Megakaryoblastic Leukemia Presenting as Acute Myelofibrosis—A Study of Four Cases With the Platelet-Peroxidase Reaction

By Barbara J. Bain, Daniel Catovsky, Maureen O’Brien, H. Grant Prentice, Emer Lawlor, T. O. Kumaran, Shaun R. McCann, Estela Matutes, and David A. G. Galton

Acute myelofibrosis (AM) or malignant myelosclerosis is a myeloproliferative syndrome in which bone marrow fibrosis is associated with a proliferation of immature myeloid cells. In four patients with typical AM, investigated by the platelet-peroxidase reaction at ultrastructural level, the blast cells were found to be megakaryoblasts. One patient, treated with the drug combination DAT, achieved a complete remission of 5 mo duration. This study supports the view that megakaryoblastic leukemia is the most frequent underlying cause of AM and proposes that it should be classified as a form of acute myeloid leukemia.

Acute myelofibrosis (malignant myelosclerosis) is a myeloproliferative syndrome characterized by diffuse marrow fibrosis and pancytopenia, but usually lacking the splenomegaly and characteristic red cell morphological changes of classical myelofibrosis. The small numbers of immature cells that are characteristically seen in the peripheral blood have been often considered to be myeloblasts, although there are few published studies establishing their nature. Breton-Gorius et al.1 described a patient with thrombocytosis associated with acute myelofibrosis in whom the peripheral blood blasts were demonstrated to be megakaryoblasts by the platelet peroxidase reaction (PPO) at ultrastructural level. Den Ottolander et al.2 reported three patients in whom the blasts were considered on morphological grounds to be megakaryoblasts, but this was not proven by ultrastructural cytochemistry. These authors3 have stressed the complete clinical and hematologic identity between acute myelofibrosis and megakaryoblastic leukemia.

In four patients with acute myelofibrosis investigated by us, the immature cells in the peripheral blood were characterized as megakaryoblasts by the PPO reaction. We discuss the hypothesis that, in the majority of cases, acute myelofibrosis might be synonymous with acute megakaryoblastic leukemia.

Case Reports

Case 1

A 74-yr-old white man presented with a 10-wk history of lethargy and exertional dyspnea and a 1-wk history of oral ulceration. He was pale with no hepatomegaly, splenomegaly, or lymphadenopathy. The blood counts showed pancytopenia (Table 1). There was anisocytosis and mild poikilocytosis with occasional tear-drop poikilocytes. Bone marrow was aspirated with difficulty from two sites. The fragments were hypocellular with the majority of cells being blasts. These were large with an eccentric round nucleus with a single large nucleolus. The cytoplasm was agranular and moderately basophilic and a minority had cytoplasmic blebs. The cytochemistry of the blasts is shown in Table 2.

A trephine biopsy specimen showed a normal structure of the bony trabeculae but no areas of normal bone marrow. One area was fatty and hypoplastic. The bone marrow was almost entirely replaced by a cellular infiltrate of fibroblasts, lymphocytes, and large undifferentiated mononuclear cells, some with prominent nucleoli. There was also collagen deposition. The few megakaryocytes seen were abnormal, having either a few nuclear lobes or 1–4 small round nuclei. Where the cellular infiltrate encroached on the fatty area, the fat cells were small and widely separated by the infiltrate. There was a dense and diffuse increase in coarse and fine reticulin fibers in the cellular area.

The patient progressively deteriorated despite antibiotics, blood and platelet transfusions, and cytototoxic chemotherapy (vincristine, cytosine arabinoside, and hydrocortisone). He died 5 wk after presentation.

Case 2

A 67-yr-old West Indian man presented with a 3-mo history of a bleeding tendency. He had suffered bruising, epistaxis, bleeding following tooth extraction, and a retinal hemorrhage. There was no hepatomegaly, splenomegaly, or lymphadenopathy. The blood counts showed pancytopenia (Table 1). There was moderate anisocytosis and mild poikilocytosis with occasional tear-drop poikilocytes. Bone marrow was aspirated with difficulty from two sites. The fragments were hypocellular with the majority of cells being blasts. These were large with an eccentric round nucleus with a single large nucleolus. The cytoplasm was agranular and moderately basophilic and a minority had cytoplasmic blebs. The cytochemistry of the blasts is shown in Table 2.

Fifteen percent of cells were undifferentiated blasts. The majority of blood and bone marrow neutrophils were hypogranular. Results of the cytochemical reactions are summarized in Table 2. Terminal transferase (TdT) levels were not raised: 0.5 U/l (normal range 0–3 U).3

The sections of the trephine biopsy showed both hypercellular and fatty hypocellular areas; the bony trabecular structure was normal. The hypercellular areas showed reduced fat spaces. The hematopoietic tissue was largely replaced by a cellular infiltrate of fibroblasts, undifferentiated mononuclear cells, and lymphocytes. Megakaryocytes were reduced in number and were almost all abnormal, being either small with few nuclear lobes, or 1–4 small round nuclei. The reticulin fibers were coarse and densely increased.

In view of the patient’s age, only supportive therapy was given. He
MEGAKARYOBLAST LEUKEMIA

Table 1. Full Blood Counts at Presentation

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Hb (g/dl)</th>
<th>WBC</th>
<th>Neutrophils</th>
<th>Lymphocytes</th>
<th>Monocytes</th>
<th>Promyelocytes or Myelocytes</th>
<th>Blasts</th>
<th>Platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.7</td>
<td>1.9</td>
<td>0.2</td>
<td>1.2</td>
<td>0.1</td>
<td>—</td>
<td>0.2</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>5.7</td>
<td>3.5</td>
<td>1.4</td>
<td>0.9</td>
<td>&lt;0.1</td>
<td>0.5</td>
<td>0.7</td>
<td>40</td>
</tr>
<tr>
<td>3</td>
<td>4.1</td>
<td>5.0</td>
<td>1.4</td>
<td>1.6</td>
<td>0.3</td>
<td>—</td>
<td>1.2</td>
<td>131</td>
</tr>
<tr>
<td>4</td>
<td>5.5</td>
<td>4.1</td>
<td>0.8</td>
<td>1.8</td>
<td>—</td>
<td>0.3</td>
<td>1.2</td>
<td>1,200</td>
</tr>
</tbody>
</table>

The blood count is shown in Table 1. The membrane phenotype of the peripheral blood blasts studied according to published methods showed them to be negative for the CALL antigen, Ia, SmIg, HuTLA, and with an antimyeloid serum. The blood count on presentation is shown in Table 1. Bone marrow could not be obtained by aspiration. A trephine biopsy showed hypercellular and hypocellular fatty areas and normal bony trabeculae. The cellular areas had sparse fat cells and islands of hemopoietic cells separated by and infiltrated with fibroblasts and mononuclear cells. Interstitial infiltration by the same cells was seen between the fat cells in the hypocellular areas. Megakaryocytes were reduced in number and were abnormal with one or several micronuclei. Both hypercellular and hypocellular areas contained an increased amount of fine reticulin, moderately dense in many areas. Collagen stains were negative.

The membrane phenotype of the peripheral blood blasts studied according to published methods showed them to be negative for the CALL antigen, Ia, SmIg, HuTLA, and with an antimyeloid serum. TdT levels assessed biochemically were normal: 1.2 U/10⁶ cells (normal range 0–3U). The patient remains reasonably well on supportive therapy, having required transfusion 3 times in the 12 wk since presentation.

Case 4

A 14-yr-old school girl presented with a 4-mo history of dyspnea, weakness, and menorrhagia. She also had had bleeding from the gums and epistaxis for 1 wk, and anorexia and weight loss (7 kg) over the previous 2 wk. On examination she was anemic and there was a pansystolic murmur over the precordium. There was no lymphadenopathy or enlargement of the liver or spleen. The blood count on presentation is shown in Table 1. There was marked anisocytosis and poikilocytosis and occasional NRBC were present. Many giant platelets were seen. The neutrophils had normal granulations, but many were Pelger cells. A bone marrow aspirate was hypercellular with some dyserythropoietic changes. Occasional abnormal megakaryocytes were seen and there were 40% blasts. Some blast cells showed abundant cytoplasm with cytoplasmic blebs. Results of cytochemical reactions are shown in Table 2. Chromosomal analysis on a nonstimulated 24-hr culture of bone marrow showed a normal karyotype, but with only 6 metaphases examined.

A trephine biopsy specimen showed normal bony trabeculae and hypercellular marrow spaces with four main patterns that merged into one another: (1) Areas of residual hemopoietic cells with fat spaces that were infiltrated to a varying degree with fibroblasts and blast cells; (2) areas of dense collections of megakaryocytes among which fibroblasts and blast cells were present; (3) areas with a loose infiltrate of fibroblasts, lymphocytes, blasts and megakaryocytes, and scarce fat cells; (4) similar areas with numerous small fat cells. Megakaryocytes were increased in all areas; they were grossly abnormal with large mononuclear forms, small forms with one or several micronuclei, and abnormally segmented forms with a few irregular nuclear lobes. There was a diffuse dense increase of fine and coarse reticulin fibers.

The patient was begun on chemotherapy with daunorubicin 65 mg i.v. once, cytosine arabinoside 130 mg i.v. 12 hourly, and 6-thioguanine 120 mg p.o. 12 hourly for 5 days (DAT protocol). Her platelet count had fallen to 374 x 10⁹/liter before chemotherapy was begun. The treatment was complicated by severe pneumonia and pancytopenia, which responded to antibiotics, and blood and platelet transfusions. Following two courses of DAT, a complete remission was obtained: Hb 9.8 g/dl, WBC 5.6 x 10⁹/liter, and platelets 246 x 10⁹/liter. The bone marrow cellularity was normal with 3% blasts. A further 4 courses of DAT were given at 4 weekly intervals. This, however, was followed by peripheral blood and bone marrow relapse. TdT, tested during relapse by the immunofluorescence method, was negative in the circulating blasts. Currently, she is being maintained on supportive therapy with blood transfusions as required.

Table 2. Cytochemical Reactions in the Blast Cells

<table>
<thead>
<tr>
<th>Light Microscopy</th>
<th>Electron Microscopy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esterases</td>
<td>MPO Cholesterol</td>
</tr>
<tr>
<td>ANAE*</td>
<td>MPO Sudan BB PAS†</td>
</tr>
<tr>
<td>Chloracetate</td>
<td>MPO PPO</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Case No.</th>
<th>ANAE*</th>
<th>Chloracetate</th>
<th>Acid Phosphatase†</th>
<th>MPO Sudan BB</th>
<th>PAS†</th>
<th>MPO PPO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ND</td>
<td>—</td>
<td>ND</td>
<td>—</td>
<td>—/+</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+/+/++</td>
<td>—</td>
<td>—/+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>—</td>
<td>—/+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>+§</td>
<td>ND</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>+</td>
</tr>
</tbody>
</table>

ND, not done; MPO, myeloperoxidase; PPO, platelet-peroxidase.
*NaF sensitive.
†Tartrate sensitive.
‡Diffuse and finely granular.
§AN butyrate esterase negative.
MATERIALS AND METHODS

Ultrastructural Studies

Peripheral blood cells from four patients were studied by ultrastructural morphology and the cytochemical techniques of myeloperoxidase and platelet peroxidase. Mononuclear cells were separated by density gradient sedimentation with Lymphoprep (Nyegaard) and washed twice with TC 199.

Transmission Electron Microscopy Morphology

Separated cells were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.0, for 2 hr, and postfixed in 1% osmium tetroxide. They were then washed in 3% agar, dehydrated in alcohol, and embedded in araldite. Thin sections were stained with alcoholic uranyl acetate and lead citrate and examined in an AE 1 6B electron microscope.

Myeloperoxidase (MPO)

The cells were fixed for 30 min in 3% glutaraldehyde in 0.1 M phosphate buffer, then incubated in dianisobenzidine (DAB) medium: 5 mg DAB in 10 ml Tris-HCl buffer containing 0.01% hydrogen peroxide, pH 7.6, for 30 min. These samples were processed and embedded as above, excluding the block staining with uranyl acetate. Sections were viewed unstained. Normal blood was processed as a positive control, and H2O2 was omitted from the incubation mixture as a negative control.

Platelet Peroxidase (PPP)

The separated, washed cells were incubated unfixed in a medium of 20 mg DAB in 10 ml of 0.5 M Ringer-Tris buffer containing 0.01% of 3% hydrogen peroxide, pH 7.3, for 1 hr at 20°C. The material was then washed 3 times in Ringer-Tris buffer, fixed for 30 min in 1.25% glutaraldehyde in phosphate buffer, and then processed in the same manner as for the MPO samples. Sections were viewed unstained, and normal platelets were included as a positive control.

Platelet Peroxidase Combined With Antiplatelet Antibody

For the cells from case 3, the PPO reaction was combined with an immunologic reaction with a monoclonal antibody AN51 raised against platelets (prepared by Dr. A. McMichael, Radcliffe Infirmary, Oxford) and a goat anti-mouse IgG coupled to gold particles (prepared by Dr. Jan de Mey, Janssen Pharmaceutica). The PPO reaction was performed first, and the cells were then fixed for 15 min at room temperature in 1.25% glutaraldehyde in phosphate-buffered saline (PBS). A quantity of 50 x 10⁶ cells were then incubated with AN51 ascitic fluid in a one-tenth dilution at 4°C for 30 min in the presence of sodium azide. After 5-min washes in PBS containing 10⁻² M sodium azide and polyethylene glycol, the cells were incubated for 30 min with goat anti-mouse IgG coupled to gold particles of 20 nm and washed 3 times as above. A control with normal mouse ascitic fluid was done simultaneously. Postfixation, dehydration, and embedding were performed as described above for EM morphology.

RESULTS

Morphology

In case 1, the blast cells had a high nuclear cytoplasmic ratio and a round or oval nucleus with a single large nucleolus. Mitochondria were small and numerous. There were long profiles of rough endoplasmic reticulum and numerous free ribosomes (Fig. 1A). A small number of blasts showed platelet demarcation membranes (Fig. 1B) and one or two electron-dense granules. The blast cells of case 2 had a more abundant cytoplasm with an irregular outline and some blebs, one or two electron-dense granules, and a slightly more condensed nuclear chromatin (Fig. 1C). A small number of micromegakaryocytes with fully developed platelet demarcation membranes were seen. In case 3 the blast cells were small with scanty cytoplasm, abundant mitochondria, and scanty short profiles of endoplasmic reticulum (Fig. 1D). The majority had no electron dense granules and a few had prominent nucleoli. A small proportion of blasts (approximately 10%) had small granules, early demarcation membranes, and cytoplasmic blebs, features suggesting megakaryocytic differentiation. In case 4, the blast cells had a round nucleus and varying degrees of chromatin condensation and moderate amounts of cytoplasm. Some cells with scanty cytoplasm and occasional granules resembling those of case 3 (Fig. 1D) were seen. A small number of micromegakaryocytes and numerous large platelets were present in theuffy coat preparation of this patient.

Ultrastructural Cytochemistry

In the four cases, the PPO reaction was positive in the nuclear envelope and endoplasmic reticulum of virtually all blast cells (Fig. 2, A–C). The cytoplasmic granules were negative. The cells with “lymphoid” appearances (scanty cytoplasm and moderate nuclear chromatin condensation) seen in cases 3 and 4 were also PPO positive, with the reaction seen predominantly in the nuclear envelope (Fig. 2B). The mitochondria were also positive due to the presence of cytochrome enzymes. The MPO reaction was negative in all blast cells (Fig. 2D). In case 2, a few promyelocytes with positive MPO reaction in the granules as well as the endoplasmic reticulum were seen. The monoclonal antibody AN51 (visualized by the gold-labeled anti-mouse Ig reagent) was positive in the platelets (Fig. 3A) and blast cells of case 3; the latter were also shown to be PPO positive (Fig. 3B).

DISCUSSION

Acute myelofibrosis is an acute myeloproliferative disorder that appears to be closely related to acute leukemia, many patients having a rising blast count terminally. The blast cells in the peripheral blood are often described as myeloblasts, although cytoplasmic granules, Auer rods, or positive reactions for myeloperoxidase or chloracetate esterase have not been demonstrated in many of the published cases. In a
Fig. 1. Transmission electron microscopy of circulating megakaryoblasts (lead citrate and uranyl acid stain). (A) Undifferentiated blast cells (case 1; x 7920). (B) Early demarcation membranes (arrow) and few electron-dense granules in a cell of case 1 (x 9360). (C) Blast with a single electron-dense granule in the cytoplasm (case 2; x 9360). (D) Blast cell with scanty cytoplasm and moderate degree of nuclear chromatin condensation, suggestive of lymphoid morphology (case 3; x 11,880).
Fig. 2. Positive PPO reaction in the nuclear membrane and endoplasmic reticulum of megakaryoblasts of case 3 (A and B; x 10,800) and case 4 (C; x 13,000); negative MPO reaction in a megakaryoblast of case 1 (D; x 9960) (unstained sections).
small number of patients, however, a positive identification of the cells as myeloblasts has been made, based on the presence of Auer rods or of cytochemical reactions characteristic of myeloblasts. In other patients, the blast cells were described as undifferentiated, although cytochemical reactions were not always carried out. In the four typical cases reported here, we have shown that blasts that were undifferentiated on light and electron microscopy morphology and most conventional cytochemical reactions, had PPO activity in the nuclear envelope and endoplasmic reticulum on ultrastructural cytochemistry and thus could be identified as megakaryoblasts. Breton Gorius et al. made the distinction between blast cells that are identified as megakaryocyte precursors only by the

PPO reaction, designated as promegakaryoblasts, and the more differentiated cells that show either specific platelet granules or early demarcation membranes, designated as megakaryoblasts. Since some intermediate cells are seen, it seems to us simpler to use the term megakaryoblast for all blast cells that can be identified as megakaryocyte precursors.

It is noteworthy that, by light microscopy cytochemistry, the megakaryoblasts in these cases were acid phosphatase and ANAE positive, the latter reaction being sensitive to NaF (Table 2). These patterns of reaction, although weaker than in leukemic monocytes, could give rise to confusion. A similar cytochemical profile was observed by den Ottolander et al. and, in a case of megakaryoblastic blast crisis of CML, by us, and is also characteristic of normal platelets and megakaryocytes (DC, unpublished observation).

Some of the blast cells, particularly in cases 3 and 4, had a “lymphoid” morphology at electron microscopy. Membrane markers and/or TdT assays performed in three cases (2, 3, and 4) failed to show a lymphoid phenotype. On the other hand, the ultrastructural morphology of the four cases showed evidence of megakaryocytic differentiation, demonstrated by early demarcation membranes in some blast cells of cases 1 and 3 (Fig. 1B) and circulating micromegakaryocytes in cases 2 and 4. Further evidence of megakaryocyte and platelet differentiation was suggested in case 4 by the bone marrow histology and the marked thrombocytosis both at diagnosis and during relapse.

It seems likely that acute myelofibrosis with proliferation of megakaryoblasts is the same condition as acute megakaryoblastic leukemia, in which fibroblast proliferation and deposition of reticulin and collagen are commonly observed. Breton-Gorius et al. described a patient with acute myelofibrosis and thrombocytosis with 70% of circulating cells being megakaryoblasts; a subsequent patient with acute megakaryoblastic leukemia had a dense reticulin network but not collagen deposition. Of the three patients with megakaryoblastic leukemia described by den Ottolander et al., two had increased reticulin but not fibrosis. Menkes et al. reported, as acute megakaryocytic leukemia, a patient with dense myelofibrosis and a proliferation of atypical megakaryocytes and blasts; the blasts were interpreted as megakaryoblasts although electron microscopy was not performed. Flandrin et al. described 11 patients with acute leukemia, highly suggestive of megakaryocytic leukemia, in all of whom there was bone marrow fibrosis; in two patients in whom the PPO reaction was done, the megakaryoblastic nature of the blasts was demonstrated. Thus, a proliferation of megakaryoblasts may be much more common than is realized in acute
myelofibrosis. This was clearly shown in our four patients where the main features was a proliferation of megakaryocyte precursor cells. Only a few immature cells, seen in case 2, were recognized as promyelocytes.

Some reported cases of acute myelofibrosis have had proliferation of the granulocyte and erythroid lines and an association with idiopathic acquired sideroblastic anemia and megaloblastosis, and with hypogranular and Pelger neutrophils. Three of our four patients showed some features of myelodysplasia, including to a varying degree the characteristic morphological abnormalities of neutrophils such as Pelger and hypogranular forms and dyserythropoiesis as well as abnormal megakaryocytes. The megakaryoblastic leukemia and myelofibrosis may thus arise against a background of myelodysplasia in some patients; however, this does not preclude the possibility that this form of leukemia might also arise de novo from an otherwise normal bone marrow. In some patients, a mixed proliferation of megakaryoblasts and myeloblasts can be seen, as shown in one of the cases of den Ottolander et al.

In acute myelofibrosis, the fibrosis may be secondary to the malignant cell proliferation rather than an intrinsic part of the disease. This is suggested by the reversal of fibrosis, which may be achieved with chemotherapy, and also by cytogenetic evidence. Van Slyck et al. showed that myeloid cells had a consistent chromosomal aberration that was not present in the bone marrow fibroblasts. Myelofibrosis is frequent not only in acute megakaryoblastic leukemia but also in megakaryoblastic transformation of Ph-positive chronic granulocytic leukemia. This suggests that the megakaryocyte line may be particularly likely to evoke marrow fibrosis. This may be mediated by a factor secreted by normal platelets, known as platelet mitogenic factor, which has been shown to stimulate fibroblast proliferation.

Although acute myelofibrosis may be a heterogeneous syndrome, in some patients the fibrosis being a response to a panmyelosis and in others to a megakaryoblastic or myeloblastic leukemia, the accumulated evidence, strongly supported by this report, suggests that frequently the proliferating blasts are megakaryoblasts. Another case studied by us with a positive PPO reaction (DC and MO, unpublished observation) will be reported separately. We conclude that megakaryoblastic leukemia is the most frequent underlying etiology of the clinical syndrome of acute myelofibrosis and should be included within the acute myeloid leukemias. Further application of ultrastructural cytochemistry will facilitate the correct diagnosis of this disease and help in its correct nosologic classification.

ACKNOWLEDGMENT

Our thanks to Professor A. V. Hoffbrand for the biochemical estimations of TdT; Drs. G. Janossy and M. F. Greaves for marker studies in cases 3 and 4; Dr. E. Law for the cytogenetic study of case 4; Dr. A. McMichael for the monoclonal antibody ANS1; Dr. J. DeMey for the goat anti-mouse IgG coupled with gold particles; and W. Hinkes for the photomicrographs.

ADDENDUM

Since this paper was submitted for publication we have demonstrated a positive PPO reaction in the blast cells of five new patients with megakaryoblastic leukemia. Four of them presented as acute myelofibrosis and one as an acute leukemia.

REFERENCES

Megakaryoblastic leukemia presenting as acute myelofibrosis -- a study of four cases with the platelet-peroxidase reaction

BJ Bain, D Catovsky, M O'Brien, HG Prentice, E Lawlor, TO Kumaran, SR McCann, E Matutes and DA Galton