Myelofibrosis With Myeloid Metaplasia: Pathophysiologic Implications of the Correlation Between Bone Marrow Changes and Progression of Splenomegaly

By Barbara C. Wolf and Richard S. Neiman

We undertook a study of 35 cases of myelofibrosis with myeloid metaplasia to assess the histopathologic findings in the bone marrow of patients with this disorder, to ascertain if changes in morphology occurred with time, and to attempt to correlate marrow findings with splenic size. We reviewed 71 bone marrow biopsies and studied 13 splenectomy specimens. Sequential bone marrow biopsies were obtained in 21 cases over intervals ranging from two to ten years (mean, 4.5 years). We noted a patchy nature and variable degree of stromal proliferation in most marrow biopsies, and were unable to demonstrate a correlation between the extent of medullary fibrosis and duration of disease, splenic weight, or degree of splenic myeloid metaplasia. We were unable to document a progression of medullary fibrosis as a cause for increasing splenomegaly. However, the alteration in the marrow stroma in this disorder is responsible for the presence of distended marrow sinusoids with intravascular hematopoiesis, a phenomenon we observed in all cases. We believe that this morphological feature, not emphasized by previous investigators, is of significance in understanding the pathophysiology of myeloid metaplasia.

M E Y E L O F I B R O S I S with myeloid metaplasia (MMM), or agnogenic myeloid metaplasia, is a chronic myeloproliferative disorder characterized by fibrosis of the bone marrow accompanied by aniso- and poikilocytosis, leukoerythroblastosis, and splenomegaly with extramedullary hematopoiesis.1 It may arise de novo or evolve from polycythemia vera in its so-called "spent phase." 2-7

A wide spectrum of morphological changes has been described in the bone marrow in MMM, ranging from hypercellularity with a slight increase in reticulin to dense fibrosis associated with virtual depletion of hematopoietic elements.1,8-12 Previous authors have stated that progressive fibrous replacement of the medullary cavity occurs during the evolution of the disease.9,12,15 However, the literature contains little supporting evidence for this hypothesis based on long-term studies of sequential biopsies.

We undertook the present study to more clearly delineate the histopathologic features of the bone marrow in MMM, to study the evolution of marrow morphology during the course of the disease, and to correlate these findings with splenic size and the extent of splenic myeloid metaplasia.

MATERIALS AND METHODS

Bone marrow biopsies were obtained from 35 patients with MMM. Criteria for diagnosis included increased medullary reticulin, splenomegaly, aniso- and poikilocytosis, and leukoerythroblastosis, i.e., the presence of immature granulocytes and erythroblasts in the peripheral blood. Relevant clinical and laboratory data were obtained from hospital charts or from attending physicians. Cases were excluded if there was any clinical or histologic evidence of metastatic carcinoma, infection, or exposure to myelotoxins that might result in secondary myelofibrosis or extramedullary hematopoiesis. In addition, patients with other myeloproliferative disorders, as determined by clinical and laboratory data, who exhibited medullary fibrosis, were excluded. None of the patients had received chemotherapy or radioactive ³²P prior to the initial diagnosis of MMM. In five of the 35 cases, the diagnosis of MMM was made during the course of pre-existing polycythemia vera. These patients developed leukoerythroblastosis and increasing splenomegaly, and bone marrow biopsies showed increased reticulin. None of these patients had erythrocytosis or an elevated hematocrit at the time that the diagnosis of MMM was made. In many cases we were able to estimate the duration of the disease prior to diagnosis, by a history of symptoms related to anemia or splenomegaly. For the purpose of this study we used the first historical reference to such symptomatology as the date of onset of the disease.

Seventy-one bone marrow biopsies were reviewed. All specimens were fixed in Zenker's fixative and processed routinely. Paraffin sections were stained with hematoxylin-eosin (H-E), the periodic acid-Schiff (PAS) reagent, Gomori's iron reaction, a modified Masson's trichrome stain, and Wilder's reticulin stain. Cellularity was estimated and recorded as hypercellular, normocellular, or hypocellular for the patient's age. We attempted to quantitate the degree of medullary fibrosis according to the Bauermeister scale.16 In 21 cases, sequential bone marrow biopsies were available. These were obtained over time intervals ranging from two to ten years, with a mean of 4.5 years. Table 1 details the intervals between biopsies in these cases. Fourteen patients received no therapy other than transfusion during the course of their disease. Five patients were treated with busulphan in an attempt to control hypersplenism, and two of these patients also received radioactive ³²P for thrombocytopenia.

We recorded observations regarding splenic size which were made upon each admission or clinic visit as estimated by physical examination. In 13 cases, splenectomy was performed because of the development of anemia or thrombocytopenia attributed to hypersplenism. The estimated duration of disease at the time of splenec-
Table 1. Sequential Marrow Biopsies

<table>
<thead>
<tr>
<th>No. of Biopsies</th>
<th>No. of Cases</th>
<th>Mean Interval (yr)</th>
<th>Range (yr)</th>
</tr>
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<tbody>
<tr>
<td>5</td>
<td>1</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>4.7</td>
<td>4-6</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>3.6</td>
<td>3-4</td>
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<tr>
<td>2</td>
<td>12</td>
<td>3.9</td>
<td>2-10</td>
</tr>
</tbody>
</table>

The 35 patients included 19 males and 16 females. Ages ranged from 22 to 87 years with a mean of 63.4 years. All were Caucasian. The diagnosis of MMM was made in 28 cases during evaluation of symptomatic or subclinical anemia and/or splenomegaly. Two other patients found to have splenomegaly presented with gastrointestinal bleeding related to thrombocytopenia and one presented with a pulmonary embolus and thrombocytosis. The duration of disease at the time of diagnosis in these cases ranged from one month to 23 years (mean, 5.2 years). In the remaining five cases MMM evolved from polycythemia vera, the interval from the initial diagnosis ranging from four to 17 years (mean, 8.6 years).

Bone Marrow Histopathology

The bone marrow sections revealed a wide range of morphological changes. Although all biopsies exhibited stromal proliferation, its degree was extremely variable. In most cases there was an inverse relationship between marrow cellularity and the amount of reticulin present. Some biopsies showed hematopoietic cellularity approaching 100%. The myeloid, erythroid, and megakaryocytic series were all increased and showed evidence of maturation, although one cell line occasionally predominated. These cellular biopsies showed only a slight increase in reticulin. Other biopsies were more fibrotic and showed depletion of the hematopoietic elements. In the majority of cases, the stroma consisted entirely of reticulin, although two biopsies showed collagenous fibrosis. We noted prominent osteosclerosis with appositional thickening of bone trabeculae in five cases, only two of which showed prominent medullary fibrosis. The remaining three marrow samples were predominantly cellular.

In spite of the variability in the degree of medullary fibrosis and cellularity, we observed three constant morphological features. Most notably, we found that the stromal proliferation was a patchy process. The majority of cases showed a striking variability in hematopoietic cellularity and reticulin pattern with a given biopsy. Some fields were cellular (Fig 1A), while others showed depletion of hematopoietic cells (Fig 1B). The amount of reticulin varied significantly from field to field, being prominent in the less cellular areas but only minimally increased in the more cellular regions. Although some investigators have stated that bone marrow fat is depleted in MMM, we found that fat cells were scattered singly and in clusters throughout both the cellular and fibrotic regions in most biopsies. Because of the marked variability in

Clinical Features

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RESULTS
hematopoietic cellularity and medullary fibrosis, we were unable to reproducibly quantitate these variables. Due to the patchiness of the reticulin pattern, we were unable to grade the overall amount present.

Another constant finding was an increased number of megakaryocytes, which often occurred in clusters (Fig 2A) and which appeared to be most numerous in areas of greatest fibrosis. Many of these cells appeared dysplastic, with bizarre nuclear configuration. Hyposegmented and hyperchromatic hypersegmented nuclei were seen, with dyssynchrony between the degree of nuclear and cytoplasmic maturation (Fig 2B).

Megakaryocytes were often the predominant cells in the hypocellular biopsies, due to depletion of the myeloid and erythroid elements. The degree of megakaryocytic proliferation did not seem to correlate with the degree of medullary fibrosis, since numerous megakaryocytes were also found in the cellular marrows. No morphological abnormalities were seen in the erythroid or myeloid series.

The third constant feature in all of our cases was the presence of distended marrow sinusoids, which frequently contained intravascular hematopoietic cells (Fig 3). Intravascular hematopoiesis was readily apparent in the hypocellular marrows, which showed large dilated sinusoids containing immature precur-

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**Fig 2.** (A) Cellular area of bone marrow in MMM showing a proliferation of large numbers of megakaryocytes (PAS stain; magnification x200). (B) High-power detail of megakaryocytes showing dysplastic forms (PAS stain; magnification x400).

**Fig 3.** (A) Representative example of bone marrow biopsy in a patient with MMM, showing distended marrow sinusoids containing clusters of intravascular cells (PAS stain; original magnification x200; current magnification x173). (B) High-power detail of distended marrow sinusoid in another of our cases, showing intravascular trilinear hematopoiesis (PAS stain; original magnification x500; current magnification x433).
sors. However, small sinusoids with intravascular hematopoietic cells could be detected using the reticulin stain even in the intensely cellular biopsies.

**Sequential Bone Marrow Studies**

We were able to demonstrate morphological progression in stromal fibrosis in only one of the 21 cases in which sequential bone marrow biopsies were obtained. In 17 of the cases, no increase in stromal fibrosis could be documented (Fig 4A through D). Four of these patients developed acute leukemia over intervals ranging from one to three years, two of whom had received chemotherapy prior to the development of leukemia. Most surprisingly, in the remaining three cases, a striking decrease in the amount of medullary fibrosis was observed, which was associated with clinical and laboratory evidence supporting the transition from MMM to polycythemia vera. (A detailed clinico-pathologic report of these three cases is to be published.)

**Splenic Size**

We found a rough correlation between splenic size, as assessed by physical examination or by weight and estimated duration of disease. All patients showed progressive splenomegaly, although the rate of splenic

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Fig 4. Representative examples of bone marrow from patients presenting with cellular (A, B) and fibrotic (C, D) marrows showing lack of progression of reticulin fibrosis. The interval between biopsies A and B is ten years and that between biopsies C and D is three years (A, B, PAS stain; magnification ×1,000; C, D, Wilder's reticulin stain; magnification ×150.)
enlargement was highly variable. The degree of splenomegaly at the time of initial diagnosis ranged from slight to massive. The larger spleens were most commonly found in patients with the longest duration of symptoms, although in occasional asymptomatic patients, massive splenomegaly was an incidental finding. Although chemotherapy is known to affect the rate of splenic enlargement, we do not believe that this was a significant factor in our cases, since only five patients were so treated and the resultant decrease in splenic size was both slight and transient. Splenic weights in the 13 splenectomized patients ranged from 300 to 3,200 g (mean, 1,420 g). Table 2 shows the relationship between bone marrow cellularity and splenic weight and disease duration. The marrows referred to were obtained up to eight months prior to splenectomy (mean, 1.4 months). The mean weight was greatest in patients with the longest average duration of disease. However, there was considerable overlap of splenic weights among these groups.

We could discern no correlation between bone marrow cellularity and fibrosis and either splenic size or clinically estimated duration of disease. Hypercellular marrow samples were obtained from patients with disease of short duration and mild splenic enlargement, as well as from patients with symptoms dating many years and massive splenomegaly. Conversely, although hypocellular fibrotic bone marrows were more usually found in patients with long-standing symptoms and prominent splenomegaly, such hypocellular biopsies were frequently obtained from patients either with a short history of clinical symptoms or with mild to moderate incidentally discovered splenomegaly. Bone marrow biopsies among the splenectomized patients showed hypercellularity with slight fibrosis in four cases, hypocellularity and prominent fibrosis in two cases, and the remaining seven cases showed moderate cellularity. As shown in Table 2, there was a wide range in both disease duration and splenic weight associated with each pattern of bone marrow histology, with considerable overlap between groups. We could not demonstrate a relationship between increasing splenic weight and disease duration with decreasing medullary cellularity. In fact, the splenic weights were greatest in cases in which the bone marrow showed marked hypercellularity. The less cellular bone marrows were not associated with smaller spleens or with disease of shorter duration.

All spleens showed trilineal extramedullary hematopoiesis (myeloid metaplasia). Hematopoietic precursors were seen in both the cords of Billroth and in the sinuses of the red pulp. Splenic hematopoiesis ranged from moderate, with precursors which were predominantly in late stages of maturation, to massive, with infiltration of the red pulp by numerous dysplastic megakaryocytes and primitive cells of the erythroid and myeloid series. There was no relationship between the bone marrow cellularity and fibrosis and the degree of splenic myeloid metaplasia or the immaturity of the hematopoietic precursors seen in that organ. The spleens obtained from the patients with hypercellular bone marrows were morphologically indistinguishable from those obtained from patients with less cellular marrows.

### Table 2. Bone Marrow Morphology in Splenectomized Cases

<table>
<thead>
<tr>
<th>No. of cases</th>
<th>Hypercellular</th>
<th>Normocellular</th>
<th>Hypocellular</th>
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<tr>
<td>Duration of disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>3–12 yr</td>
<td>1–13 yr</td>
<td>1–3 yr</td>
</tr>
<tr>
<td>Mean</td>
<td>7.5 yr</td>
<td>4.0 yr</td>
<td>2.0 yr</td>
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<tr>
<td>Splenic weight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>1,400–3,200 g</td>
<td>300–1,900 g</td>
<td>1,100–1,300 g</td>
</tr>
<tr>
<td>Mean</td>
<td>2,333 g</td>
<td>1,093 g</td>
<td>1,200 g</td>
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DISCUSSION

MMM is a disease in which fibrosis of the bone marrow, aniso- and poikilocytosis, leukoerythroblastosis, and splenomegaly occur in the absence of infection, exposure to myelotoxins, metastatic carcinoma, or other hematologic disorders that might result in secondary myelofibrosis and extramedullary hematopoiesis.9,13,16 The medullary stromal proliferation often results in a “dry tap,” that is, the inability to aspirate the marrow. Bone marrow biopsy is therefore essential to confirm the diagnosis of MMM.1,13,19,21

We studied bone marrow biopsies from 35 patients with MMM. Increased reticulin, trilinear hematopoiesis, and clusters of dysplastic megakaryocytes were found in all specimens. Although stromal proliferation was evident in all cases, we found a wide range in the degree of medullary fibrosis and hematopoietic cellularity, in agreement with previous authors.1,8,12 However, we were also impressed with the variability in the degree of fibrosis within a single biopsy. We found hypercellular areas with a mild increase in reticulin alternating with densely fibrotic areas that were depleted of hematopoietic cells. Although the patchy nature of medullary fibrosis has been mentioned by others,22 we believe that its importance in creating difficulties in assessing the progression in fibrosis in this disorder has not been emphasized.

Previous authors have referred to “stages” of bone marrow histopathology in MMM9,12,14 and have postulated that a progressive fibrosis occurs in the course of the disease.9,12,15 The degree of splenomegaly in MMM has been shown to correlate with the duration of disease as estimated by historical parameters. Ward
and Block \textsuperscript{11} noted that hypercellular marrows were most commonly found in patients with mild splenomegaly, while fibrotic marrows were usually associated with prominent splenomegaly. These authors implied that the bone marrow morphology in MMM progressed from the cellular pattern to hypocellularity with fibrosis. However, they acknowledge that their study provided little evidence to support this hypothesis. Fourteen of their patients had more than one biopsy and only one of these showed a change in histologic pattern. These authors emphasized the need for long-term studies. Pitcock \textit{et al} \textsuperscript{18} reviewed sequential biopsies from 15 patients with MMM and observed a progression of medullary fibrosis in only one. In some instances, they noted a slight decrease in fibrosis. However, only three patients were observed for a period of greater than two years. Our findings, based on sequential studies extending over a significantly greater interval of observation than either that of Ward and Block or of Pitcock \textit{et al}, do not provide evidence of a progression of fibrosis in most cases of MMM. Moreover, in agreement with observations of Lohman and Beckmann,\textsuperscript{22} we noted a decrease in fibrosis in some cases, which, in our study, was associated with clinical and laboratory evidence of conversion to polycythemia vera, a disorder conventionally thought to precede MMM.

Our findings fail to substantiate the belief that a progression of fibrosis occurs in the bone marrow in most cases of MMM. Although we also found a rough correlation between the duration of disease and splenic size, we could not correlate decreasing medullary cellularity and increasing reticulin with clinically estimated splenic size or splenic weight. In contrast to Ward and Block’s observations, the largest spleens in our study were obtained from patients with hypercellular bone marrows, providing evidence against the hypothesis that a progressive fibrosis and myeloid atrophy occurs in the course of the disease. Our findings are supported by Bentley and Herman,\textsuperscript{23} who used a digital image processing technique to quantitate medullary fibrosis in MMM. They found no correlation between the degree of fibrosis and either splenic size or duration of disease. We also noted no predictable relationship between marrow morphology and microscopic estimates of the degree of splenic extramedullary hematopoiesis. This finding has been corroborated in other studies.\textsuperscript{11,18} In addition, we could detect essentially no change in the degree of bone marrow cellularity and fibrosis in 16 of 17 patients in whom sequential biopsies were obtained and who did not develop acute leukemia, in spite of a progressive increase in splenic size.

Clinical experience gained from studying patients with polycythemia vera who evolved to “spent phase,” as well as laboratory studies demonstrating progressive insolubilization of collagen,\textsuperscript{24} support the belief that marrow fibrosis progresses in MMM. However, several lines of evidence suggest that the fibroplasia in this disorder is not simply incremental. These include (1) our observations of the patchy nature of the fibrosis, (2) our inability to demonstrate a provable increase in fibrosis over a longer period of observation than that reported by previous authors, and (3) morphological and clinical evidence in three of our cases of reversion to polycythemia vera. Although we could not document progressive fibrosis in MMM, we cannot be certain that our results were not partly related to sampling error. However, such error would be minimized in cases in which multiple biopsies were studied. Although progressive fibrosis of the medullary cavity must logically occur as part of the natural history of MMM, the factors governing its progression appear to be complex, and its rate must be extremely variable, and often very slow.

We believe that our ability to invariably demonstrate distended sinusoids with intravascular hematopoietic cells in bone marrow biopsies from patients with MMM is of potential importance in understanding the pathogenesis of splenic myeloid metaplasia and the apparent lack of relationship between the degree of splenomegaly and medullary fibrosis. Although prominent sinusoids have been noted by other investigators, intravascular hematopoiesis had only rarely been mentioned.\textsuperscript{1,14,17} The two most widely accepted theories to explain myeloid metaplasia in this disease are the compensatory theory\textsuperscript{12} and the myelostimulatory theory.\textsuperscript{29,41} The compensatory theory, which states that splenic hematopoiesis occurs in compensation for a failing progressively fibrotic bone marrow, can be disproved by the results of this study, as well as those of others.\textsuperscript{9,11,21} The myelostimulatory theory, which states that splenic hematopoiesis is similar to that seen in polycythemia vera and results from a reversion of hematopoiesis to embryonic sites, has been disproved by our previous demonstration that neither the human fetal spleen nor spleens in patients with P vera contain significant hematopoiesis.\textsuperscript{25,26} The latter observation is supported by those of Rappaport.\textsuperscript{1} We believe that the demonstration of distended sinusoids with intravascular hematopoiesis on the bone marrow provides an explanation for the leukoerythoblastosis and so-called myeloid metaplasia in the spleen in MMM. The altered stromal microenvironment of the bone marrow provides hematopoietic cells access to the circulation. These cells tend to remain within the vascular spaces of
the bone marrow either because of pinching off of the
marrow sinusoids by the stromal fibrosis or because of
their cohesive nature. Once these immature hematopoietic cells enter the peripheral blood, they are
filtered out by the spleen. Progressive accumulation of
the hematopoietic cells in the spleen is therefore a
function of time and explains the fact that progressive
splenomegaly appears to be independent of the extent
of bone marrow fibrosis.

ACKNOWLEDGMENT
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