructural examination if congenital dyserythropoietic anaemia is suspected
Investigation of unexplained thrombocytopenia	Only if MDS is suspected	
Investigation of pancytopenia (including suspected aplastic anaemia)	Yes	Cytogenetic analysis if MDS is suspected; appropriate culture if mycobacterial infection or leishmaniasis is suspected; bone marrow is a useful source of DNA if investigation for Pearson's syndrome is required; cytogenetic analysis if a haemophagocytic syndrome is suspected†
Investigation of a leucoerythroblastic blood film and suspected bone marrow infiltration	Yes	Cytogenetic analysis if a haematological neoplasm is suspected; if an abnormal infiltrate is found, immunophenotyping and cytogenetic analysis may be useful; cytogenetic analysis is indicated if a small cell tumour of childhood is suspected because the demonstration of certain specific cytogenetic abnormalities can confirm the diagnosis
Investigation of suspected acute leukaemia	No*	Cytogenetic and possibly molecular genetic analysis; immunophenotypic analysis unless cells are clearly myeloid
Assessment of remission status after treatment of acute leukaemia	No*	Follow up cytogenetic analysis is only occasionally useful; molecular genetic analysis may be indicated for assessment of minimal residual disease
Investigation of suspected MDS or myelodysplastic/myeloproliferative disorder	Yes	Cytogenetic analysis; investigation of colony forming units if juvenile myelomonocytic leukaemia is suspected
Investigation of suspected chronic myeloid leukaemia	No*	Cytogenetic analysis; molecular genetic analysis is not indicated because it can be performed, when necessary, on peripheral blood cells
Follow up of chronic myeloid leukaemia	No	Cytogenetic analysis
Investigation of suspected myeloproliferative disorder (polycythaemia rubra vera, essential thrombocythaemia, idiopathic myelofibrosis, or systemic mastocytosis)	Yes	Cytogenetic analysis; investigation of colony forming units (erythropoietin independent burst forming units) may be useful but in most centres is not a routine diagnostic test
Investigation of chronic lymphocytic leukaemia	Yes	Immunophenotyping is not indicated because it can be performed easily on the peripheral blood
Investigation of suspected non-Hodgkin's lymphoma	Yes	If an abnormal infiltrate is present, immunophenotyping, molecular analysis and cytogenetic analysis may be needed
Diagnosis and follow up of hairy cell leukaemia	Yes	Immunophenotyping, unless there are sufficient circulating cells for it to be performed on peripheral blood cells; tartrate resistant acid phosphatase stain if detailed immunophenotyping is not available
Staging of low grade non-Hodgkin's lymphoma (if the results of investigation will alter management)	Yes	Immunophenotyping, unless there are sufficient circulating cells for this to be done on blood cells; cytogenetic and molecular genetic analyses are sometimes useful if the specific type of non-Hodgkin's lymphoma has not already been determined
Investigation of multiple myeloma	Generally indicated	Cytogenetic analysis may be useful because demonstration of poor prognosis abnormalities may influence management; immunophenotyping is only needed if cytology of the aspirate is not diagnostic and if it is not certain whether or not a monoclonal plasma cell population is present
Staging of high grade non-Hodgkin's lymphoma (in those cases where results of the investigation will alter management)	Yes	
Investigations of suspected storage disease	Not essential	
Investigation of fever of unknown origin	Yes	Cultures for mycobacteria and also, if there is a possibility of previous exposure, for leishmania and histoplasma
In suspected chromosomal disorders in neonates when rapid confirmation is required	No	Cytogenetic analysis (may produce results in 1 day cf. several days if cultured peripheral blood lymphocytes are used)
Confirmation of normal bone marrow if bone marrow is being aspirated for allogeneic transplantation	No	

\*Unless there is difficulty obtaining a good aspirate.

†Because Epstein-Barr virus related haemophagocytic syndrome may be associated with a clonal proliferation of neoplastic T cells.

MDS, myelodysplastic syndrome.

#### MICROCYTIC ANAEMIA

The importance of bone marrow aspiration in the investigation of microcytic anaemia is often not appreciated. Misdiagnosis of anaemia of chronic disease as iron deficiency is common and it is not infrequent for such patients to be subjected to an extensive search for a source of blood loss without a firmly based diagnosis of iron deficiency. If results of biochemical assays are equivocal it is important to perform a bone marrow aspirate to ensure an accurate diagnosis before embarking on other investigations.

#### ACUTE LEUKAEMIA

Although it is often possible to establish a diagnosis of acute leukaemia from peripheral blood examination, bone marrow aspiration should nevertheless be carried out. This is both

because the likelihood of successful cytogenetic analysis is higher if bone marrow cells are used and because a baseline is needed for comparison with bone marrow aspirates performed during treatment. In addition, bone marrow aspiration permits the assessment of trilineage dysplasia, which may be of prognostic relevance.

#### CHRONIC MYELOID (GRANULOCYTIC) LEUKAEMIA

In chronic myeloid leukaemias, a bone marrow aspirate usually offers little diagnostically useful information beyond that which can be gleaned from a careful examination of the peripheral blood. However, as for the acute leukaemias, cytogenetic analysis is more often successful when performed on the bone marrow and aspiration is therefore indicated

for this purpose. Trephine biopsy is not usually necessary. However, if the accelerated phase of the disease or blast transformation is suspected, bone marrow aspiration and trephine biopsy are both important.

#### CHRONIC LYMPHOCYTIC LEUKAEMIA

The diagnosis of chronic lymphocytic leukaemia can usually be made without difficulty from the peripheral blood features and the immunophenotype. A bone marrow aspirate is therefore not essential in patients with early stage disease, particularly in elderly patients in whom treatment may never become necessary. Bone marrow assessment is indicated before treatment is undertaken. This should always include a trephine biopsy because the bone marrow aspirate gives very little information beyond that already available from examination of the blood, whereas the trephine biopsy permits an accurate assessment of the extent of infiltration and gives information of prognostic importance. During follow up of intensive treatment of chronic lymphocytic leukaemia there is little point in performing a bone marrow aspirate alone because there may be residual disease detectable only by trephine biopsy.

#### NON-HODGKIN'S LYMPHOMA

Diagnosis of non-Hodgkin's lymphoma is usually based on a lymph node biopsy. However, in patients with bone marrow involvement, diagnosis can be reliably based on cytology, immunophenotyping, and the pattern of bone marrow infiltration. When there are circulating lymphoma cells, immunophenotyping can be performed on the peripheral blood and the bone marrow aspirate is of little importance. The trephine biopsy is much more important because it permits an assessment of the pattern and extent of infiltration, which is of both diagnostic and prognostic relevance, and may demonstrate lymphoma when no abnormal cells have been detected in the blood or the bone marrow aspirate. However, if circulating lymphoma cells are not present, the detailed immunophenotyping that is possible on cells in a bone marrow aspirate can help in both diagnosis and classification of non-Hodgkin's lymphoma and the aspirate is then a useful supplement to the trephine biopsy. A bone marrow biopsy performed in patients with low grade lymphoma sometimes shows unexpected high grade transformation, which necessitates a different therapeutic approach.

#### MULTIPLE MYELOMA AND MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE

Bone marrow aspiration is indicated in suspected multiple myeloma. A trephine biopsy is usually also performed. Because infiltration is often focal, it is sometimes essential for a diagnosis. In other patients it provides a baseline for comparison with follow up biopsies. Whether bone marrow examination should be carried out in patients when "monoclonal gammopathy of undetermined significance" (MGUS) is suspected is controversial. If such patients are referred to a haematologist then a bone

marrow aspirate is often performed. However, it should be remembered that at least 1% of patients over the age of 60 years have a paraprotein and over the age of 70 years the figure rises to 3%. Furthermore, investigation of serum immunoglobulins is often performed without a clear clinical indication. It seems reasonable not to perform a bone marrow examination if a low concentration paraprotein has been detected almost incidentally in a patient who does not have anaemia, bone pain, hypercalcaemia, or other relevant clinical features. However, if investigation is undertaken then both a bone marrow aspirate and a trephine biopsy should be performed. A crush preparation of bone marrow fragments (see below) is useful in the investigation of suspected MGUS and multiple myeloma.

#### AUTOIMMUNE THROMBOCYTOPENIC PURPURA

Some controversy surrounds the role of bone marrow aspiration in suspected autoimmune thrombocytopenic purpura. In children, the American Society of Haematology (ASH) guidelines suggest that bone marrow aspiration is not usually needed.<sup>2</sup> The guidelines of the British Paediatric Haematology Group recommend bone marrow examination for children whose disease does not remit within two to three weeks or if treatment, especially with corticosteroids, is planned.<sup>3</sup> More recently, Lilleyman commented "the consensus has swung against this (that is, bone marrow aspiration), provided the history and the clinical picture is entirely typical of acute onset ITP and the peripheral blood is entirely normal apart from profound and isolated thrombocytopenia". However, he continues, "the threshold for marrow examination should be low if there is the slightest clinical doubt".<sup>4</sup>

In adults with acute onset of thrombocytopenia, treatment is usually indicated and in British haematological practice a pretreatment bone marrow aspiration is usually thought to be indicated. If autoimmune thrombocytopenic purpura appears very likely, trephine biopsy is not needed. In adults with moderately severe chronic thrombocytopenia, investigation is indicated to establish a diagnosis, even if immediate treatment does not appear to be indicated. If autoimmune disease appears very likely, only an aspirate is required but, if a myelodysplastic syndrome is suspected, a trephine biopsy is also needed.

American practice appears to differ somewhat from that in the UK, with the ASH guidelines suggesting bone marrow aspiration only in patients above the age of 60 years.<sup>2</sup>

#### Site of aspiration

The need for a trephine biopsy is an indication for selecting the iliac crest, usually the posterior superior iliac spine, as the site for aspiration. The anterior iliac crest is also satisfactory, at least in thin patients. If only an aspirate is needed either the ilium or the first part of the body of the sternum can be used. The iliac crest is safer but it is often preferable to use the sternum in very obese or immobile patients. Sternal aspiration may also be necessary if the

pelvis has been irradiated. Sternal aspiration is safe if proper procedures are followed (see below). In children, the posterior superior iliac spine is usually a suitable site for aspiration. Even in babies this site is often suitable and it is rarely necessary to use the tibia.

If clinically indicated, bone marrow aspiration can also be carried out at other sites—for example, at sites of focal bone marrow disease identified by scanning procedures or radiology.

#### **Planning the bone marrow aspirate**

Although it may seem self evident, it is necessary to plan a bone marrow aspiration to ensure not only that the procedure is safe and causes minimal discomfort to the patient but also that all appropriate specimens are taken into the correct anticoagulant and despatched to the correct laboratory. The appropriate supplementary tests may be better determined after the bone marrow aspirate has been examined. Therefore, it is often useful to take a 10 ml sample into preservative free heparin and stain and examine a single film rapidly before deciding whether supplementary investigations are indicated and, if so, which are most likely to be informative.

#### **Procedure for aspiration and preparation of films**

The procedure of bone marrow aspiration is well described in standard textbooks and so will not be described in detail. However, certain important points will be discussed.

##### **CHOICE OF NEEDLE**

Disposable needles are preferable (see below). All of the standard needles are satisfactory for aspiration biopsy of the iliac crest. It is acceptable to remove the guard, if necessary, to provide a greater length of needle in obese patients. For sternal aspiration the needle selected should have a guard that screws along the needle—for example, a Klima needle—because the guard cannot slip during the procedure. A needle with a guard secured by a side screw—for example a Salah needle—is less satisfactory because the guard can slip, allowing the needle to penetrate further than was intended. Occasionally, in a very obese patient, it is necessary to use a trephine biopsy needle, which is generally longer than an aspiration needle, to obtain an aspirate from the iliac crest.

##### **OPERATOR AND PATIENT SAFETY**

The patient should be questioned with regard to allergy to latex, local anaesthetic, or any antiseptics or sedatives that might be used.

Sterile gloves should be worn and an aseptic technique must be used. Gloves are for the protection of the operator as well as the patient. Because of the high incidence of allergy in individuals exposed to latex, the gloves selected should ideally be non-powdered and non-latex. If latex gloves are used they should have a low amount of extractable latex proteins; there is evidence that corn starch glove powder aerosolises latex protein, leading to inhalation of potential allergens by the operator and others.

If either the operator or the patient is allergic to latex, gloves chosen should be vinyl, neoprene, or nitrile. On safety grounds, it is preferable to use disposable needles. This not only eliminates the risk of transmission of infection to the current or future patients but also avoids the necessity for cleaning non-disposable needles, a procedure that may be hazardous for staff.

When aspirating from the sternum, it is of vital importance to aspirate only from the first part of the body of the sternum (or from the manubrium) and to judge the depth carefully. The needle that is used for infiltrating with local anaesthetic can be used to measure the depth of the periosteum, the guard then being set so that the needle will penetrate only 5–6 mm beyond the depth of the periosteum. Particular care must be exercised in patients with suspected multiple myeloma and in elderly patients who may have osteoporosis; inadvertent penetration through the sternum is more likely in these patients if the needle guard is not set correctly or if a needle with an insecure guard is used. When aspirating from the posterior superior iliac spine, the guard can safely be set so that the needle can penetrate up to 1 cm beyond the surface of the bone.

Bone marrow aspiration and, in particular, trephine biopsies should not be carried out single handedly. A trained assistant can prepare slides. Alternatively, the operator can prepare slides while the assistant applies pressure to the wound to ensure adequate haemostasis. The assistant should, of course, wear gloves for his/her protection.

Bone marrow aspiration is sometimes required in patients with thrombocytopenia or abnormal coagulation tests. Excessive bruising or bleeding is very unlikely in patients with thrombocytopenia if five minutes of firm pressure is applied at the end of the procedure. However, as a precaution and assuming that the procedure was performed on the posterior iliac crest, the patient should lie on his/her back for a further 10–15 minutes to apply more prolonged pressure. Bruising is more likely if a patient has a coagulation defect or is anticoagulated. When possible, any clotting defect should be corrected before aspiration is performed.

##### **PATIENT COMFORT**

An appropriate explanation of the procedure is essential for patient confidence and for very anxious patients it is better to avoid the sternum. Adequate local anaesthesia is important to minimise pain. The skin, subcutaneous tissues, and periosteum should be infiltrated, with particular attention being paid to the periosteum. It is important to wait until the local anaesthetic has had time to take effect and to test for adequacy of anaesthesia before proceeding. The patient must be warned of the possibility of suction pain before it occurs and be reassured that it will be brief. If suction is distressing, slowing the rate of aspiration is indicated. No sedation is necessary in adults who are having only an aspirate performed unless they are unusually anxious. In children, particularly those requiring repeated bone

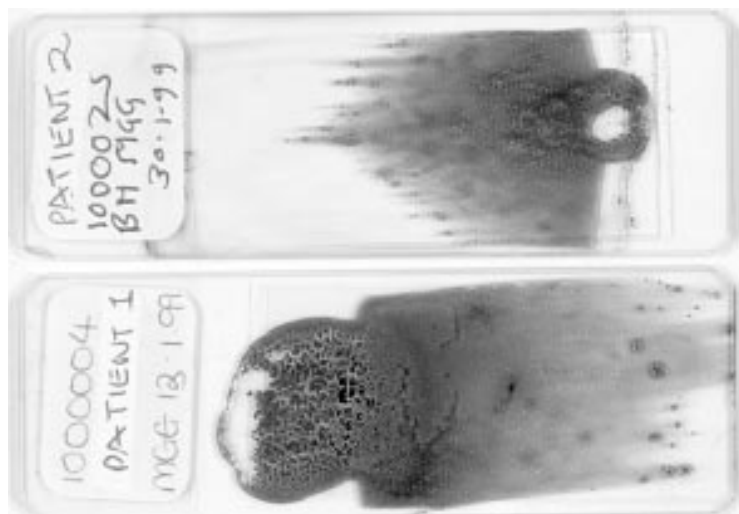


Figure 1 Stained bone marrow films showing a film of appropriate length, spread towards the frosted end where the label is applied (patient 1), and a film that is too long and has been spread, incorrectly, away from the frosted end where the label is applied (patient 2).

marrow aspirations and other procedures during the treatment of acute leukaemia, it is preferable to perform the procedure under a general anaesthetic.

#### PATIENT CONSENT

When bone marrow aspiration is to be performed under a general anaesthetic or heavy sedation it is essential that an explanation is given in advance and that a consent form is signed. For other patients, local practice should be followed with regard to signing a consent form. Most hospitals consider that oral consent after an appropriate explanation is sufficient for conscious patients. Seeking written consent may merely increase the patient's apprehension.

#### TECHNICAL CONSIDERATIONS

When aspirating from the sternum, the needle should be inserted slightly to one side of the midline because bone marrow is less cellular in the midline. The needle should be inserted at right angles to the surface of the bone. In carrying out a biopsy from the posterior superior iliac spine, the needle should be inserted into the bone in the direction of the anterior superior iliac spine, to avoid penetration of the sacroiliac joint. The patient can be in the lateral decubitus position or, if thin, can be prone. The anterior iliac crest is technically less satisfactory but can be used if there is some reason not to use the posterior iliac crest. If the patient is thin enough for the ilium to be grasped between the thumb and finger of the left hand a direct approach through the thick cortex into the anterior superior iliac spine is possible. Otherwise a lateral approach just below and behind the anterior superior iliac spine can be used. For either approach the patient should be supine.

The minimum amount of bone marrow needed for the tests indicated should be aspirated because the greater the volume of marrow aspirated the more dilution by peripheral blood occurs. If a large volume of bone

marrow is needed—for example, for cytogenetic analysis or immunophenotyping—it is useful to aspirate about 0.25 ml first, this being used for spreading films, and then to use a second syringe to obtain a further sample. Ideally, films should be spread immediately without any anticoagulant being added. However, if there has been any difficulty in aspiration and it is thought that the specimen might clot rapidly, part of the aspirate should be put into anticoagulant (see below). Glass slides with a frosted end for labelling should be used. The spreader should be narrower than the width of the glass slide to facilitate examination of the edges of the bone marrow film. A small drop of marrow should be placed about 1 cm from the end of the slide and spreading should be towards the frosted end (fig 1). This ensures that it will be possible to examine the thinnest part of the film, where cytological details are optimal, while the slide is firmly positioned on the stage. Spreading away from the frosted end towards the end of the slide may mean that it is very difficult to examine an important part of the film by high power because the slide is not stable on the microscope stage. A plastic pipette should be available in case the first films spread suggest that the marrow is very dilute and it is necessary to concentrate fragments. Various techniques can be used. A large drop of bone marrow can be put on one end of a slide and the liquid part of the marrow can be aspirated from one edge of the drop, using the pipette, before the residual more cellular marrow is spread. Alternatively, a large drop of bone marrow can be put in the centre of a slide and the liquid part can be poured off on to a gauze square or can be aspirated with the pipette. Any fragments present are then visible and can be picked up with the edge of the spreader and spread on another slide. In some circumstances it can be useful to make a crush preparation. A drop of bone marrow containing fragments is placed in the middle of a slide and a second slide is then placed on top of the first. The slides are dragged away from each other, in the direction of the long axis of the slide, so that any fragments present are crushed. This technique is particularly important in suspected multiple myeloma, when neoplastic cells are sometimes present in larger numbers in fragments than in trails. It would probably also be a useful technique in systemic mastocytosis, in which mast cells often remain within fragments. If no bone marrow has been aspirated into the syringe there may nevertheless be a small drop in the needle; attempts to express this should be made by reinserting the trocar into the aspiration needle. When bone marrow aspiration is being performed for suspected acute promyelocytic leukaemia, the aspiration and spreading should be done as rapidly as possible to avoid clotting of the sample. It is particularly useful to have an assistant under these circumstances. The first 0.1–0.2 ml aspirated should be spread immediately, without prior manipulation, while an assistant applies a second syringe and aspirates further bone marrow for cytogenetic analysis.

If it is necessary to anticoagulate bone marrow before spreading films, a salt of EDTA (for example, K<sub>2</sub>EDTA) should be used, with the amount of EDTA being appropriate for the volume of bone marrow. This is most easily achieved using a small bottle intended for paediatric blood samples. Films should be spread without delay to avoid storage artefact. Bone marrow samples for supplementary tests should be anticoagulated with preservative free heparin if cytogenetic analysis is required. For immunophenotyping, either heparin (not necessarily preservative free) or EDTA is satisfactory. For molecular genetic analysis, EDTA is satisfactory, although preservation of DNA may be better in heparin if there is any delay in transport of the specimen. If ultrastructural examination is required, a drop of bone marrow should be put directly into glutaraldehyde.

The slides should be labelled immediately the films are made, using a pencil to write on the frosted end of the slide. Minimum labelling requirements at this stage are the patient's surname and the date.

Any residual bone marrow aspirate can be left in the syringe until it clots and can then be teased out into formal saline and submitted for histological examination. Alternatively, the bone marrow can be expelled immediately into formalin, which disperses the particles; they can then be collected as a cytoblock or into a filter. Either of these techniques increases the likelihood of detecting focal lesions and the presence of abnormal cells trapped within fragments; the absence of clotted blood with the second technique gives a better sample for cutting and provides sections that are more convenient to examine. If a trephine biopsy is being carried out at the same time as the aspirate these procedures are redundant.

If it is not clear whether or not supplementary investigations are indicated, part of the bone marrow should be anticoagulated with preservative free heparin. A single bone marrow film should be examined and, depending on the findings, the specimen can then be despatched for whatever investigations appear relevant. For example, if an aspirate were being done because of a leucoerythroblastic anaemia and the bone marrow film showed carcinoma cells cytogenetic analysis would not be indicated whereas, if the film suggested a possible haematological neoplasm, cytogenetic analysis should be done. If preservative free heparin is used as the anticoagulant the marrow specimen will be suitable for any of the usual supplementary tests.

#### **Processing and staining of bone marrow films**

Bone marrow slides should be thoroughly dry before they are fixed or artefactual changes occur. For example, the nuclear contents appear to leak into the cytoplasm of erythroblasts, an appearance that can be misinterpreted as dyserythropoiesis. When dry, the films should be thoroughly fixed in methanol or whichever fixative is indicated for specific cytochemical stains. The methanol should be fresh

so that there is no artefact introduced by water absorbed into the methanol. Bone marrow films should be stained with a Romanowsky stain, such as May-Grünwald-Giemsa or Wright-Giemsa. They can be stained in an automated staining machine or manually. If urgent examination is needed only one film should be fixed and stained because drying may be inadequate. The remainder of the slides are processed when thoroughly dry.

A film with at least one fragment, preferably more, should be used for a Perls' stain (for assessment of iron stores) on all initial bone marrow biopsies. This stain can generally be omitted from subsequent procedures. All iron stains should be performed together with a control slide, containing fragments, from a patient known to have iron in bone marrow fragments.

Once stained, a cover slip should be applied and the slide should be labelled with the patient's name and the date (not omitting the year). Usually a laboratory number is also allocated and included on the label.

#### **Assessing and reporting bone marrow films**

Unless a very recent blood count and film is available a venous blood sample should be obtained at the same time as the bone marrow aspirate. A blood film should already have been examined carefully in the prebiopsy assessment of the patient but should be examined again as part of the assessment of the bone marrow aspirate. A reticulocyte count, performed on the day of the bone marrow aspiration, can be useful—particularly for determining whether erythroid hyperplasia is associated with effective or ineffective erythropoiesis. The films should first be examined under low power ( $\times 10$  objective) to assess the number of fragments, the cellularity, and the number of megakaryocytes, and also to detect any low incidence abnormal cells such as carcinoma cells. The films should then be examined in detail using a  $\times 40$  or  $\times 50$  objective. There should be a systematic assessment of the cellularity and contents of fragments, megakaryocyte number, and morphology and cytological features of other lineages. Fine cytological details should be assessed using an oil immersion  $\times 100$  objective. A differential count should be performed in the trails behind several fragments because this will be the part of the film minimally diluted by peripheral blood. At a minimum, several hundred cells should be counted, with the granulocyte and erythroid series, lymphocytes, and plasma cells being enumerated and a myeloid to erythroid cell ratio being calculated. Depending on the indication for the bone marrow aspiration, this may be all that is required. For example, a more detailed differential count would not be required if the bone marrow features were those of iron deficiency or megaloblastic anaemia. However, if the features were those of acute leukaemia or a myelodysplastic syndrome a 500 cell differential count should be performed, and all cell types should be enumerated. Similarly, if the features were

those of multiple myeloma with 80% of myeloma cells being present, a detailed differential count would not be necessary. However, if myeloma or MGUS was suspected but the number of plasma cells was only moderately increased, 500 cells should be counted and the count should include several different bone marrow films. A focal increase in cells of one particular lineage should be noted. Both blast cells and myeloma cells are sometimes irregularly distributed so that a differential count should be performed in the area where abnormal cells are most numerous. The report might then read—for example, “overall plasma cells are 10% of bone marrow cells but in the trails behind several fragments they comprise 30–40% of cells”. The quantitative and qualitative assessment of cells in Romanowsky stained films should be followed by assessment of the iron stain and any cytochemical stains. If stainable iron is absent from fragments but there are only small numbers of fragments available for assessment this should be noted. The report might read—for example, “no storage iron detected but only one fragment available for assessment”.

If a patient has had previous bone marrow aspirations performed, comparison should be made with previous films to assess disease progression or treatment response.

The report of the bone marrow films should include the clinical details, the major features of the blood count, the results of blood film examination, and the bone marrow findings. It is useful to include the white cell count, haemoglobin concentration, platelet count, mean cell volume, and reticulocyte count as a routine, but in selected patients other important blood count abnormalities should be recorded. The report should then include details of the aspiration procedure, specifically the site of aspiration, whether aspiration was easy or difficult, and whether or not bone texture was normal. The report on the bone marrow films should differentiate factual statements from opinion. The body of the report should include an assessment of cellularity and a systematic description of each lineage. The myeloid to erythroid cell ratio and the salient features of the differential count should be given. The report should include a list of other investigations that have been performed—for example, “trephine biopsy and immunophenotyping to follow”, so that clinical staff are aware of any investigations that are still pending. Finally, the report should have a summary or conclusion in which it is appropriate to express an opinion and, if necessary suggest further tests. If it is possible to make a definite diagnosis this should be done. Varying levels of

certainty might be expressed as follows: (1) “the findings are those of multiple myeloma” or “the diagnosis of multiple myeloma is confirmed”; (2) “the bone marrow features are compatible with multiple myeloma but are insufficient in themselves to establish the diagnosis” or “the bone marrow findings support a diagnosis or multiple myeloma but should be assessed in the light of clinical, radiological, and other laboratory features”; (3) “the bone marrow aspirate does not support a diagnosis of multiple myeloma but results of the trephine biopsy are still outstanding” or “the bone marrow aspirate shows no features suggestive of multiple myeloma”.

If a bone marrow aspirate fails or yields only peripheral blood it should nevertheless be reported so that a record of the attempt exists in the laboratory and in the patient records. The site of attempted aspiration and the bone texture should be reported.

Stained bone marrow slides should be stored as long as is economically feasible, preferably for 20 or 30 years. A blood film should always be stored with the bone marrow slides. It is also useful to store unstained, unfixed slides for a shorter period in case further investigation is subsequently indicated, or more stained slides are required—for example, if the patient is transferred to another hospital or is entered in a clinical trial. Unstained slides also provide a source of positive control slides for iron stains. Unfixed slides constitute a biological hazard and, once thoroughly dry, are best stored in sealed plastic containers.

### Standard operating procedure

Laboratories should have a written, signed and dated standard operating procedure for bone marrow aspiration.

### Training

A bone marrow aspiration is a potentially hazardous procedure. All operators must be trained and the procedures they perform must be supervised until competence is assured. Those trained to carry out this procedure may include not only medical practitioners but also specialist nurses.

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