Examination of the bone marrow

Introduction

An abnormal full blood count is a common finding in many different medical conditions; however, the cause may often not be immediately apparent. The history and examination of the patient, together with other tests such as the blood film, may help towards the diagnosis. Occasionally it is necessary to further investigate the patient with a bone marrow examination to determine the origin of the abnormalities. The indications for this are described in *Table 1*.

Both liquid marrow and solid trephine biopsies may be taken to provide a huge amount of additional information to help with the diagnosis of many different haematological and non-haematological conditions (Brown et al, 2006).

Bone marrow procedure

In the first few years after birth, blood cell production is widespread in most of the bones in the body; however, in later life haematopoietic tissue is confined to the sternum, skull, long limb bones, pelvis and vertebrae. The marrow is therefore usually obtained from the posterior iliac crest which is an easily accessible and safe area. The sternum was previously a commonly attempted site, but because of the potential risk of cardiac trauma is usually only used now for extremely obese patients. Disposable single-use aspirate and trephine needles (rather than re-usable needles) are used.

The patient is positioned in the left lateral position with the hips flexed. The anterior iliac crest is identified and the ridge of bone followed to determine the posterior crest. The surface of the skin, subcutaneous tissues and bone surface are anaesthetized with local anaesthetic. The aspirate needle is then inserted through the skin to the bone, and the needle is then advanced with a twisting motion until the marrow cavity is entered. A quick aspiration with a syringe will usually acquire the liquid marrow which can then be placed onto glass slides for morphology, or into appropriate containers for other investigations.

The aspirate needle is removed and if necessary a trephine can then be performed with a larger, wider bore needle. Again it is inserted into the periostium and the inner needle is removed before the hollow outer needle is advanced a further 2–3 cm. When the needle is removed the core of bone should remain and this can be placed in formalin for histological examination. In general the bone marrow procedure is safe with only local pressure briefly needed on the marrow site and a sterile dressing to cover the area. Prolonged bleeding or pain is infrequent and can usually be treated with conservative measures.

Bone marrow aspiration

Morphology

The liquid marrow is spread onto a glass slide and left to dry. It can be then stained with a variety of dyes for morphological inspection under light microscopy. Initially the small particles of marrow and their trails are inspected at low power for the cellularity of the sample and the presence of megakaryocytes. At higher magnification the morphology of the individual cells can be seen, together with the distribution and relative proportions of the different cell lines. All the normal stages of cell development should be present, and usually there is a slight preponderance of myeloid cells as compared to erythroid or lymphoid cells (*Figure 1*).

An increase in marrow cellularity can be caused by a variety of conditions such as marrow infiltration, myelodysplasia or any of the myeloproliferative disorders. A marrow infiltrated with an acute leukaemia will in general have over 20% leukaemic blasts and a corresponding reduction in the normal marrow cells. The blasts are often medium to large cells, with morphological features such as open chromatin and nucleoli present.

The presence of the fine, needle-like cytoplasmic Auer rods will suggest a diagnosis of acute myeloid leukaemia. In chronic lymphocytic leukaemia, however, there will be an increase in small, mature lymphoid cells with little cytoplasm; while chronic myeloid leukaemia marrows have an increase in all of the stages of myeloid

Table 1. Indications for bone marrow investigations

Bone marrow aspiration	Unexplained thrombocytopaenia, neutropaenia or anaemia
	Pancytopaenia
	Blood film suggesting acute leukaemia or myelodysplasia
	Suspected myeloma, myeloproliferative disorders
	Leucoerythroblastic anaemia
	Monitoring of leukaemia response to therapy
	Staging of lymphoma
	Assessment of iron status or sideroblastic anaemia (needs Perls stain)
	Investigation of suspected storage disease
	Investigation of pyrexia of unknown origin (plus cultures for mycobacteria, leishmania or histoplasma)
Bone marrow trephine	Suspected lymphoma
	Myelofibrosis
	Myeloma
	Metastatic carcinoma
	Aplastic anaemia

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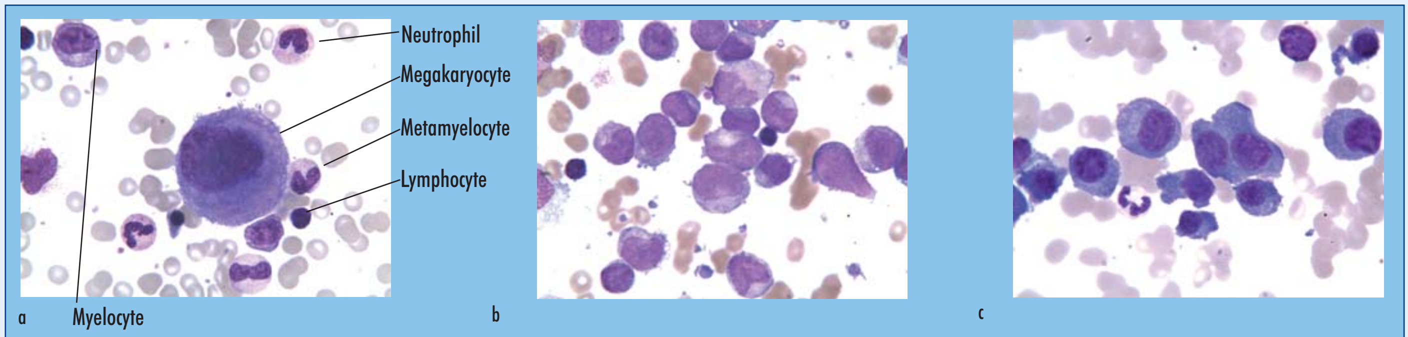


Figure 1. Bone marrow morphology. a. Normal aspirate. b. Acute myeloid leukaemia showing multiple leukaemic blasts. c. Myeloma showing multiple plasma cells including one binucleate cell.

cells, plus basophils and eosinophils. Lymphoproliferative diseases can also have marrow involvement, demonstrating lymphoid cells with a variety of morphological appearances. For example in Burkitt's lymphoma the affected lymphoid cells will be densely blue stained with a vacuolated cytoplasm morphology, while in myeloma the malignant plasma cells will be increased, and these show an eccentric circular nucleus and a pale peri-nuclear golgi zone area.

Hypocellularity can be caused by other conditions such as aplastic anaemia or myelofibrosis. In marrow aplasia there is a decrease in the total amount of marrow cells as a result of underproduction, although the cells look morphologically normal, while in myelofibrosis there is an increase in reticulin or collagen in the marrow, so reducing the available normal haematopoietic areas.

Immunophenotyping

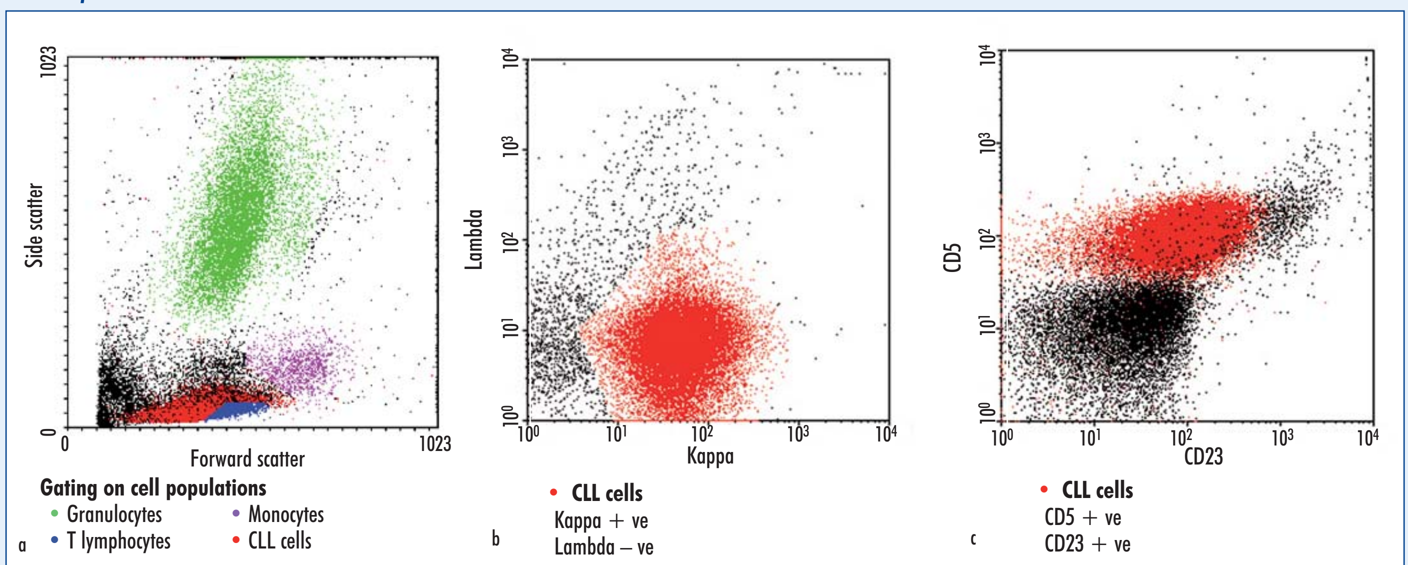
The leucocytes obtained from the marrow sample express a variety of proteins on their cell surface, and the distribution and pattern of these markers can be used to help with the diagnosis of many blood disorders. The cells are incubated with monoclonal antibodies coupled to fluorescent markers. The cells are then analysed by a flow cytometric machine where they pass through a narrow aperture and a light source from a laser shines on the cells. The pattern of the scatter of light and fluorescence is used to gain information about the physical properties of the cells.

Large numbers of cells can be analysed rapidly, and by incubating the samples with a variety of different antibodies many diseases can be diagnosed. For example the patterns of markers can differentiate between myeloid and lymphoid leukaemias, or between the different types of lymphoma (*Figure 2*). Immunophenotyping can also help with the classification of the disease, e.g. differentiating between the different types of acute myeloid leukaemias.

Cytogenetics and fluorescent in-situ hybridization

Cytogenetics is the study of chromosomes and the disorders relating to them (*Figure 3*). In haematology there are often acquired disorders of the chromosomes in the malig-

Figure 2. Immunophenotyping of chronic lymphocytic leukaemia (CLL). A two-dimensional plot can be obtained depending on fluorescence of each cell (individual dots). Clusters of cells can be gated, and these groups of cells analysed with different markers to determine their characteristics. These markers are usually given a cluster of differentiation (CD) number. The plots demonstrate some typical patterns obtained from a patient with CLL (usually CD5, 19, 20, 23 positive): (a) the total nucleated cells in the sample. The lymphoid cells are gated (red). b. The lymphoid cells are clonal (positive for kappa, negative for lambda in this sample). c. These cells are positive for CD5 and 23.



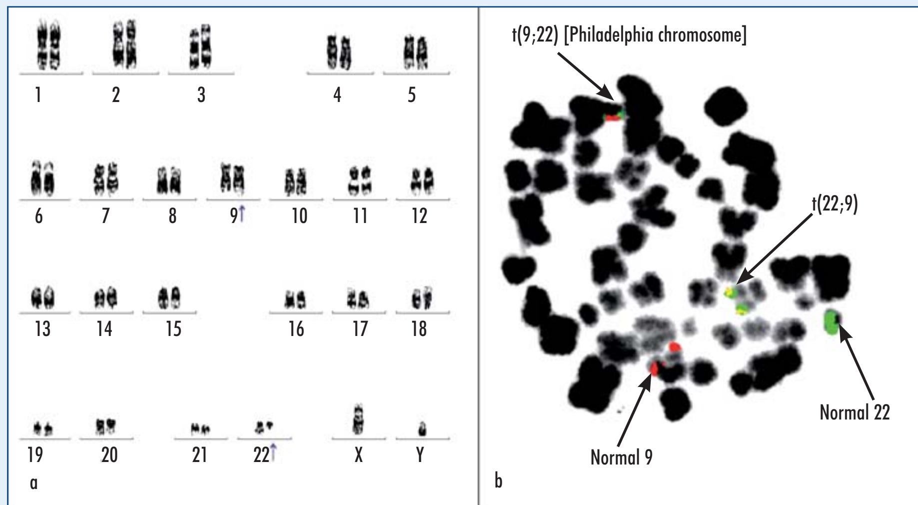


Figure 3. Cytogenetic analysis. a. Karyotype demonstrating t(9;22). b. Fluorescent in-situ hybridization (FISH) demonstrating t(9;22).

nant cells which can give both diagnostic and prognostic information about individual cases. Initially staining metaphase samples with dyes such as trypsin and Giemsa produces chromosomal banding which can be used to produce cytogenetic maps. These karyotypes can then be used to detect chromosomal abnormalities such as translocations, deletions and additions. For example in acute myeloid leukaemia patients with abnormalities such as t(15;17), t(8;21) and inv (16) are classed as having a good prognosis, while those with monosomy 7, and 11q23 abnormalities are classified as poor prognostic markers which tend to respond less well to conventional therapy.

A further investigation that can be performed by the cytogenetic laboratory is the fluorescent in-situ hybridization (FISH) technique. DNA has a characteristic intrinsic property that when present in the single-stranded state it can anneal or hybridize with its complementary sequence. Specific DNA probes labelled with fluorochromes can be incubated with the patient's samples and analysed by fluorescence microscopy. The presence of the specific DNA areas can be used for diagnostic purposes.

Molecular

To analyse samples for mutations at the molecular level, nucleic acids are isolated from the patient's white cells and the specific molecular region of interest is amplified – the process of polymerase chain reaction (PCR) (*Figure 4*). Samples are heated to denature the DNA, then primers of interest are annealed to the single strand DNA before polymerization. By repeating this process small quantities of DNA of interest can be greatly increased. These can then be separated and analysed by gel electrophoresis.

There are many different molecular defects that can be detected by molecular techniques. For example in the Philadelphia chromosome t(9;22), the breakpoint cluster region (BCR) gene from chromosome 22 is juxtaposed to the Abelson gene from chromosome 9. This leads the chimeric fusion protein BCR-ABL which is diagnostic of chronic myeloid leukaemia and can be detected by conventional cytogenetics or molecular techniques.

Other molecular defects that can be analysed include the FLT3 tyrosine kinase mutations. FLT3 is found on chromosome 13q12 and is involved in a complex array of proliferation and cell death pathways. Defects of FLT3 can occur as internal tandem duplications of the nucleotide and are one of the commonest molecular abnormalities found in acute myeloid leukaemia, approximately 22% in newly diagnosed adults (Levis and Small, 2003). The presence of this defect is associated with a worse prognosis (Kottaridis et al, 2001).

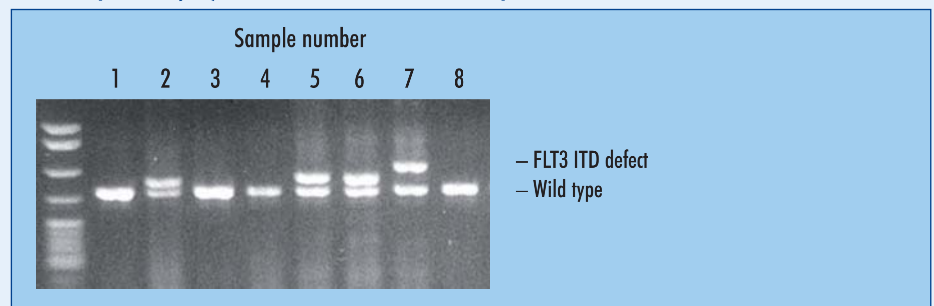
If a molecular defect is found in the patient's sample at diagnosis then this can also be used to accurately monitor the response of the disease to therapy, and to look for relapse. This is called minimal residual disease monitoring. It can be performed by cytogenetic or immunophenotyping tests and these have a sensitivity of picking up 1 abnormal cell in 100–1000 normal cells. However, molecular testing has a far higher sensitivity of 1 in 10 000–100 000 cells. Therefore if the defect persists in the marrow cells after therapy, the disease is not in remission. Alternatively if the molecular defect is rediscovered after therapy has previously cleared the marrow, then the patient may be relapsing and early salvage therapy may be appropriate.

Gene array

The ability to analyse the molecular expression of multiple individual genes from marrow samples is a newly developed technology. It is currently used for research, but it has the potential to be an important tool to provide further diagnostic and prognostic information. Gene chips can contain over 20 000 single-stranded DNA oligonucleotide gene sequences attached to the chip surface. The leucocytes from a marrow sample are purified and cRNA produced which is then incubated with a gene chip. The washed chips are then scanned for fluorescence.

The relative expression of each gene can be measured and analysed to determine

Figure 4. Polymerase chain reaction results of eight acute myeloid leukaemia patients, showing FLT3 internal tandem duplication (ITD) molecular defect detected in samples 2, 5, 6 and 7.



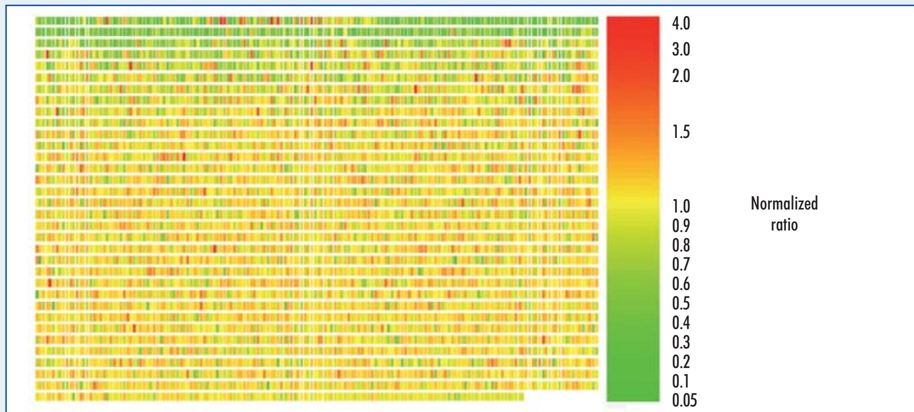


Figure 5. A sample gene array from an acute myeloid leukaemia sample. Each gene is represented by a coloured square. A normalized ratio of <1.0 (green) demonstrates under-expression, while >1.0 (red) shows over-expression.

which genes are under, or over expressed, and so to provide a gene fingerprint for the sample (*Figure 5*). The gene profiles obtained can help with the diagnosis of the different marrow malignancies, for example differentiating myeloid from lymphoid disorders. It can also provide additional information to help with the prognosis of the disease. In the future an individual gene array expression profile may be acquired from each haematological malignancy case, which may allow the tailoring of specific therapy for the patient.

Trephine

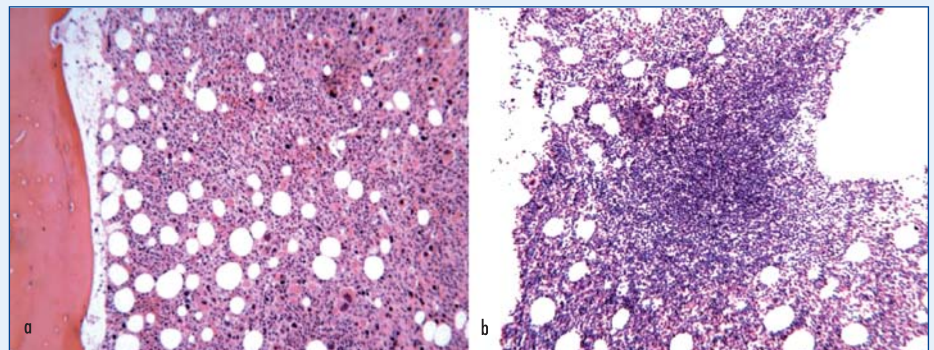
The aspiration samples consist of liquid marrow, but the trephine biopsy is a core of bone which is decalcified and then cut into thin histological slices. It provides a great deal of information about the architecture and cellularity of the marrow, together with further evidence of marrow infiltration (Bain, 2001) (*Figure 6*). The relative distribution of each cell line can be assessed, such as increased megakaryocytes in essential thrombocythaemia, or erythroid hyperplasia in polycythaemia vera.

Marrow infiltration can be seen in some conditions which can be diffuse as in acute leukaemia, or nodular as with some types of lymphoma. Non-haematological malignancies such as breast or prostate cancers can occasionally metastasize to the marrow and be viewed.

Further analysis of the marrow can be performed using different staining techniques. A Giemsa stain can help look for mast cells, while the silver stain will demonstrate any increase in marrow reticulin as in myelofibrosis. Immunohistochemical staining of the marrow can also be used when focal marrow lesions are found by conventional staining. The trephine sample can be cut into many thin sections, and incubated with a panel of monoclonal antibodies. These slides can also be viewed by light microscopy and the pattern of antigens expressed by the cells in the sample can help with diagnosis.

The different classes of non-Hodgkin's or Hodgkin's lymphoma can be distinguished from each other, as can haematological from non-haematological tumours. This technique differs from immunophenotyping flow cytometry in that it is performed on solid rather than liquid samples.

Figure 6. Bone marrow trephine. a. Normal trephine – range of cell types with fat spaces interspaced. b. Focal infiltration non-Hodgkin's lymphoma, with surrounding normal cellularity.



KEY POINTS

- Many disorders will lead to abnormal findings in the full blood count.
- If the cause is not apparent then investigation of the bone marrow may be necessary.
- Liquid aspirates and solid trephines can be acquired.
- These can be sent for a variety of investigations including morphology, immunophenotyping, cytogenetics and molecular tests.

Conclusions

A huge amount of information can be gained by the analysis of the bone marrow from patients. This can help with diagnosis of a condition, help with prognostic information, and be used for minimal residual disease testing. **BJHM**

Conflict of interest: none.

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