Myelofibrosis in Chronic Granulocytic Leukemia

By Harvey R. Gralnick, John Harbor and Charles Vogel

In 181 patients with chronic granulocytic leukemia (CGL) 39 patients demonstrated myelofibrosis (MF) during the course of their disease. These groups can be divided into two groups: one group initially presenting with the clinical and laboratory findings of CGL but with concomitant MF and a second group in whom MF developed late in the course of CGL. The occurrence of MF with CGL was associated with three findings: (1) elevation of the leukocyte alkaline phosphatase, (2) a very poor prognosis and (3) the frequent association of MF and blastic transformation of CGL. MF should be considered an integral part of CGL and when present should be considered part of the terminal phase of CGL and treated accordingly.

Although myelofibrosis (MF) has been observed to accompany chronic granulocytic leukemia (CGL), the significance in the natural history of the leukemia process is unclear. In the present paper the characteristics of 39 patients with documented CGL associated with MF are examined. In addition, a group of eight patients with increased reticulin in their bone marrows will be presented. The 39 patients with MF and CGL could be divided into two groups: seven patients presenting with the classical clinical and laboratory findings of CGL but with concomitant MF and 32 patients in whom MF developed late in the course of CGL. The object of the present study is to show the close relationship between MF and CGL, to correlate some of the biochemical, laboratory and clinical changes with alterations in the nature and course of CGL and to define the nature of the terminal phase of CGL.

Materials and Methods

Case Material

The clinical records and bone marrows of 181 patients with CGL seen at the National Cancer Institute (NCI) in the period 1959 to early 1968 were examined. Each patient had, on an average, five bone marrow examinations. Some were aspirates alone but, since 1962, bone marrow needle biopsies have been performed, in addition to bone marrow aspirations. Thirty-nine patients demonstrated MF at some time during the course of the disease. Each of the 39 had at least one Westerman-Jensen or Vim-Silverman

From the Hematology Service and the Nuclear Medicine Department, Clinical Center, National Institutes of Health, Bethesda, Md., and the Solid Tumor Service, Medicine Branch, National Cancer Institute, Bethesda, Md.


Harvey R. Gralnick, M.D.: Hematology Service, CPD, Clinical Center, National Institutes of Health, Bethesda, Md. John Harbor, M.D.: Dept. of Nuclear Medicine, Georgetown University, Washington, D.C.; formerly Nuclear Medicine Department, Clinical Center, National Institutes of Health, Bethesda, Md. Charles Vogel, M.D.: Lymphoma Treatment, Kampala, Uganda; formerly Solid Tumor Service, Medicine Branch, National Cancer Institute, Bethesda, Md.
MYELOFIBROSIS IN LEUKEMIA

Fig. 1.—(A) Hypercellular bone marrow with marked myeloid hyperplasia from a patient with untreated chronic granulocytic leukemia. × 100. (B) Bone marrow smear from the same patient demonstrating myeloid hyperplasia with granulocytic cells in all stages of maturation. × 400.

needle biopsy of bone marrow. Nineteen patients with MF were males and 20 were females. The mean age at time of diagnosis of CGL was 42.6 years and ranged from 15–58 years. In addition, since early 1968 marrow biopsies have been performed on an additional 30 newly diagnosed, untreated patients with CGL.

Laboratory Methods

Bone marrow specimens were fixed in B-5 fixative (mercuric chloride, sodium acetate, and concentrated formaldehyde) for 2 hours. The bone biopsies were decalcified by the rapid “Decal” (Scientific Products, Washington, D.C.) method for 1 hour. The tissues were then processed on the Technicon (Technicon Co., Chauncey, N.Y.), embedded in Paraplast (Thomas, Philadelphia), and sectioned at 5 μ. The sections were routinely stained with hematoxylin and eosin. In addition, all biopsy sections were stained with a modified Hortega-Foot method1 to demonstrate reticulin and with Masson trichrome1 to demonstrate collagen. Bone marrow aspirates and peripheral blood smears were air dried and prepared with a modified Romanowsky stain.

In the last 30 consecutive untreated patients with CGL, bone marrow biopsy sections were routinely stained for reticulin and collagen.

Bone marrow cytogenetics were performed on all patients.2 Leukocyte alkaline phosphatase was measured initially quantitatively3 and later by a semiquantitative method.4 All patients in this study had multiple determinations of routine hematologic and clinical chemistry values. Serum B12 assays were performed in some patients.

Bone marrow scans were performed 1 hour after intravenous administration of 3 mCi of 99mTc-sulfide colloid.5 Free 99mtechnitium was always less than 2 per cent by radiochromatography. A scintillation camera fitted with a 4000-hole low energy collimator was used for scanning. Anterior views were obtained over the shoulders, pelvis, knees and ankles. Posterior views were obtained over the cervical and lumbosacral spine areas. Each scintiphoto was integrated for 200 seconds regardless of the number of counts collected.

RESULTS

Bone Marrow Interpretation

Typical bone marrow histology of CGL has been characterized as hypercellular with marked myeloid hyperplasia and varying degrees of megakaryocytosis (Fig. 1.) In contrast to this pattern the bone marrow histology of the 39 patients with CGL and MF demonstrated a dense fibrous reaction con-
Fig. 2.—(A) Bone marrow from a patient with chronic granulocytic leukemia with broad bands of fibrous tissue filling the marrow cavity. × 100. (B) Hortega-Foot stain of this marrow showing marked increase of reticulin fibers. × 100.

Reticulum cells and reticulin fibers (Fig. 2). Reticulin strains were positive in all patients and collagen strains were positive in all but two. The marrow was usually hypocellular with scattered lymphocytes, plasma cells and megakaryocytes. A plethora of large round cells which appeared to be megakaryocytes were present in several patients' marrows. Dilated sinusoids and capillaries were common. No specific histologic changes could be found in trabecular bone.

In the 39 patients the degree of MF could be classified as diffuse in 29 and focal in 10 in bone marrow biopsies. In four patients it was possible to observe focal MF progress to diffuse involvement in subsequent marrow biopsies. In six patients focal MF did not progress to diffuse involvement. In one patient with focal fibrosis, no MF could be demonstrated in subsequent marrow specimens.

Once diffuse MF was present, the only change in marrow cellularity or cell content noted in subsequent specimens was an infiltration and, at times, an almost complete replacement by immature myeloid cells characteristic of blastic transformation. In these bone marrows myelofibrosis was still present and the amount of reticulin and collagen did not appear to be altered from previous marrows. In the group of patients with focal myelofibrosis, fibrous reaction was equally dense where present but was interspersed with areas of normal to hypercellular marrow characteristic of CCL.

None of the 30 newly diagnosed untreated patients with CGL demonstrated appreciable myelofibrosis in marrow biopsy sections at the time of diagnosis, but, in eight of the 30, moderate to marked increases in reticulin fibers could be demonstrated with reticulin stains and in these slight increases in collagen were seen. This condition will be referred to as minimal reticulin fibrosis. In these eight patients bone marrow was easily aspirated.

Examination of peripheral blood smears in patients with CGL and MF did not reveal the erythrocyte morphology typical of agnogenic myeloid metaplasia. Occasional tear drop cells with anisocytosis and poikilocytosis were seen, and rare circulating nucleated red blood cells were present.
Table 1.—Clinical Features of 32 Patients With CGL and Late Onset of MF

| Clinical Characteristics: Patients With MF at Time of Initial Diagnosis of CGL |
| Mean duration from Dx of CML to Dx MF | 36 months |
| Range | (7-131) months |
| Mean duration from Dx of MF to demise | 4.9 months |
| Range | (1-27) months |
| Mean total survival from Dx of CML | 41 months |
| Range | (10-132) months |
| Patients with blastic transformation | 22 |
| Confirmed antemortem | 22 |
| Confirmed postmortem | 5 |
| Not confirmed | 5 |
| Patients receiving busulfan | 27 |
| Patients with bone pain | 14 |
| Patients with radiographic bone lesions | 5 |

Dx, diagnosis.

Clinical Characteristics: Patients With MF at Time of Initial Diagnosis of CGL

None of the seven patients in this category had received prior myelosuppressive chemotherapy and there was no previous history of exposure to radiation or myelotoxic agents. The mean duration of symptoms prior to diagnosis in these patients was 7.7 months (range 2-16). Clinical symptomatology was in no way different from that in CGL without MF. Easy fatigueability, anorexia, weight loss and left upper abdominal "fulness" were typical complaints; one patient had bone pain but no radiographic bone lesions. All patients had splenomegaly and five had hepatomegaly on physical examination; two patients had prominent lymphadenopathy.

Four patients died with a mean survival of 8.8 months (range 3-15) after the diagnosis of CGL with MF was established. These patients had developed blastic transformation 1, 2, 3, and 12 months after diagnosis.

Patients With MF as a Late Complication of CGL

One patient in this group of 32 had a past history of radium implantation for dysmenorrhea, a second had received Tapazole before the diagnosis of CGL was established, and a third had been exposed to mustard gas during World War I. Clinical symptomatology and physical findings (Table 1) at the time of diagnosis of CGL were no different from those noted in other series of patients with CGL. The clinical course of the chronic phase of their disease was generally uncomplicated except for rare episodes in some patients of drug-induced bone marrow hypoplasia, with secondary infections or minor hemorrhagic complications. Twenty-two patients received Busulfan (Burroughs Wellcome, Tuckahoe, N. Y.) as the primary form of therapy and five others received this drug at some time during the clinical course. Of the five patients who never received Busulfan, one received 32P and four dibromonanitol (DBM). At varying intervals (7-131 months) after the initial diagnosis of CGL there was a change in the clinical and hematological status of these patients, often with recurrence of earlier symptomatology. Increasing splenic enlargement, anemia, thrombocytopenia and development of resistance to previously effective therapy were the predominant problems. In
addition, 14 of the 32 patients had significant bone pain, some with bone
tenderness; five of these 14 patients had radiographic bone lesions; three
were purely osteolytic, one mixed osteoblastic and lytic, and one predomi-
nantly sclerotic. Because of a change in the clinical course in these patients,
iliac crest of sternal marrow aspirations were attempted but marrow was
rarely obtained. Bone marrow biopsies established the presence of myelo-
fibrosis shortly before (2–3 months) or at the time of these varied clinical
changes.

While overall survival from diagnosis of CML to death is comparable to
other series of patients with CML (mean 41 months), survival after develop-
ment of MF was poor (mean 4.9 months). Only one patient lived for more
than 1 year after the presence of MF was established. This patient had focal
increase in reticulin which persisted unchanged until her death. The most
significant clinical problem in these patients (late onset MF) was ascertain-
ing the presence of blastic transformation. Blastic transformation was con-
formed antemortem in 22/32 patients. In one of these patients the diagnosis
was made by lymph node aspiration and in two others by tibial or humeral biopsy
after bone marrow scanning. Postmortem examinations were done on 9/10
patients who died without antemortem proof of blastic transformation. In
five of these patients blastic transformation was detected by bone marrow
sections from several sites. Postmortem sections of vertebrae and sternum
from patients with MF and blastic transformation contained focal areas of
hypercellular marrow in a background of dense myelofibrosis. These hyper-
cellular areas were composed primarily of large immature myeloid cells con-
sistent with myeloblasts. The other five patients with MF who could not be
proven to have blastic transformation developed peripheral blood and/or
bone marrow pictures characterized by granulocytic dyspoiesis and immatu-
ritiy but with less than 50 per cent myeloblasts in marrow specimens.

Laboratory Studies

Although approximately 10 per cent of patients with CGL seen at the
NIH do not have the Philadelphia chromosome, all 39 patients with CGL and
MF were Ph’ positive (Table 2. None of the seven patients presenting with
CGL and MF had any chromosome abnormalities other than a single Ph’
chromosome. However, eight of the 32 patients who developed MF as a late
complication had two Ph’ chromosomes. One of these eight had lymphad-
enopathy and all had concomitant blastic transformation, established either
antemortem or postmortem. Of these eight patients, six were studied cyto-
genetically more than once; five had a single Ph' chromosome at the time of
diagnosis of CGL but converted to the double Ph' cell line at the time of
development of MF. The sixth still had a single Ph' chromosome at the time
MF was detected on multiple iliac crest biopsies. She subsequently developed
blastic transformation with a double Ph' chromosome but was found to have
easily aspirated sternal bone marrow while still showing MF in iliac crest
biopsies.

None of the CGL patients presenting with MF had significant thrombocy-
topenia or leukopenia, but five had hemoglobin values less than 9.0 Gm. per
cent without antecedent bleeding episodes. Of those patients developing MF
as a late complication of CGL, 18 of 32 had hemoglobin values less than 9.0
Gm. per cent at the time of diagnosis of MF. Thrombocytopenia was likewise
significant in these patients with 18 of 32 having platelet counts less than
100,000/cu. mm. at the time of diagnosis of MF. In fact, most of these
patients had counts less than 20,000/cu. mm. and required platelet trans-
fusions. Ten patients had WBC counts less than 10,000/cu. mm. with four
of these below 4000/cu. mm.

Vitamin B\textsubscript{12} levels were done in eight patients at the time of diagnosis
of CGL and the values were elevated in all. While there were abnormalities
of serum uric acid and renal and liver function tests in many patients, no
consistent abnormalities of these parameters were noted. These abnormalities
could not be related to changes in the course of the disease.

Leukocyte alkaline phosphatase (LAP) was measured in 30 of the 39 pa-
tients with MF and CGL. The initial scores were low in 25, normal in two,
and elevated in three. Of the seven patients who presented with CGL and
MF, six had low or zero LAP scores and the other patient had the Philadel-
phia chromosome, high vitamin B\textsubscript{12} levels and a high LAP. In the group of
patients who developed MF late, 19 of 23 tested at presentation had low LAP
scores, two had normal scores and two had high scores. Of these 19 patients
with initially low LAP scores, 11 had new LAP determinations when they
developed MF. Eight had normal or elevated scores and three maintained
low LAP scores. LAP determinations were performed at the time of diagnosis
of MF in six additional patients (not tested at time of diagnosis of CGL).
Two had low LAP scores and four had elevated scores.

**Marrow Scanning**

Sixteen patients had bone marrow scans. The scans could be classified ac-
cording to three patterns: (1) peripheral extension without central depression,
(2) peripheral extension with central depression, and (3) generalized depres-
sion. Two patients had generalized marrow expansion (Fig. 3A) charac-
terized by normal or increased marrow activity in the adult marrow sites:
pelvis, shoulder and lumbarosacral spine and expansion of activity into juvenile
sites, i.e., distal femur, humerus, knees and ankles. The sternal marrow is
seldom well visualized because of scatter from adjacent liver and spleen
which sequester 80–90 per cent of the administered dose. Both of these
patients had bone marrow biopsy specimens characterized by granulocytic
Fig. 3.—(A) Pattern of marrow expansion. There is extension of marrow into the humerus, distal femur, knees and ankles. (B) Pattern of marrow depression with expansion. Absence of marrow in adult sites with extension into juvenile sites: humerus, distal femur, knees and ankles. (C) Pattern of marrow depression. Absence of marrow activity in both adult and juvenile sites.

hyperplasia and hypercellular marrow, with no evidence of increased reticulin.

Seven patients showed a pattern of marrow depression with expansion (Fig. 3B) characterized by decreased activity in the adult marrow sites and increased activity in juvenile sites. Three of these seven had biopsies (two tibial, one humeral) where scans showed active marrow. Two of these biopsy specimens revealed distinct islands of immature myeloid cells (Fig. 4) dispersed within predominantly fibrous tissue. Apparently this marrow component was present in sufficient quantity to sequester a significant amount of injected colloid. The third specimen revealed only myelofibrosis. Seven patients were characterized by generalized bone marrow depression (Fig. 3C). All had predominantly myelofibrotic biopsy specimens except for one patient whose biopsies showed only minimal reticulin fibrosis.

DISCUSSION

MF occurring in the course of CGL has been observed by others but the prevalence of this finding and its prognostic implications have not previously been established. In the present series 39/181 (22%) patients with CGL were found to have significant MF on antemortem needle biopsies. Bone marrow biopsies in these patients with CGL have helped to define the marrow characteristics of CGL, particularly in the preterminal phase. These studies show that MF is more common than previously recognized in CGL and that diffuse fibrosis heralds an accelerated phase with rapid demise.
The 32 patients who developed MF late in the course of CGL had initial clinical and laboratory findings and over-all survival duration (46 months) comparable to those of other large series.12-13 The finding of MF was an ominous prognostic sign for the group of 32 patients who developed MF late in the course of CGL. They had a mean survival of 4.9 months after the diagnosis of myelofibrosis was established. In this late onset group, the survival was the same whether the patient had focal or diffuse MF. In the seven patients with MF detected concomitantly with CGL the prognosis was poor and blastic transformation occurred early in the course of the disease. None of the patients had osteosclerosis on bone roentgenograms or the peripheral red blood cell morphology characteristic of agnogenic myeloid metaplasia.

In an additional 30 untreated patients studied prospectively, none demonstrated myelofibrosis on bone marrow biopsy; however, in eight patients minimal reticulin fibrosis was found with special stains. It may be significant that 22 per cent of our 181 patients with CGL developed MF and that 27 per cent of 30 untreated new patients demonstrate increased reticulin. This latter group of patients will help to determine if minimal reticulin fibrosis is the forerunner of MF.

In the past, most studies of the terminal phases of CGL have been based on peripheral blood findings.14,15 Consequently, the frequent occurrence of MF in bone marrow was not detected. In a recent study of the terminal phase of CGL,15 a group of patients developed a significant change from the benign clinical course of CGL but without demonstrable blastic transformation. The possibility of MF causing or being associated with these changes was not mentioned.

In the present study the diagnosis of coexisting blastic transformation was often difficult to prove but was definitely demonstrated either ante- or post-mortem in 27 of 32 patients with late MF and in four of seven patients with MF at onset of CGL. In the other five patients with “late” onset of MF, bone marrow and/or peripheral blood demonstrated varying degrees of granulocytic abnormalities and immaturity which by classical criteria were not sufficient
to be designated blastic transformation. The rapidly deteriorating clinical status and poor response to chemotherapy in these five patients was not different from that seen in patients with blastic transformation. From the bone marrow findings it is apparent that the concept of the terminal phase of CGL should be expanded from blastic transformation to include MF with granulocytic dyspoiesis and immaturity but lacking the number of myeloblasts characteristic of blastic transformation.

The terminal phase of CGL is associated with changes in laboratory parameters. Anemia and thrombocytopenia were reported as the commonest early findings in the terminal phase of CGL. Anemia and thrombocytopenia were commonly seen and could not be attributed to chemotherapeutic effects.

The development of the double Ph' chromosome has been associated with atypical myeloid maturation, preterminal phase of CGL, cases of CGL with prominent lymphadenopathy and blastic transformation. In the present series, eight patients who developed MF late in the course of CCL had double Ph' chromosomes; all were subsequently found to have blastic transformation. It is apparent that the presence or development of a double Ph' or polyploid Ph' chromosome, although not specific for blastic transformation or MF, indicates an acceleration of the course of CCL.

The leukocyte alkaline phosphatase is characteristically low or absent in CCL but may return toward normal when remission is induced. In contrast, in myeloid metaplasia with myelofibrosis the leukocyte alkaline phosphatase is usually normal or elevated. In the group of patients with late MF there was a concomitant increase in the LAP values, but almost half of the patients in this group subsequently developed blastic transformation. A LAP score which is initially low and changes to normal or high values in conjunction with changes in the clinical course is an indicator of an accelerated phase of CCL.

In order to evaluate the presence of blastic transformation and MF in patients with CCL, only bone marrow needle biopsies are now performed at the NIH. Even with this technique the potential sampling error is large. Bone marrow scanning has aided in choosing biopsy sites and assessing marrow distribution in CCL. Scans were correlated with bone marrow biopsies. In two of three patients with depression in adult sites but expansion into juvenile sites, bone marrow biopsies of the humerus and tibia revealed focal areas of active myelopoiesis consistent with blastic transformation. In the third patient marrow biopsy revealed only MF. In our experience there is no clear correlation between the severity of MF and the depression of uptake in bone marrow scans.

The reasons for the high incidence of MF in CCL are not clear. Some have implied a relationship between chemotherapy, particularly Busulfan, and the late development of MF. Busulfan has been noted to cause dysplasia and fibroplasia of the lungs, skin, ovary and cervix. Logically, Busulfan might be expected to cause fibroplasia in bone marrow, but five patients in the present series had not received Busulfan and seven patients never
received any chemotherapy prior to the discovery of MF. It seems that Busulfan is not necessary to produce MF, but it remains possible that the drug can intensify the tendency to fibrosis in CCL.

In this report we have been concerned with fibroblastic proliferation in CCL and resultant MF. This fibroblastic proliferation usually appears late in the disease, but may present early in the course of the disease. Thirty-nine patients with CCL had concomitant MF. The three most consistent findings in these patients were (1) the elevation of the LAP at the time that MF appeared; (2) the poor prognosis when MF was present; and (3) the frequent association of MF and blastic transformation.

REFERENCES


Myelofibrosis in Chronic Granulocytic Leukemia

HARVEY R. GRALNICK, JOHN HARBOR and CHARLES VOGEL