Bone marrow fibre content in acute myeloid leukaemia before and after treatment

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SUMMARY Bone marrow fibre content was studied in 34 patients with acute myeloid leukaemia at presentation and subsequently after chemotherapy and at complete remission. The findings indicate that: (1) some degree of marrow fibrosis is present in about one third of the patients with acute myeloid leukaemia at presentation; (2) effective antileukaemia treatment may result in reversal of marrow fibrosis; and (3) increase in marrow fibre content at diagnosis does not affect haemopoietic regeneration after treatment and achievement of complete remission.

Bone marrow trephine biopsy in patients with haematological malignancies has shown that focal or diffuse increases in marrow reticulin may be found in many of these conditions, including acute leukaemias.¹⁻⁴ Kundel *et al*² reported that as many as 30% of patients with acute leukaemia, both lymphoblastic and myeloblastic, have a diffuse increase in marrow reticulin. A much higher incidence of marrow fibrosis has been reported by others⁵ in both these conditions. Patients with reticulin fibrosis of their marrows have been considered to have poorer prognoses than those without such increases.²

The present study was carried out on material from 34 patients with acute myeloid leukaemia treated with intensive chemotherapy. The frequency, extent, and type of fibrosis at the time of initial diagnosis, the effect of cytotoxic therapy on the marrow fibre content, and the effect of fibrosis on prognosis were assessed and the possibility of haemopoietic regeneration in the presence of severe marrow fibrosis was examined. The question of acute or malignant myelosclerosis as a distinct entity separate from acute leukaemia with marrow fibrosis was also considered.

Material and methods

Bone marrow trephine biopsies were obtained from 34 patients with acute myeloid leukaemia at diagnosis and at varying intervals during the course of intensive remission induction chemotherapy and after its completion. One to four specimens were obtained from each patient during the first eight weeks of observation. All patients in this group were

treated with one or two courses of the RATE protocol (daunorubicin, cytosine arabinoside, 6-thioguanine, and epipodophyllotoxin) (VP16-213).⁶ Initially, all the trephine biopsy specimens were obtained with the 11G (regular adult) Jamshidi needle from the posterior iliac crest and later some of the biopsies were obtained with a new bone marrow biopsy needle.⁷ The biopsies were then processed in methyl-methacrylate⁸⁻⁹ from which 3 μ m sections were cut, without decalcification, with a tungsten carbide knife in a Jung's high performance microtome (Autocut). Sections were softened in 96% ethanol and were then attached to gelatin coated slides by gentle brushing with a soft brush. The methacrylate was dissolved from the sections by immersion in benzene, and the sections were stained with May-Grunwald-Giemsa and Gomori's reticulin stain.

The bone marrow fibre content in each section was visually assessed and graded according to the scoring system described previously.¹⁰ The type of bone marrow fibre was mainly reticulin in grades 1 and 2, a combination of reticulin and collagen in grade 3, and mainly collagenous in grade 4. No increase in marrow reticulin was recorded as grade 0 and any focal increase was recorded separately.

Results

Seventy one specimens from 34 patients were studied: 34 were obtained at diagnosis and 37 were obtained after chemotherapy and after complete remission.

The bone marrow fibre content at the time of initial diagnosis of acute myeloid leukaemia in 34 cases

Bone marrow section from a case of acute myeloid leukaemia. (a) Showing considerable increase (grade 3) in bone marrow fibre content at diagnosis. Gomori's reticulin stain. Original magnification $\times 600$. (b) Showing considerable reduction in the bone marrow fibre content after treatment. Gomori's reticulin stain. Original magnification $\times 600$. (c) Complete remission showing good haemopoietic regeneration. A mild degree of marrow fibrosis can be seen. Gomori's reticulin stain. Original magnification $\times 600$.

Table 1 Bone marrow fibre content in patients with acute myeloid leukaemia at the time of initial diagnosis, after chemotherapy, and at complete remission

Stages of the disease	No of cases studied	Grade of fibrosis			
		0	1	2	3
At diagnosis	34	22 (65%)	9 (26%)	0	3 (9%)
After chemotherapy	23	18*	3	1	1
At complete remission	16	14	1	1	0

*Five cases showed focal increase from grade 1 (+) to grade 3 (+++).

Table 2 Relation between the degree of marrow fibrosis and remission rate

Response to treatment	No of patients*	Grade of fibrosis	
		0	1-3
Complete remission	16	11/17 (65%)	5/9 (56%)
No remission	10	6/17 (35%)	4/9 (44%)

*Eight patients who died in the first four weeks, during induction treatment, due to unrelated causes were excluded.

was as follows: grade 0 in 22 (65%), grade 1 in nine, and grade 3 in three cases (Table 1). One of the nine cases with grade 1 fibrosis was classified as M_1 , one as M_2 , two as M_4 , three as M_5 , and one as M_6 . Two of the three cases with grade 3 fibrosis were classified as M_1 and one as M_4 according to FAB criteria. When the initial degree of marrow fibrosis (at the time of diagnosis) was considered in relation to prognosis a slight excess in complete remission rates was noted in those with little or no fibrosis (grade 0) compared with those with grade 1-3 marrow fibrosis (Table 2). This difference, however, was not significant.

After intensive chemotherapy the bone marrow fibre content was decreased by at least one grade in five cases, including one of grade 3 fibrosis (Figure (a) and (b)). In 18 cases there was no change in the bone marrow fibre content. One of these 18 cases had grade 3 marrow fibrosis and did not show any change after two courses of chemotherapy. An increase (from grade 1 to grade 3) of a focal nature was seen in five cases, although the overall fibre content remained unchanged in all these cases except one which showed some reduction.

When the capacity for haemopoietic regeneration was analysed in relation to the bone marrow fibre content, it was noted that in six of nine cases with increased marrow fibrosis (grade 1 in five and grade 3 in one) good haemopoietic regeneration occurred after chemotherapy with complete remission in all six of them. Haemopoietic regeneration in these six patients, including one with grade 3 marrow fibrosis (Figure (c)), suggests that increase in the bone marrow fibre content does not affect haemopoietic recovery after treatment and achievement of complete remission.

Discussion

The bone marrow in acute myeloid leukaemia is usually dominated by massive infiltration by blast cells and the diagnosis is made from examination of peripheral blood and bone marrow aspirate. Bone marrow trephine biopsy is not routinely used to establish a diagnosis. As a result the evidence of marrow fibrosis in acute myeloid leukaemia is not adequately documented, the effect of chemotherapy on the marrow fibre content remains unclear, and the concept that marrow fibrosis in acute myeloid leukaemia carries a poor prognosis is still upheld.

The present study shows that at least some increase in fibre content is present in 35% of the patients with acute myeloid leukaemia at presentation. The overall incidence of marrow fibrosis was lower in this group of patients than in those previously reported^{2,5}. No particular morphological type of acute myeloid leukaemia in the FAB classification¹¹ had increased marrow fibrosis in comparison with the other types.

The pathogenesis of marrow fibrosis in patients with acute leukaemia is not clear, but it is generally considered to be reactive.¹² Burston and Pinniger¹ proposed that the increase in the bone marrow fibre content was a physiological response to increased haemopoietic activity. This theory has, however, been questioned by Sanerkin³ since highly cellular marrows may have a normal bone marrow fibre content. The present findings confirm the view of Sanerkin since 65% of the patients with acute myeloid leukaemia in this series had highly cellular bone marrows but normal fibre content.

The presence and importance of marrow fibrosis in malignant disorders of the blood and lymphoid tissue have become more widely recognised^{2,5,12-15} and have raised doubts about the validity of the distinction between malignant myelosclerosis and acute granulocytic leukaemia first proposed by Lewis and Szur.¹⁶ In the present study three patients presented with features similar to those of malignant myelosclerosis and showed considerable (grade 3) marrow

fibrosis and circulating blast cells. The most frequent type of leukaemia associated with the syndrome of malignant myelosclerosis is megakaryoblastic leukaemia,¹⁷ but as has been shown in this study it could also be seen in other types of acute myeloid leukaemia.

The finding of increased marrow reticulin in patients with acute leukaemia has been regarded as being associated with poor prognosis, attributable to the persistence of reticulin and consequent possible interference with normal repopulation of the marrow after therapeutic aplasia.² Cytotoxic therapy was successful in inducing complete remission in six of nine cases of acute myeloid leukaemia with increased (grade 1-3) bone marrow fibrosis; the bone marrow fibre content decreased concurrently in three of the cases, including one with grade 3 fibrosis. Two of the three patients who did not achieve complete remission also showed reduction in the bone marrow fibre content after treatment but they failed to regenerate adequately and died of intercurrent infection. These findings confirm the view of Hann *et al*¹⁸ and Manoharan *et al*⁵ that the occurrence of marrow fibrosis does not necessarily change the overall prognosis of patients with acute leukaemia and that effective treatment may result in resolution of the increased marrow reticulin and thus help the repopulation of the bone marrow by normal regenerating haemopoietic cells. Reversal of marrow fibrosis has also been reported¹⁹ in acute megakaryoblastic leukaemia after remission induction and consolidation chemotherapy followed by bone marrow transplantation.

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