Chronic Idiopathic Myelofibrosis
Clinicopathologic Features, Pathogenesis, and Prognosis

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• Context.—Chronic idiopathic myelofibrosis (CIMF) is a clonal myeloproliferative disease characterized by panmyelosis with intact maturation, progressive bone marrow fibrosis, and multiorgan extramedullary hematopoiesis.

Objective.—This review article aims to summarize the recent updates regarding the clinicopathologic features, molecular pathogenesis, cytogenetic abnormalities, diagnostic criteria, new diagnostic ancillary tests, and prognostic factors of CIMF.

Data Sources.—Important relevant articles indexed in PubMed/MEDLINE (National Library of Medicine) through the end of 2005 and referenced medical texts.

Conclusions.—Because CIMF has a variety of clinical presentations, diagnosis may be challenging; the prefibrotic stage of CIMF has always been a challenging disease for pathologists to diagnose accurately. The recently proposed European Clinical and Pathological criteria can be helpful in the diagnosis of CIMF, especially in its prefibrotic stage. The enumeration of CD34-positive cells in the peripheral blood and the presence of circulating endothelial progenitor cells are the new important ancillary tests for the diagnosis of a small subset of patients with CIMF with atypical presentation. The recent discovery of the new mutation affecting the Janus tyrosine kinase 2 (JAK2V617F), more frequently observed in patients with polycythemia vera, is seen in approximately 35% to 57% of patients with CIMF. This mutation can serve as another diagnostic tool. Important factors affecting prognosis in CIMF are anemia, age of the patient, white blood cell count, degree of fibrosis, and number of blasts in the peripheral blood.

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Chronic idiopathic myelofibrosis (CIMF) is a clonal stem cell defect characterized by panmyelosis with intact maturation, progressive bone marrow fibrosis, and splenomegaly with multiorgan extramedullary hematopoiesis. The disease is known by other names, such as agnogenic myeloid metaplasia, myelosclerosis with myeloid metaplasia, idiopathic myelofibrosis, megakaryocytic myelosis, and osteomyelosclerosis. These different names applied to the same entity indicate the features of the disease most striking to the various observers. According to the World Health Organization classification, CIMF is categorized in the spectrum of chronic myeloproliferative disorders that includes chronic myelogenous leukemia (Philadelphia chromosome positive: t(9;22)(q34;11), BCR/ABL), polycythemia vera (PV), essential thrombocythemia (ET), chronic neutrophilic leukemia, and chronic eosinophilic leukemia/hypereosinophilic syndrome.

EPIDEMIOLOGY

The incidence of CIMF is estimated to be 0.3 to 1.5 per 100,000 individuals per year.2,3 It occurs most commonly in the seventh decade (rare reports of familial and nonfamilial cases in younger patients, even infants, have been described)4,5 and there is equal incidence in men and women.7 Although the etiology of idiopathic myelofibrosis is unknown, myelofibrosis can be a secondary process resulting from hematologic or nonhematologic conditions (Figure 1).8–10 Myelofibrosis develops in 25% to 50% of patients with PV11,12 and in 2% to 3% of patients with ET.13 The secondary myelofibrosis is far more common than CIMF, but it is important to exclude these conditions before making the diagnosis of CIMF.

PATHOGENESIS

Marrow Fibrosis

The cause of the excessive marrow fibrosis observed in this disorder remains unclear. Platelets, megakaryocytes, and monocytes are thought to secrete several cytokines, such as transforming growth factor-β, basic fibroblast growth factor (bFGF), epidermal growth factor, and platelet-derived growth factor, which may result in fibroblast proliferation and dysregulation of extracellular matrix formation.7,14

A recent study has shown that patients with CIMF have abnormal and elevated neutrophil or eosinophil emperipolesis through megakaryocytes.15 Once in the megakaryocyte, the neutrophil releases proteolytic enzymes, resulting in the death of both cells.15 This causes the release of transforming growth factor-β, and platelet-derived growth factor from the alpha granules of megakaryocytes. Platelet-derived growth factor stimulates the proliferation of fibroblasts, mesenchymal, and smooth muscle cells.
Transforming growth factor-β is responsible for the extracellular matrix formation. Additionally, circulating megakaryocytes and platelets from patients with CIMF contain increased levels of bFGF, a potent angiogenic factor and a growth factor for fibroblasts. This, together with elevated platelets, megakaryocytes, and serum bFGF observed in patients with progressive fibrosis, suggest that bFGF takes part in both angiogenesis and fibrosis.

Neoangiogenesis

Neoangiogenesis, as a result of the production and release of angiogenic factors, is an important feature of CIMF; however, neoangiogenesis is also observed in a broad spectrum of tumors and therefore is not specific to CIMF. The increase in bone marrow vasculature in CIMF has been shown to correlate with increased spleen size and to be an independent risk factor for overall survival. A number of angiogenic growth factors, including bFGF and vascular endothelial growth factor, are thought to be the causative factors for this neoangiogenesis. One study has suggested that the endothelial proliferation and growth of capillary blood vessels in the bone marrow may be the result of upregulations of microvascular transforming growth factor-β type 1 receptor and bFGF overexpression. It appears that neoangiogenesis is caused by angiogenic cytokines, which are likely produced by the abnormal megakaryocytes in CIMF marrow.

Abnormal Stem Cell Trafficking

Chronic idiopathic myelofibrosis is also characterized by increased number of circulating CD34-positive hematopoietic stem cells, which is likely caused by abnormal stem cell trafficking. Studies have shown that CD34-positive cell mobilization is caused by proteolytic environment. The elevated levels of neutrophil elastase and matrix metalloproteinase in the plasma of patients with CIMF suggest that these proteolytic activities are a product of a malignant clone. However, the elevated plasma levels of neutrophil elastase and matrix metalloproteinase are nonspecific, as these also can be found elevated in PV.

Furthermore, vascular cellular adhesion molecule is cleaved by the neutrophil proteases in the bone marrow following hematopoietic stem cell or hematopoietic progenitor cell mobilization and is therefore elevated following such mobilization. Xu et al., in their study, demonstrated high levels of soluble vascular cellular adhesion molecule compared with the concentration in the plasma of patients with PV or control patients. These results suggest that the elevation of functional neutrophil elastase activity and the associated cleavage of vascular cellular adhesion molecule are characteristic of patients with CIMF and may play a role in the abnormal CD34-positive cell mobilization that characterizes CIMF as compared with PV.

Extramedullary Hematopoiesis

The origin of extramedullary hematopoiesis in CIMF remains unclear. The activation of stem cells dormant in the spleen and liver since fetal life has been hypothesized to be the cause of extramedullary hematopoiesis. According to this hypothesis, CIMF would recapitulate ontogenesis by a reversion to fetal distribution of hematopoietic activity, resulting in the expansion of hematopoiesis within the central marrow cavity and the extension of this hematopoietic tissue to the marrow cavities of extramedullary sites. However, there is a fundamental difference in hematopoiesis in the spleens of adult patients with CIMF from what occurs in fetal life. Although there is extensive hematopoiesis in the spleens of patients with CIMF, the fetal spleen contributes only marginally to hematopoiesis and contains numerous late erythroid precursors but few early erythroid or granulocytic cells.

However, a recent study by O’Malley et al. has shown that allelic losses or loss of heterozygosity were common in extramedullary hematopoiesis found in spleens of patients with chronic myeloproliferative disorders and acute myeloid leukemia. Comparison of spleen and bone marrow specimens from the same patients demonstrated additional loci of loss of heterozygosity in the spleen compared with the marrow. Their findings suggest that the
extramedullary hematopoiesis may result from the same stem cells from marrow that circulate and reside in the spleens.

**Other Potential Key Molecules Involved in the Pathogenesis of CIMF**

Globulin transcription factor-1 (GATA-1) is the founding member of a transcription factor family that regulates growth and maturation of a diverse set of tissues and may have a role in CIMF pathogenesis. GATA-1 is expressed primarily in hematopoietic cells and is essential for proper development of erythroid cells, megakaryocytes, eosinophils, and mast cells. In the absence of GATA-1, megakaryocytes accumulate in the bone marrow and develop nuclear and cytoplasmic abnormalities and often fail to undergo endomitosis. Gurbuxani et al. have documented the mutagenesis of GATA1 as an early event in children with Down syndrome who develop acute megakaryoblastic leukemia and transient myeloproliferative disorder. In Down syndrome, there is a 46-fold higher incidence of acute myeloid leukemia (with acute megakaryoblastic leukemia accounting for at least half the cases) and transient myeloproliferative disorder, a preleukemia, which may be present in 10% of infants with Down syndrome. In this study, acquired mutations in GATA1 were detected in most patients with Down syndrome and with acute megakaryoblastic leukemia, and in nearly every patient with transient myeloproliferative disorder. A study of rodent models demonstrates that mice with carriers of a mutant GATA1 gene showed megakaryocytic hyperplasia, bone marrow fibrosis, and extramedullary hematopoiesis. The characteristic presence of dysplastic megakaryocytes is a common finding in CIMF, in the hematologic manifestation of Down syndrome, and in the GATA-1 mouse model. Furthermore, reduction of the expression of GATA-1 in subpopulations of megakaryocytes of patients with CIMF has been reported. However, no mutations of GATA-1 have been documented in patients with CIMF until now.

The receptor for thrombopoietin (c-Mpl), a humoral regulator of megakaryocytic maturation. The decreased expression of c-Mpl in platelets was first reported in a group of patients with ET. Subsequently, Molitero et al. showed that c-Mpl was first found to be decreased in all 34 patients with PV and in 13 of 14 patients with myelofibrosis. However, recent reports have shown that c-Mpl is found to decrease in the platelets of approximately 50% of patients with CIMF, PV, or ET. Furthermore, decreased expression of c-Mpl was also found in hereditary thrombocytopenia caused by a TPO gene mutation, suggesting that this alteration can also be caused by different molecular mechanisms.

Retinoic acid receptor-beta 2 (RARbeta2) is a member of the nuclear receptor superfamily of transcription factors that mediates the effects of retinoids. Jones et al. analyzed the expression of RARbeta2 in CD34-positive cells from 17 patients with CIMF using quantitative polymerase chain reaction. The results showed that the expression of RARbeta2 was significantly decreased in 100% of the patient samples compared with that in CD34-positive cells from 10 healthy individuals. Using methylation-specific polymerase chain reaction, they found hypermethylation of RARbeta2 in 16 (89%) of 18 patients, and the methylated form of the gene was absent in CD34-positive cells from healthy individuals. These findings suggest that RARbeta2 acts as a tumor suppressor gene in CIMF and that epigenetic changes are the most significant determinants of RARbeta2 gene activity in these patients.

Most recently, JAK2/STAT pathway molecules have also been shown to play an important role in chronic myeloproliferative disorder pathogenesis. Further details are discussed in the Molecular Genetics section.

**CLINICAL MANIFESTATIONS**

Approximately 30% of patients with CIMF are asymptomatic at presentation, and the diagnosis is suggested by abnormal blood findings or incidentally discovered splenomegaly. In the remaining cases there is an insidious onset of symptoms that are often secondary to hypercatabolic state (fever, weight loss, and night sweats) and peripheral blood abnormalities (fatigue and dyspnea resulting from anemia and bleeding, and petechiae resulting from thrombocytopenia and/or abnormal platelet function). Gout (which may precede actual disease by 10 years) and renal stones occur because of hyperuricemia and are fairly common.

Splenomegaly (90% of patients) is the main physical finding, and nearly 50% of the patients have hepatomegaly. In the initial, prefibrotic stage of CIMF, minimal splenomegaly is present in only approximately 15% of the patients, along with the previously mentioned nonspecific signs and symptoms. Remarkable history in this group of patients is thromboembolic episodes and hemorrhage.

**MORPHOLOGY**

The classic diagnostic features for CIMF (Table 1) include leukoerythroblastosis with poikilocytosis of red blood cells (teardrop forms) in blood smear, varying degrees of fibrosis with atypical megakaryocytic hyperplasia in the bone marrow, osteosclerosis, splenomegaly and less dramatic hepatomegaly (extramedullary hematopoiesis), and absence of Philadelphia chromosome or BCR/ABL gene rearrangement. But the morphologic findings at the time of diagnosis can be quite variable depending on the stage of CIMF (ie, the prefibrotic or fibrotic stage). The prefibrotic stage, also known as the cellular phase, accounts for 20% to 25% of cases, and the fibrotic stage accounts for 70% to 80% of cases (Table 2).

**Prefibrotic Stage**

In the prefibrotic stage, the abnormal peripheral blood findings include variable mild anemia, borderline to slight leukocytosis, and frequently elevated platelet count. Leukoerythroblastosis, dacryocytes, atypical platelets, and circulating megakaryocytes may be present in low numbers. Basophilia and eosinophilia are found in 10% to 30% of CIMF cases.

The bone marrow in the prefibrotic stage of CIMF is usually hypercellular. There is a prominent, left-shifted,
granulocytic proliferation. The megakaryocytic proliferation is characterized by abnormal growth and paratrabe-
cular clustering.41,43,44 The atypical, immature megakaryo-
cytes exhibit marked pleomorphism ranging from giant megakaryocytes to atypical micromegakaryocytes. Defec-
tive nuclear-cytoplasmic differentiation, dense or coarse chromatin, with bulky, clumsy, and irregular-looking (‘‘cloudlike’’ or ‘‘balloon-shaped’’) lobulations of the megakaryocytic nuclei are characteristic findings (Figures 2 and 3).42 The megakaryopoiesis is the most prominent hallmark and key feature to diagnose prefibrotic CIMF. Reticulin fibrosis is absent or minimal. Well-marginated focal lymphoid aggregates are seen in one third of patients with CIMF, and less frequently in patients with other chronic myeloproliferative disorders.43

### Fibrotic Stage

In the fibrotic stage of CIMF, marked anemia is present but the granulocyte count and platelet count may be low, normal, or elevated. The leukoerythroblastic picture (immature granulocytic and erythroid precursors with variable amounts, 1% to 10%, of blasts) (Figure 4) is prominent, with numerous teardrop cells (Figure 5). The presence of 10% to 19% blasts in the bone or blood marrow is defined as an accelerated phase of CIMF, and 20% or more blasts in the blood or bone marrow indicates an acute leukemic transformation.1 The platelet morphology is abnormal, with large hypogranular forms seen. Circulating megakaryocytes and micromegakaryocytes are also frequently seen.46–49

The bone marrow is usually normocellular to hypocel-
lular. Progressive reticulin fibrosis, highlighted by reticu-
lin stain, is present in the fibrotic stage and with advanc-
ing disease (Figure 6). There is progressive loss of hematopoietic elements, often resulting in only clusters of atyp-
al megakaryocytes persisting within the dense reticulin or collagen fibrosis. Increased number and distention of marrow sinuses with intrasinusoidal hematopoiesis (Figure 7) is also a characteristic finding.46–49 The atypical megakaryocytes, which have the same abnormal morphology as in the prefibrotic stage, are not only the predom-
inant elements in the marrow but also within the extramedullary hematopoietic cells. In addition to the pro-
gressive fibrosis, increasing osteosclerosis can develop, characterized by broad and irregular bony trabeculae (Figure 8).41,43,44

### Extramedullary Hematopoiesis

Although extramedullary hematopoiesis may be seen in a number of sites,46 the most common sites are the spleen and liver (Figure 9).25,50,51 Myeloid metaplasia, which by definition means the presence of proliferating hematopoietic cells and their progenies outside the bone marrow, constantly affects the spleen and liver in CIMF. In the spleen, there is expansion of the red pulp by erythroid, granulocytic, and megakaryocytic (predominant lineage) cells that are located mainly in the sinuses. Extramedul-
lar hematopoiesis is also seen in the hepatic sinuses. Fibrosis is seen in both spleen and liver.

### DIAGNOSIS OF CIMF

There is a new proposed European Clinical and Path-
ological criteria (Table 3)52 that consists of a set of clinical and pathologic criteria for diagnosing CIMF and distin-
guishing CIMF from ET, other myeloproliferative disor-
ders, and myelodysplastic syndrome. The diagnosis of prefibrotic CIMF has been very challenging. Some patients with prefibrotic CIMF who presented with subtle clinical findings have been categorized as having ‘‘unclassifiable’’ myeloproliferative disorders, or those patients presenting with pronounced thrombocythemia (>500 × 10^9/L) may have been given a diagnosis of essential thrombocytemia (false ET). It is especially important to differentiate between essential thrombocytemia (true ET) and thrombocytemia caused by prefibrotic CIMF because the life expectancy is lower (8.9%) in ET compared with prefibrotic CIMF, which ranged up to 32.3%.35 It is essential to mention that the unique abnormal megakaryocytic proliferation and clustering is one of the requirements for the diagnosis of CIMF by European Clinical and Pathological criteria (Table 3). This criteria system has categorized CIMF into 4 stages (Table 3): prefibrotic CIMF, early CIMF, manifest CIMF, and full-blown, end stage CIMF. Another set of diagnostic criteria, developed by the Italian Society of Hematology, is commonly used for differentiating CIMF from other chronic myeloproliferative disorders and

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### Table 2. Comparison of the Prefibrotic and the Fibrotic Stages of Chronic Idiopathic Myelofibrosis

<table>
<thead>
<tr>
<th>Findings</th>
<th>Prefibrotic Stage</th>
<th>Fibrotic Stage</th>
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<tbody>
<tr>
<td><strong>Clinical</strong></td>
<td></td>
<td></td>
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<tr>
<td>Physical examination</td>
<td>Minimal to no splenomegaly and hepatomegaly</td>
<td>Moderate to marked splenomegaly and hepatomegaly</td>
</tr>
<tr>
<td>Blood cell measurements</td>
<td>Variable peripheral blood findings, but usually:</td>
<td>Marked anemia</td>
</tr>
<tr>
<td></td>
<td>Mild anemia</td>
<td>Low, normal, or elevated white blood cells</td>
</tr>
<tr>
<td></td>
<td>Mild to moderate leukocytosis</td>
<td>Low, normal, or elevated platelets</td>
</tr>
<tr>
<td></td>
<td>Mild to marked thrombocytosis</td>
<td></td>
</tr>
<tr>
<td><strong>Morphologic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral blood morphology</td>
<td>Minimal or no leukoerythroblastosis</td>
<td>Leukoerythroblastosis</td>
</tr>
</tbody>
</table>
| Bone marrow               | Hypercellular     | Prominent anisopoikilocytosis with dacryo-
|                          | Granulocytic proliferation | cytes (teardrop cells) |
|                          | Megakaryocytic proliferation with atypia and par-
|                          | atrabecular clustering | Prominent megakaryocytic proliferation and |
|                          | Minimal or absent reticulin fibrosis | with atypia |
| **Extramedullary**        |                   |               |
| Hematopoiesis             |                   |               |
| |                  | Leukoerythroblastosis |
| |                  | Prominent anisopoikilocytosis with dacryo-
| |                  | cytes (teardrop cells) |
| |                  | Normocellular or hypocellular |
| |                  | Prominent megakaryocytic proliferation and |
| |                  | with atypia |
| |                  | Definite reticulin and/or collagen fibrosis |
| |                  | Dilated marrow sinuses with intrasinusoidal |
| |                  | hematopoiesis |
| |                  | Osteosclerosis |

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ANCILLARY TESTS

Although the diagnostic criteria suggested by various societies can be used to diagnose CIMF in most patients with the disease, it remains challenging to render an accurate diagnosis of CIMF in a small subset of patients with atypical presentation. In these cases, additional tests are needed for accurate diagnosis.

Circulating CD34-Positive Cells

The enumeration of circulating CD34-positive cells in peripheral blood may be useful in the workup of patients with Philadelphia chromosome (Ph) negative chronic myeloproliferative disorders. CD34 is a phenotypic marker of hematopoietic stem and progenitor cells expressed by 1% to 3% of the human bone marrow cells and by 0.05% of circulating nucleated cells. Recently, it was reported that the median number of CD34-positive cells in peripheral blood in CIMF (91.6 × 10^6/L) was 360 times higher than the healthy population (median, 0.25 × 10^6/L) and 18 to 30 times higher than in a selected population of patients with other Ph-negative chronic myeloproliferative disorders (median, 5 to 6 × 10^6/L). In the latter study, the receiver operating the characteristic curve analysis showed that 15 × 10^6/L was a criterion for CD34-positive cells resulting in a complete discrimination between CIMF and other Ph-negative chronic myeloproliferative disorders. In this study, the cells in EDTA-anticoagulated blood were counted and the phenotype of cells was studied by flow cytometry analysis. The antibody panel used was CD45-FITC (fluorescein isothiocyanate)/CD34-PE (phycoerythrin)/LDS751 and CD38-FITC/CD34-PE/CD45-PerCP (peridinin chlorophyll protein). For the calculation of absolute CD34-positive cells, the corrected number of CD34-positive events was divided by the average total number of CD45-positive events from CD45-FITC/CD34-PE/LDS751 (laser dye styryl) staining and this value was multiplied by the absolute number of granulocytes count. A minimum number of 100 CD34-positive events and 100,000 CD45-positive events were gathered for the quantification of CD34-positive cells by flow cytometry. By doing so, an important prognostic correlation was seen between the number of circulating CD34-positive cells and blast transformation. Patients with more than 300 × 10^6/L circulating CD34-positive cells had a 50% probability of developing blast transformation by 11 months. Therefore, monitoring CD34-positive cells would allow not only reliable differentiation of CIMF from other Ph-negative chronic myeloproliferative disorders, but prospective monitoring could be of clinical importance to the disease from myelodysplastic syndromes with marrow fibrosis; these criteria are listed in Table 4. However, criteria proposed by the Italian Society of Hematology does not define the prefibrotic stage of CIMF.

Regarding the grading of fibrosis, a panel of experienced European pathologists has introduced a grading system for myelofibrosis composed of 4 categories. This system assesses the quantity and quality (reticulin/collagen) of the fiber content in areas of hematopoiesis only (Table 5). The degree of fibrosis should be assessed by disregarding the lymphoid nodules and vessels as well as the fibers framing the adipocytes. Areas of prominent scleredema/scarring should be included in the overall grading.

**Figure 2.** Low-power view of a bone marrow biopsy section illustrates atypical megakaryocytic hyperplasia (hematoxylin-eosin, original magnification ×20).

**Figure 3.** High-power view of a bone marrow biopsy section illustrates a cluster of pleomorphic megakaryocytes with coarse chromatin, with bulky, clumsy, and irregular-looking lobulations of nuclei (hematoxylin-eosin, original magnification ×60).

**Figure 4.** Peripheral smear shows a nucleated red blood cell and mature and immature granulocytes (Wright stain, original magnification ×60).
Figure 5. Peripheral smear shows dacryocytes (teardrop cells) (Wright stain, original magnification ×100).

Figure 6. Reticulin stain of a bone marrow biopsy specimen shows grade 1 fibrosis (original magnification ×20).

Figure 7. Bone marrow biopsy specimen from a patient with chronic idiopathic myelofibrosis, illustrates a dilated sinus (hematoxylin-eosin, original magnification ×40).

Figure 8. Advanced chronic idiopathic myelofibrosis. Prominent osteosclerosis is seen in this bone marrow biopsy specimen (hematoxylin-eosin, original magnification ×10).

Figure 9. Prominent extramedullary hematopoiesis in spleen, involved by trilineage hematopoiesis (hematoxylin-eosin, original magnifications ×40 [A] and ×60 [B]).
The quality of reticulin staining should be assessed by keeping the other 4 optional criteria when splenomegaly is absent are the criteria.

C2 Intermediate clinical stage

Anemia grade 2: hemoglobin > 10 g/dL.

Definitive leukoerythroblastic blood picture and/or teardrop erythrocytes

Splenomegaly

No adverse sign†

C3 Advanced clinical stage

Anemia grade 3: hemoglobin < 10 g/dL plus 1 or more adverse sign†

* Fiber density should be assessed in hematopoietic (cellular) areas. The quality of reticulin staining should be assessed by keeping the detection of normal staining in vessel.

Although the increase of CD34-positive cells in the peripheral blood is highly specific for CIMF, it is not highly sensitive for diagnosing CIMF. There have been a few subsequent studies in which a small population of patients with CIMF with normal CD34-positive cells in the peripheral blood was observed.60,61 For example, Arora et al56 showed that 14% of the patients with myelofibrosis (both CIMF and post-ET and post-PV myelofibrosis) had a normal peripheral blood CD34 count.

### Circulating Endothelial Progenitor Cells

Circulating endothelial progenitor cells have been associated with vascular injury, repair, or malignancy.60,61 In a recent study, circulating endothelial progenitor cells were measured by phenotypic analysis using flow cytometry in peripheral blood cells using FITC-conjugated anti-CD34, PE-conjugated anti-CD133, and PerCp-conjugated anti-VEGFR2. The CD34-positive cells were electronically gated and the percentage of cells coexpressing CD133 and VEGFR2 was evaluated. Detection of endothelial progenitor cells by flow cytometry was defined by the presence of at least 0.03% nucleated cells coexpressing the three antigens over the background fluorescence. Results were expressed as a percentage of CD34-positive cells that co-expressed CD133 and VEGFR2. On the basis of the peripheral blood nucleated cell count, the absolute number of C34-positive CD133/VEGFR2-positive cells was also calculated.62 The results of this study showed that CD34-positive, CD133-positive, and VEGFR2-positive endothelial progenitor cells were detectable in unselected peripheral blood cells of 50.9% of CIMF patients, 37.5% of control

### Table 3. European Clinical and Pathologic Criteria for the Diagnosis of Chronic Idiopathic Myelofibrosis (CIMF)*

<table>
<thead>
<tr>
<th>Stage</th>
<th>Clinical Criteria</th>
<th>Pathologic Criteria</th>
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<tbody>
<tr>
<td>A1</td>
<td>No preceding or allied other subtype of myeloproliferative disorders CML or MDS</td>
<td>B1 Megakaryocytic and granulocytic myeloproliferation and relative reduction of erythroid precursors. Abnormal clustering and increase in atypical giant- to medium-sized megakaryocytes containing clumsy (cloudlike) lobulated nuclei and definitive maturation defects.</td>
</tr>
<tr>
<td>C1</td>
<td>Early clinical stages Normal hemoglobin or slight anemia, grade 1: hemoglobin &gt; 12 g/dL Slight or moderate splenomegaly on palpation or &gt;11 cm on ultrasound scan or CT thrombocytemia, platelets in excess of 400, 600, or even 1000 × 10^9/L</td>
<td>Staging of CIMF MF0 (prefibrotic stage CIMF): not reticulin fibrosis MF1 (early CIMF): slight reticulin fibrosis MF2 (manifest CIMF): marked increase in reticulin and slight to moderate collagen fibrosis MF3 (overt CIMF): advanced collagen fibrosis MF3: Osteosclerosis (endophytic bone formation) and decreased cellularity</td>
</tr>
<tr>
<td>C2</td>
<td>Intermediate clinical stage Anemia grade 2: hemoglobin &gt; 10 g/dL Definitive leukoerythroblastic blood picture and/or teardrop erythrocytes</td>
<td></td>
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<tr>
<td>C3</td>
<td>Advanced clinical stage Anemia grade 3: hemoglobin &lt; 10 g/dL plus 1 or more adverse sign†</td>
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<table>
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<tr>
<th>Necessary Criteria</th>
<th>Optional Criteria</th>
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<tr>
<td>Diffuse bone marrow fibrosis</td>
<td>Splenomegaly</td>
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<tr>
<td>Absence of t(9;22) chromosome or BCR/ABL rearrangement in peripheral blood</td>
<td>Anisopellicity with teardrop erythrocytes</td>
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<tr>
<td></td>
<td>Circulating immature myeloid cells</td>
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<tr>
<td></td>
<td>Circulating nucleated red cells</td>
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<tr>
<td></td>
<td>Clustered marrow megakaryocytes</td>
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<td></td>
<td>Myeloid metaplasia</td>
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* Combination of A1 + B1 establishes diagnosis of CIMF; any other criterion confirms CIMF. Four clinicopathologic stages of CIMF: (1) prefibrotic CIMF: A1, B1, C1, + MF0; (2) early CIMF: A1, B1, C1, + MF1; (3) manifest CIMF: A1, B1, C2, + MF2 or MF3; and (4) end stage, full-blown CIMF: A1, B1, C3, + MF3 or MF>3. CML indicates chronic myelogenous leukemia; MDS, myelodysplastic syndrome; and CT, computed tomography.

† Age, >70 years; hemoglobin, <10 g/dL; myeloblasts in peripheral blood, >2%; erythronormoblasts in peripheral blood, >2%; leukocytosis, >20 × 10^9/L; thrombocytopenia, <300 × 10^3/L; severe constitutional symptoms; massive splenomegaly; and cytogenetic abnormalities.
patients, and 21% of healthy participants. Patients with CIMF had a median of 0.26% endothelial progenitor cells, significantly higher than that in healthy controls (median, 0%) and in patients with other Ph-negative chronic myeloproliferative disorders (median, 0.1%). In 14.5% of CIMF patients, the numbers of endothelial progenitor cells were greater than the highest value found in patients with other Ph-negative chronic myeloproliferative disorders. More importantly, this study revealed that the increased numbers of circulating endothelial progenitor cells were also associated with younger patient age and a diagnosis of prefibrotic CIMF. Thus, circulating endothelial progenitor cells are elevated in patients with CIMF in the early stage of the disease and may be a good marker for diagnosing prefibrotic CIMF if the results are validated by further studies.

**CYTOGENETICS**

Chromosomal abnormalities are detected in approximately half the patients with chronic idiopathic myelofibrosis, but no specific cytogenetic defect has been identified. The most common chromosomal abnormalities include del(13q), del(20q), partial trisomy 1q, and trisomy 8.6,8,63 Abnormalities of chromosomes 7, 9, and 12 are also reported.67 A recent study suggests that der(6)t(1;6)(q21–23;p21.3) is a specific chromosome abnormality in CIMF and might harbor gene(s) specifically associated with CIMF.64 Deletions affecting chromosomes 7 and 5 also occur but may be associated with prior cytotoxic therapy.1 At the time of leukemic transformation, 90% of the cases will have cytogenetic clonal abnormalities.65

Al-Assar et al66 studied comparative genomic hybridization versus conventional cytogenetics in patients with CIMF and suggested that genomic aberrations were more common than previously reported by conventional cytogenetic analysis. According to this study, gains of 9p are the most common cytogenetic aberration (50% of the cases), and may play a crucial role in the pathogenesis of CIMF.66 Chromosome 9p contains JAK2, a gene recently identified to have a critical gain of function mutation (see following discussion). It would be of interest to study whether or not these patients carry such a mutation. If these patients lack the JAK2 mutation, the gain of 9p may represent another way of upregulating the JAK2 function leading to CIMF. It is also likely that a fluorescent in situ hybridization probe for chromosome 9p may be developed in the future for the diagnostic application.

**MOLECULAR GENETICS**

The recent detection of JAK2 mutation may be a potential major breakthrough for understanding the pathobiology of chronic myeloproliferative disorder.67 JAK2 (V617F) is a somatic point mutation resulting in the substitution of valine by phenylalanine at codon 617. Janus kinase 2 (JAK2) is a cytoplasmic protein-tyrosine kinase that is recruited by ligand binding to cytokine receptors where it is activated by transphosphorylation and, in turn, phosphorylates critical tyrosine residues on the receptor that become docking sites for STAT (signal transducer and activation of transcription) proteins.68 JAK2 plays an essential role in definitive erythropoiesis because it is the primary tyrosine kinase activated by erythropoietin.69 In the past several months, at least 7 studies have independently described a close association between an activating JAK2 (V617F) mutation and Ph-negative chronic myeloproliferative disorders. The mutational frequency in patients with CIMF is approximately 35% to 57%.67,70–75 Screening for JAK2 (V617F) mutation is not specific enough to be used as a tool for the diagnosis of CIMF; however, it may be a useful test to diagnose prefibrotic CIMF and to differentiate CIMF from myelofibrosis resulting from secondary causes (Figure 1).

**DIFFERENTIAL DIAGNOSIS**

During the prefibrotic stage, distinguishing CIMF from PV or ET can be challenging. Chronic myeloproliferative disorders, and neoplastic and inflammatory disorders associated with marrow fibrosis can be difficult at times to differentiate from fibrotic stage of CIMF.

Up to 40% patients with chronic myelogenous leukemia may display marrow reticulin or collagen fibrosis68,69,77 and marked megakaryocytic hyperplasia such as CIMF; however, the megakaryocytes are small and hypolobated, whereas those of CIMF are large and pleomorphic.68,77 In addition to the morphologic findings, the presence of Philadelphia chromosome or the BCR-ABL fusion gene is diagnostic for chronic myelogenous leukemia.69,77 In practice, cytogenetic or molecular studies should be done in all cases of chronic myeloproliferative disorders.

Twenty percent of patients with CIMF can have marked thrombocytosis, even in excess of 1000 × 10^9/L.26,46,80 In such cases, differentiating prefibrotic CIMF from ET can be difficult. Differences in megakaryocyte morphology may provide a useful clue. In ET, the megakaryocytes have deeply lobulated and hyperlobulated nuclei, abundant mature cytoplasm, and smooth nuclear contours.69,75 The pleomorphic, bizarre, and atypical megakaryocytes of CIMF are not seen in ET.61 In the fibrotic stage of CIMF, the differentiation from ET is easier because leukoerythroblastic blood smear with teardrop red blood cells and marrow fibrosis associated with this stage are not features of ET.

The red blood cell parameters can be helpful in distinguishing the polycythemic stage of PV from the prefibrotic stage of CIMF. In PV, the red blood cell mass is increased. Because of the increased numbers of erythroid precursors, the myeloid-erythroid ratio is usually decreased. In contrast, most patients with CIMF are anemic and the bone marrow shows a higher myeloid-erythroid ratio than in PV. The megakaryocytes in PV are pleomorphic, grouped together, and have deeply lobulated nuclei, but lack the atypical and dysplastic features seen in CIMF. Serious blood loss or iron deficiency may obscure some of these features; however, an iron trial may be required for the recognition of PV. Postpolycythemic myelofibrosis and myeloid metaplasia is characterized by a leukoerythroblastic blood smear, with teardrop red blood cells and splenomegaly. Reticulin and collagen fibrosis are the hallmark features of postpolycythemic myelofibrosis and myeloid metaplasia.4,62 Therefore, postpolycythemic myelofibrosis and myeloid metaplasia is morphologically indistinguishable from CIMF.

Fibrosis of variable degrees is seen in the bone marrow of 30% of patients with chronic myelomonocytic leukemia.63 Such patients may also have splenomegaly and marked megakaryocytic hyperplasia with dysplasia, which might make it difficult to distinguish from CIMF. The presence of peripheral blood and marrow monocytosis, and erythroid and granulocytic dysplasia should lead one to suspect chronic myelomonocytic leukemia.

Approximately 15% to 20% of patients with myelodyso-
plastic syndrome show a significant increase in reticulin fibers. The fibrosis is often focal, and rarely is overt collagen deposition noted. The focal fibrosis and megakaryocytic abnormalities seen in myelodysplastic syndrome with fibrosis can cause difficulties in distinguishing myelodysplastic syndrome with fibrosis from CIMF. A diagnosis of myelodysplastic syndrome with fibrosis should be favored when there is multilineage dysplasia in the blood and marrow, a significant increase in reticulin fibers, and no significant hepatosplenomegaly. In contrast, in CIMF, although there is megakaryocytic atypia and significant hepatosplenomegaly, rarely is there granulocytic and erythrocytic dysplasia present. Classification can sometimes be problematic, especially when patients present with leukoerythroblastic blood smear with teardrop red blood cells, hepatosplenomegaly, fibrosis of the bone marrow, and severe trilineage dyspoiesis (features of both CIMF and myelodysplastic syndrome). Although such cases could be representing the accelerated myelodysplastic phase of chronic myelogenous leukemia, some investigators have suggested that such cases are a unique transitional group between myelodysplastic syndrome and chronic myeloproliferative disorders.

Acute panmyelosis with myelofibrosis, also known as acute myelofibrosis, acute myelosclerosis, and acute myelodysplasia with myelofibrosis, is a very rare form of acute myeloid leukemia that is characterized by bone marrow fibrosis and acute panmyeloid proliferation. Acute panmyelosis with myelofibrosis is a fulminant fatal course. The blood morphology shows pancytopenia with circulating blasts. Patients have minimal or absent splenomegaly. Although multilineage dysplasia is present, in contrast to myelodysplastic syndrome with fibrosis, there is marked increase in reticulin fibrosis. In CIMF, the megakaryocytes have a typical, markedly contorted nuclei with condensed nuclear chromatin. Splenomegaly is a prominent feature. In acute panmyelosis with myelofibrosis, the megakaryocytes usually have nonlobated or hypolobated nuclei with dispersed nuclei. Splenomegaly is minimal or absent. Additionally, a significant increase in the percentage of blasts in the marrow would exclude the diagnosis of CIMF.

Any acute leukemia may be associated with marrow fibrosis, particularly those with megakaryocytic differentiation. To make a diagnosis of acute leukemia, the percentage of blasts in the blood or marrow should be more than 20%.

**PROGNOSIS**

The median length of survival for patients with CIMF is approximately 3.5 to 5.5 years from diagnosis (range, 1–15 years). The main causes of death are infection, hemorrhage, cardiac failure, thrombosis, transformation to acute leukemia, and postsplenectomy complications. Renal failure and hepatic failure have also been reported as causes of death.

Incidence of leukemic transformation occurs in approximately 5% to 30% of patients within the first 10 years. One recent study showed leukemic transformation to be fatal in 98% of the cases, with a median survival period of 2.6 months. In most cases, the leukemic transformation was myeloid in origin, with the most common subtype (according to French-American-British classification) being M7 (acute megakaryoblastic leukemia). The prognostic factors in CIMF are anemia, the age of the patient, white blood cell (WBC) count, and the number of blasts in the peripheral blood. Although there have been many discrepancies in the studies regarding the prognostic significance of these factors, everyone has agreed on the degree of anemia as being the most important prognostic factor for CIMF. A hemoglobin level of less than 10 g/dL is associated with poor prognosis. Additionally, older age is associated with the worse prognosis. The WBC count as a prognostic factor is controversial. Some studies have associated a high count with an adverse outcome, and some studies have implicated low WBC count with a worse prognosis. The increased number of CD34-positive blasts in peripheral blood has been associated with an unfavorable patient risk group. A study showed that patients with CIMF presenting with CD34-positive cell count of $300 \times 10^9/L$ in the peripheral blood had a 50% risk of developing acute leukemia within 11 months of diagnosis. The relationship of abnormal karyotyping with prognosis is controversial, with some studies supporting and other studies not favoring it as a prognostic factor. Thrombocytopenia has been associated with adverse outcome in a few studies.

Currently, several systems to predict the prognosis have been reported; these are summarized in Table 6. Using the hemoglobin level and leukocyte count, Dupriez et al proposed a scoring system (the Lille scoring system) that had 3 distinct prognostic groups. The low-risk group (hemoglobin > 10 g/dL and WBC between $4 \times 10^9/L$ and $30 \times 10^9/L$) had a median survival of 93 months, whereas those in intermediate risk group (hemoglobin < 10 g/dL or WBC < $4 \times 10^9/L$ or > $30 \times 10^9/L$) and high-risk group (hemoglobin < 10 g/dL and WBC < $4 \times 10^9/L$ or > $30 \times 10^9/L$) had a median survival of 26 months and 13 months, respectively. This scoring system is very attractive because of its simplicity and discriminating power. It has been widely used to stratify patients in clinical tri-

<table>
<thead>
<tr>
<th>Table 6. The Different Prognostic Scoring Systems</th>
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<tbody>
<tr>
<td><strong>Lille System (Dupriez et al., 1996)</strong></td>
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<tr>
<td>---------------------------------------------</td>
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<tr>
<td>Hemoglobin, &lt;10 g/dL</td>
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<tr>
<td>Leukocytes, &lt;4000/μL or &gt;30 000/μL</td>
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<tr>
<td>Each presence scores 1 point</td>
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<tr>
<td>Median survival, mo</td>
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<tr>
<td>Score 0: 93</td>
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<td>Score 1: 26</td>
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<td>Score 2: 13</td>
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<tr>
<td>Score 3: 10</td>
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<tr>
<td>Score 4: 185.3</td>
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**Abbreviations:** WBC, white blood cell; MF, myelofibrosis; MF0, myelofibrosis, low risk; MF1, myelofibrosis, intermediate risk; MF2, myelofibrosis, high risk; MF3, myelofibrosis, very high risk.


