Bone marrow histology 3: Value of bone marrow core biopsy in acute leukaemia, myelodysplastic syndromes, and chronic myeloid leukaemia

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Introduction

The standard approach to the diagnosis of acute leukaemia and myelodysplasia has been based on the morphology and percentage of malignant haemopoietic cells in peripheral blood and in an aspirated sample of bone marrow.

The FAB group reports on acute leukaemia have emphasised the value of additional information gained from cytochemistry,¹ and immunological studies and cytogenetics are increasingly being used to provide an accurate diagnosis in acute leukaemia² and myelodysplasia.

Aspirated bone marrow only provides a small sample which is diluted with sinusoidal blood and provides no information on the architectural changes occurring within the marrow cavity as a result of the leukaemic process. An adequate core biopsy sample permits a more accurate analysis of the degree of involvement of the marrow by the leukaemic process and may provide additional information on the response of normal marrow elements to the malignant infiltrate. Despite these advantages marrow core biopsy is not widely accepted as an essential requirement in the investigation of haemopoietic malignancies. One reason for this may be the view that decalcification and paraffin-wax embedding of such biopsy specimens produces distortion and shrinkage, resulting in difficulty in interpreting cellular morphology. This problem can be overcome by using plastic embedding of biopsy specimens,³ but satisfactory morphological details may also be obtained with improved methods of processing paraffin-wax embedded material,^{4,5} with the additional advantage that such processing also allows the use of immunocytochemical techniques.

This review will assess the value of marrow core biopsy as a routine diagnostic procedure in the investigation of acute leukaemia, myelodysplasia, and chronic myeloid leukaemia.

Acute myeloblastic leukaemia (AML)

Aspirated bone marrow permits accurate diagnosis and FAB classification in most cases of AML. The value of a core biopsy specimen is that it provides a more accurate assessment of total marrow cellularity and blast cell numbers both of which are usually numerically greater in biopsy samples than in aspirated marrow. Biopsy material also allows for accurate quantitation of normal marrow components such as plasma cells, lymphocytes, mast cells, eosinophils and macrophages which are sometimes

found in increased numbers in acute leukaemia.⁶ Whether this additional information is of any prognostic value is at present uncertain.

The newer techniques of processing core biopsy specimens make recognition of cytomorphological features easier, but the appearances of cells are often different from those seen in Romanowsky stained marrow smears. Despite these differences it is usually possible on marrow section to distinguish accurately between acute myeloblastic and lymphoblastic leukaemia, but morphological classification into the AML FAB subtypes can be difficult and a comparison of biopsy specimens and aspirates resulted in agreement in only 57% of cases.⁷

Enzyme histochemistry is routinely used for diagnosis on marrow aspirates but is limited on paraffin-wax embedded sections to the detection of chloroacetate esterase by the Leder procedure.⁸ The use of plastic embedding procedures allows for a wide range of histochemical reactions, including chloroacetate esterase in myeloid cells (fig 1), α -naphthyl butyrate esterase in monocyte/macrophage cells, alkaline phosphatase in fibroblast-like reticulum cells and acid phosphatase in megakaryocytes, monocytes, and T lymphocytes.⁹ The usefulness of the information obtained from marrow core enzyme histochemistry requires further evaluation, although the confirmed value of chloroacetate esterase in determining cells of the myeloid series indicates that the Leder procedure should be routinely used on marrow biopsy specimens.

Monoclonal antibodies detecting myeloid antigens are now available for use on paraffin wax sections of marrow biopsy specimens and have been evaluated in the immunophenotyping of AML.^{10,11} Positive reactivity is obtained with a range of antibodies but because AML subtypes exhibit a heterogenic phenotype a single antibody rarely produces diagnostically useful information with the exceptions of anti-glycophorin C in erythroleukaemia and both anti-platelet glycoprotein III and anti-factor VIII related antigen in megakaryoblastic leukaemia. Immunophenotyping of marrow sections remains at the research stage, but it is likely that this technique will be of increasing value in routine diagnostic work.

HYPOPLASTIC AML

In the hypoplastic variant of AML core biopsy of marrow is essential for an accurate diagnosis. The incidence of this condition is about 5% of all cases of AML. The diagnostic features are a hypocellular marrow (less than

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50% cellularity) with more than 30% primitive myeloid cells (fig 2). It is essential that the cellular morphology is carefully assessed in all hypoplastic core biopsy specimens to avoid mistakes in diagnosis, particularly between hypoplastic AML and hypoplastic anaemia. Antecedent myelodysplastic features are found in one third of cases and there is often a history of previous exposure to leukaemogenic agents.^{12 13} The pathogenesis of the hypocellularity is uncertain, but serial biopsy specimens usually have a constant hypocellular pattern, indicating that this condition is a true variant of AML.

Megakaryoblastic leukaemia (FAB M7) and acute myelofibrosis

Are these two terms describing separate entities or are they the same disease? Both are characterised by absence of clinical splenomegaly, pancytopenia, and a rapidly fatal course. Blast cells may be present in the peripheral blood and are characterised as megakaryoblasts by a positive platelet peroxidase reaction at ultrastructural level. An adequate marrow aspirate cannot usually be obtained and diagnosis requires core biopsy with features of increased numbers of blast cells, increased

megakaryocytes of atypical morphology, and a dense increase in reticulin (figs 3 and 4).

In a comparative study of FAB M7 and acute myelofibrosis there were similar morphological findings with the exception of more prominent lymphocytic aggregates in acute myelofibrosis. These diseases can be separated into different entities by immunohistochemical reactivity to monoclonal anti-factor VIII related antigen. In FAB M7 the blast cells in the core biopsy specimen are positive; in acute myelofibrosis they are negative.¹⁴ This finding, together with a previous report of heterogeneity of the blast cells in acute myelofibrosis,¹⁵ suggests that acute myelofibrosis results from a proliferation of more primitive stem cells.

Increased marrow reticulin fibres may also be associated with cellular dysplastic changes, but this can be considered as a separate entity and will be discussed in the section dealing with myelodysplastic syndromes.

Acute lymphoblastic leukaemia (ALL)

In most cases of ALL core biopsy provides only limited additional information compared with standard diagnostic techniques used on aspirated marrow or peripheral blood. Marrow

Figure 1 Bone marrow core biopsy specimen from a case of acute myelomonoblastic leukaemia (FAB M4). Positive staining with naphthol AS-D chloroacetate esterase reaction in myeloblasts (Leder's stain).

Figure 2 Bone marrow core biopsy specimen from a case of hypoplastic AML showing fatty hypocellular marrow with foci of blast cells (haematoxylin and eosin).

Figure 3 Bone marrow core biopsy specimen from a case of megakaryoblastic leukaemia (FAB M7) with prominent atypical megakaryocytes and blast cells (haematoxylin and eosin).

Figure 4 Pronounced fibrosis in a case of megakaryoblastic leukaemia (FAB M7) (Gomori reticulin stain).

Figure 5 Bone marrow core biopsy specimen from a case of MDS showing ALIP and occasional dyserythropoietic features (↓) (haematoxylin and eosin).

Figure 6 Bone marrow core biopsy specimen from a case of CML showing paratrabecular blast cells and promyelocytes with maturing myeloid cells in the intertrabecular marrow space (haematoxylin and eosin).

cellularity and blast cell numbers may be more accurately assessed on core biopsy but FAB classification is very difficult.⁷ Increased amounts of reticulin are found in about 70% of cases of ALL. There is a strong association between an increase in reticulin and lymphoblasts expressing B cell lineage markers, but whether marrow fibrosis is of any prognostic importance is at present uncertain.¹⁶ There are also a few reported cases of ALL which on core biopsy fulfil the diagnostic criteria for hypoplastic acute leukaemia.¹³ These must be differentiated from the more commonly reported cases of transient marrow aplasia which may precede the development of common ALL.¹⁷ Bone marrow necrosis can sometimes be suspected in the marrow aspirate of ALL at presentation or relapse because cellular features are lost and background staining is amorphous. In such situations a trephine biopsy specimen is required for confirmation.¹⁸

Currently, the newer immunohistological techniques are limited in the additional information they can provide and their use is restricted to the detection of T cell ALL with polyclonal anti-CD3.¹⁰

Myelodysplastic syndromes (MDS)

The MDS comprise a group of diseases characterised by morphological abnormalities in myeloid, erythroid, and megakaryocytic cell lines. The FAB cooperative group¹⁹ have defined, by cytological criteria, five subtypes: refractory anaemia (RA), refractory anaemia with ring sideroblasts (RARS); refractory anaemia with excess of blasts (RAEB); refractory anaemia with excess of blasts in transformation (RAEB-t); and chronic myelomonocytic leukaemia (CMML). Recognition of these subtypes may be difficult in a core biopsy specimen,²⁰ although improved accuracy of diagnosis can be achieved if peripheral blood blast cell and monocyte counts are also known.²¹ A core biopsy specimen does allow a disturbed marrow cellular spatial orientation to be recognised, resulting in the presence of myeloid precursor cells away from their normal position along endosteal surfaces. This feature is termed abnormal localisation of immature precursors (ALIP) and is defined as the presence of three or more myeloblasts or promyelocytes clustering centrally in the marrow (fig 5). There must be more than three ALIPs per section before they can be considered to be of diagnostic importance,²⁰ and it is important to recognise that aggregates of immature erythroid and megakaryocytic cells, as demonstrated by immunochemistry, may also mimic the appearance of an ALIP (pseudo-ALIP).²²⁻²³ Although ALIP is typically associated with MDS, it is not unique to this group of diseases and can sometimes be seen in marrow treated with chemotherapy in AML and chronic myeloid leukaemia, and also following bone marrow transplantation.²² Does the finding of ALIP have prognostic as well as diagnostic importance? There seems to be an

association between ALIP and shorter patient survival, not only in RAEB, RAEB-t, and CMML where ALIP is most common, but also in RA and RARS where, by definition, there is no excess of myeloblasts in the marrow aspirate.²²⁻²⁴

Dysmegakaryopoiesis is found in up to 80% of cases of MDS²⁵ and abnormal megakaryocytic morphology may be easier to detect in a core biopsy specimen than in aspirated marrow. As well as having diagnostic importance the severity of dysmegakaryopoiesis may also indicate a poorer prognosis.²⁶ Characteristic features are hypo- and hyperlobulated megakaryocytes and megakaryoblasts with a high nuclear : cytoplasmic ratio, delicate nuclear chromatin, and one or more nucleoli.²⁰ Micromegakaryocytes are frequently present and although they can be recognised in a haematoxylin and eosin preparation, they are more easily seen after staining with periodic acid schiff (PAS) or by immunochemistry using monoclonal antibody anti-CD61 directed against glycoprotein IIIa.²⁵ The relatively rare 5q-syndrome is suspected from the finding of a uniform population of monolobulated micromegakaryocytes.²⁰

Dyserythropoiesis is present in most cases of MDS,²⁰ normally manifest as megaloblastic maturation or as defective maturation in which the erythron is composed of cells at the same stage of development. A predominance of proerythroblasts can also be seen.²⁰⁻²⁶

Accurate quantitation of marrow blast cells is required for a correct subtyping of MDS and for differentiation from acute leukaemia. Although quantitation is more difficult in a core biopsy specimen than in aspirated marrow, a good concordance in blast cell estimation between core and aspirate samples has been reported.²¹

A core biopsy specimen is of special value in the detection of increased concentrations of marrow reticulin, present in about 50% of samples of MDS.²⁰⁻²¹⁻²⁶ This is usually of moderate degree, although several recent reports have described cases of myelodysplastic syndrome associated with a pronounced degree of fibrosis.²⁷⁻²⁹ The prognostic importance of this finding is at present unclear because it is suggested that this condition may be associated with an increased²⁷ or a decreased²⁸⁻²⁹ survival. A biopsy specimen is also important for accurate diagnosis of the rare MDS hypoplastic variant, characterised by pancytopenia, marrow hypoplasia, and a low incidence of progression to acute leukaemia.³⁰

The assessment of a biopsy sample in MDS requires not only consideration of the dyshaemopoietic features but should also include quantitation of eosinophils and plasma cells. Eosinophilia, defined as greater than eight eosinophils per microscopic field ($\times 400$ magnification), has been recorded in a quarter of cases and may be associated with improved survival. Plasmacytosis of more than two plasma cells per microscopic field ($\times 400$ magnification) is seen in 18% of all MDS

cases, and in as many as one half of the CMML subtype, although no prognostic value has been attached to this finding.^{21 26}

Chronic myeloid leukaemia (CML)

The diagnosis of CML is based on typical morphological features of excess myeloid activity in blood and bone marrow aspirate, a low leucocyte alkaline phosphatase score, the presence of the Philadelphia chromosome [t(9;22(q34;q11)] and the molecular consequences of the Ph translocation with formation of the BCR-ABL fusion gene on chromosome 22. A core biopsy specimen provides only limited additional diagnostic information although it has been suggested that two subtypes can be identified. One has proliferation of predominantly granulocytic precursor cells, the other a mixed proliferation of granulocytic and megakaryocytic lines (chronic megakaryocytic-granulocytic myelosis).³¹ The suggestion was made that these morphological subtypes show differences in clinical behaviour, but more recent work has shown no clinical, evolutionary, or prognostic differences between the two groups.³²

The characteristic morphological pattern in chronic myeloid leukaemia is of blast cells and promyelocytes located in the paratrabeular and perivascular region with increasing proportions of more mature myeloid cells in the central intertrabeular spaces (fig 6). Accurate determination of blast cells and promyelocytic numbers may provide independent prognostic information. Patients with pronounced infiltration (four to eight layers of blasts and promyelocytes) compared with minimal infiltration (one to three layers) have an increased probability of early blast cell transformation.³³

An advantage of marrow biopsy in CML is that it allows marrow fibrosis to be assessed. In newly diagnosed patients up to half have increased amounts of reticulin in more than 50% of the cellular areas of the biopsy specimen. Increased fibrosis is strongly associated with poor prognostic features such as splenomegaly, anaemia, increased peripheral blood and marrow blasts, and additional karyotypic abnormalities, and it has therefore been suggested that fibrosis can be a useful prognostic factor at diagnosis.³⁴ In sequential biopsy specimens taken during the course of the disease many patients show a progressive increase in marrow fibrosis which correlates with the number of megakaryocytes, and this too is associated with a worsening prognosis.³⁵

Conclusion

A core biopsy specimen complements the peripheral blood and marrow aspirate findings in providing additional information for the diagnosis and assessment of prognosis in acute leukaemias, myelodysplastic syndromes, and chronic myeloid leukaemia. In recommending the routine use of core biopsy it is essential that adequate, well processed samples are available for morphological interpretation and for the

assessment of newer cytochemical and immunohistological techniques. Also important is a greater awareness of the benefits of close cooperation between histopathologists and haematologists in ensuring that maximal information is gained from bone marrow samples in haematological malignancies.

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