

## Chronic Myeloproliferative Diseases: Laboratory Support of Diagnosis and Management

### Clinical Background

Chronic myeloproliferative diseases (cMPDs) are clonal stem cell disorders characterized by proliferation of 1 or more of the granulocytic, erythrocytic, myelomastocytic, or megakaryocytic cell lines. These diseases collectively have an incidence of 6 to 9 per 100,000 population annually.<sup>1</sup> This Clinical Focus describes the various cMPDs and the use of laboratory testing for diagnosis and management.

The cMPDs include chronic myelogenous leukemia (CML), essential thrombocythemia (ET), polycythemia vera (PV), chronic idiopathic myelofibrosis (CIMF), chronic eosinophilic leukemia (CEL)/hypereosinophilic syndrome (HES), systemic mastocytosis (SM), chronic neutrophilic leukemia (CNL), and 8p11 myeloproliferative syndrome (MPS). All cMPDs exhibit at least 1 of the features listed in Table 1.

cMPDs typically occur in adults 40 to 60 years old and are uncommon in people <20 years old. Frequently, the onset is insidious and the clinical course indolent. Patient complaints may include fatigue and lethargy, weight loss, abdominal discomfort, easy bruising, night sweats, and swollen, painful joints. Physical examination may reveal pallor, enlargement of the spleen or liver, and petechiae.

Distinguishing between the cMPDs is often difficult because of the overlap of clinical and laboratory findings. For example, most cMPDs result in organomegaly, leukocytosis, and excessive megakaryocyte proliferation. Each cMPD begins with effective hematopoiesis resulting in circulating mature blood cells, but eventually progresses to ineffective hematopoiesis and bone marrow failure or, potentially, acute leukemia. Table 2 details hematologic characteristics of the various cMPDs, including those considered diagnostic by the World Health Organization (WHO).

The finding that constitutive (unregulated) protein-tyrosine kinase (PTK) activity is associated with cMPDs has led to the development of molecularly targeted therapies. For example, imatinib mesylate (Gleevec®) is a potent PTK inhibitor and prevents cellular proliferation in *bcr/abl*-positive patients. Imatinib is the treatment of choice for CML with a reported 76% complete cytogenetic response (CCR) rate in chronic-stage patients.<sup>5</sup> Imatinib has also been used to treat other cMPDs including PV,<sup>6</sup> CEL/HES,<sup>7</sup> and certain subtypes of SM.<sup>4</sup>

Table 1. Clinical and Laboratory Features of cMPDs<sup>1-4</sup>

Feature	CML	ET	PV	CIMF	CEL/ HES	SM	CNL	8p11 MPS
Overproduction of 1 or more blood cell lines with dominance of a transformed clone	+	+	+	+	+	-	+	+
Increased fibrosis in bone marrow	≤40%	-	+/-	+	-	-	-	-
Increased cellularity in bone marrow	+	+/-	+	+/-	+	+	+	+
Chromosomal abnormalities*	100%	≈5%	≈15%	≈35%	?	?	≈10%	100%
Thrombotic and/or hemorrhagic complications	-	+	+	+	-	+	≈27%	-
Extramedullary hematopoiesis (EMH) by liver and/or spleen	+	+/-	+	+	-	-	-	-
Transformation to AML	≈70%	<5%	≈10%	≈18%	-	<5%	-	+
Overlapping clinical features	+	+	+	+	+	+	+	+

CML, chronic myelogenous leukemia; ET, essential thrombocythemia; PV, polycythemia vera; CIMF, chronic idiopathic myelofibrosis; CEL, chronic eosinophilic leukemia; HES, hypereosinophilic syndrome; SM, systemic mastocytosis; CNL, chronic neutrophilic leukemia; AML, acute myeloid leukemia; 8p11 MPS, 8p11 myeloproliferative syndrome

\*Does not include somatic mutations

Table 2. Chronic Myeloproliferative Disease Incidence and Hematologic Characteristics, Including WHO Diagnostic Criteria (Bolted) <sup>1,3</sup>

	Incidence (per 100,000)	M:F	Peripheral Blood Characteristics	Bone Marrow Characteristics
Chronic Myelogenous Leukemia	1–1.5	≈1.7:1	<p><b>Positive for Ph and/or bcr/abl</b> Leukocytosis (median WBC count ~170 x 10<sup>9</sup>/L) Predominance of neutrophils in different stages of maturation Absolute basophilia; eosinophilia ± Platelet count N or ↑ Mild anemia ±</p>	<p><b>Positive for Ph and/or bcr/abl</b> Hypercellularity due to ↑ neutrophils at different stages of maturation Pseudo-Gaucher cells and sea-blue histiocytes ± Blasts &lt;5%; ↑ eosinophils Small megakaryocytes Reticulin fibers ↑ in up to 40% of patients</p>
Essential Thrombocythemia	1–2.5	1:1	<p><b>Platelet count ≥600 x 10<sup>9</sup>/L for ≥2 months</b></p>	<p><b>Predominantly megakaryocyte proliferation with mostly mature, enlarged forms</b></p>
Polycythemia Vera	0.8–1.0	≈1.7:1	<p>↑ <b>hemoglobin (&gt;18.5 g/dL in men; &gt;16.5 g/dL in women) and hematocrit OR</b> ↑ <b>RBC mass (&gt;25% above mean normal predicted value)</b> WBC count &gt;12 x 10<sup>9</sup>/L* Platelet count &gt;400 x 10<sup>9</sup>/L</p>	<p>↑ <b>cellularity with triineage proliferation</b> Stainable iron ±</p>
Chronic Idiopathic Myelofibrosis	0.5–1.5	1:1	<p>Prefibrotic stage: • Mild to marked thrombocytosis • Mild to moderate leukocytosis • Mild anemia</p> <p>Fibrotic stage: • <b>Moderate to marked anemia</b> • <b>Leukoerythroblastosis</b> • <b>Prominent RBC poikilocytosis with teardrop shapes</b></p>	<p>Prefibrotic stage: • ↑ cellularity • <b>Neutrophilic proliferation</b> • <b>Megakaryocyte proliferation with abnormal forms</b> • Minimal or absent reticulin fibrosis</p> <p>Fibrotic stage: • ↓ cellularity • <b>Presence of reticulin and/or collagen fibrosis</b> • Dilated marrow sinuses with intraluminal hematopoiesis • Atypical and prominent megakaryocyte proliferation • New bone formation (ie, osteosclerosis)</p>
Chronic Eosinophilic Leukemia/Hyper-eosinophilic Syndrome	Unknown	≈9:1	<p><b>Persistent eosinophilia ≥1.5 x 10<sup>9</sup>/L for ≥6 months</b> <b>Myeloblasts &lt;20%</b></p>	<p>↑ <b>eosinophils</b> <b>Myeloblasts &lt;20%</b></p>
Systemic Mastocytosis	Unknown	≈1:2	<p>Anemia ± Leukopenia or leukocytosis ± Thrombocytopenia or thrombocytosis ±</p>	<p><b>Multi-focal clusters or aggregates of mast cells (&gt;15/ aggregate)</b> Staining for tryptase may confirm the diagnosis Mast cells may be spindle-shaped and may have reniform or indented nuclei</p>
Chronic Neutrophilic Leukemia	Unknown, but rare	1:1	<p><b>WBC count &gt;25 x 10<sup>9</sup>/L</b> <b>Segmented neutrophils and bands &gt;80%</b> <b>Immature granulocytes &lt;10%</b> <b>Myeloblasts &lt;1%</b></p>	<p>↑ <b>cellularity with granulocytic hyperplasia</b> <b>Myeloblasts &lt;5% of nucleated marrow cells</b> <b>N neutrophilic maturation pattern</b></p>

M:F, male to female ratio; Ph, Philadelphia chromosome; ↑, increased; N, normal; ↓, decreased; ±, may be present

\*In the absence of fever or infection

## Chronic Myelogenous Leukemia

Patients may present with splenomegaly, but more commonly CML is detected with a routine complete blood count (CBC) in asymptomatic patients. The hallmark of CML is the presence of the Philadelphia chromosome (Ph) and/or the *bcr/abl* fusion gene.<sup>1</sup> CML progresses through 3 sequential phases (chronic, accelerated, and blast), each of which is progressively more resistant to therapy. Thus, early diagnosis and treatment is imperative.

## Essential Thrombocythemia

Although some patients present with symptoms of vascular occlusion or hemorrhage, ET is asymptomatic in more than 50% of patients and is identified fortuitously with a routine CBC that reveals an elevated platelet count.<sup>1</sup> Thrombocytosis due to secondary causes such as systemic infections, inflammatory conditions, bleeding, or malignancy must be ruled out before clonal ET can be diagnosed. Thrombosis and hemorrhage are the most frequent clinical complications in patients with ET.

## Polycythemia Vera

The most serious complications of PV are thrombosis, hemorrhage, and hypertension. Approximately 25% of patients present with venous or arterial thrombosis, myocardial ischemia, or stroke.<sup>1</sup> Major complaints at diagnosis include headache, dizziness, visual disturbances, and numbness/tingling. Erythromelalgia (vasodilation with burning), pruritus, and gout may also be present. About 70% of patients have plethora or splenomegaly and 40% have hepatomegaly.

## Chronic Idiopathic Myelofibrosis

CIMF, also known as agnogenic myeloid metaplasia, is characterized by anemia, progressive splenomegaly and bone marrow fibrosis, and multi-organ extramedullary hematopoiesis (EMH). Up to 30% of patients with CIMF are asymptomatic at diagnosis and CBC findings or splenomegaly seen during a routine physical examination trigger the diagnostic workup.<sup>1</sup> The remainder present with signs of EMH, which accounts for many of the peripheral blood findings in fibrotic CIMF. Major causes of morbidity and mortality include acute myeloid leukemia (AML), which develops in 5% to 30% of CIMF patients; bone marrow failure due to hemorrhage or infection; thromboembolic events; portal hypertension; and cardiac failure.<sup>1</sup>

## Chronic Eosinophilic Leukemia/Hypereosinophilic Syndrome

In about 10% of CEL or HES patients, hypereosinophilia is detected incidentally and no symptoms are apparent.<sup>1</sup> Other patients may experience constitutional symptoms including fever, fatigue, cough, angioedema, muscle pains, pruritus, and diarrhea. Tissue infiltration by eosinophils, especially in the heart, skin, nervous system, and lungs may lead to more serious symptoms. Organ involvement, especially in the heart, is the most severe complication of CEL and HES.

## Systemic Mastocytosis

Clinical symptoms of SM can be grouped into 1) constitutional symptoms; 2) skin conditions such as pruritus and urticaria; 3) mast cell mediator-related features

including abdominal pain, flushing, headache, and respiratory symptoms; and 4) bone-related complaints including fractures and bone and joint pain.<sup>3</sup> Symptoms range from mild to life-threatening depending on the degree of organ involvement. SM should be distinguished from cutaneous mastocytosis, a childhood disorder usually confined to the skin that frequently shows spontaneous regression.<sup>4</sup>

## Chronic Neutrophilic Leukemia

Splenomegaly, the most constant clinical feature, is caused by neutrophilic infiltration and may be symptomatic.<sup>1</sup> Most patients have hepatomegaly and 25% to 30% report a history of bleeding.

## 8p11 Myeloproliferative Syndrome

8p11 MPS is a rare disorder (<50 cases) characterized by gene changes involving chromosome 8.<sup>8</sup> Presenting symptoms may include lethargy, weight loss, fevers, night sweats, lymphadenopathy, and splenomegaly. Bone marrow findings include marked eosinophilia, which is also seen in peripheral blood. Other CBC results include marked leukocytosis with mostly neutrophils, metamyelocytes, and myelocytes; basophilia and thrombocytosis are typically absent. Unlike other cMPDs, 8p11 MPS progresses to high-grade T-cell lymphoblastic lymphoma in 60% to 70% of patients and rapidly transforms to AML.

## Individuals Suitable for Testing

- Individuals with the hematologic abnormalities listed in Table 2, with or without clinical symptoms of cMPDs
- Individuals being monitored following diagnosis of a cMPD

## Test Availability

Table 3 lists tests used for diagnosis and management of cMPDs.

## Test Selection and Interpretation

### Chromosome Abnormalities

Because of the clonal nature of the cMPDs, detecting chromosomal abnormalities and somatic mutations is important for diagnosis, treatment selection, and monitoring. In the case of CML, a specific chromosomal abnormality (ie, Ph+) is considered diagnostic. No such specific abnormality or molecular marker has been identified for the other disorders. However, recurring abnormalities that are not disease-specific have been associated with the non-CML disorders (Table 4). Detection of these abnormalities meets the WHO requirement for establishing clonality and thus diagnosing a cMPD.<sup>13</sup>

Baseline bone marrow karyotyping data are used to identify clonal evolution, which is the appearance of a genetic abnormality not present previously. Clonal evolution is associated with a poor prognosis and, in CML, is indicative of passing from the chronic to the accelerated or blast phase (Table 4). Additionally, identification of a chromosomal abnormality rules out a non-malignant reactive disorder.

cMPD-associated somatic mutations have been identified using polymerase chain reaction (PCR) techniques (Table 5). Identification of such mutations meets the WHO diagnostic criterion for establishing clonality and may be useful in selecting therapy and evaluating prognosis.

Table 3. Tests Available to Support Chronic Myeloproliferative Disease Diagnosis and Management\*

Test Code	Assay	Clinical Use
16031X	ABL Kinase Domain Mutation in CML, Plasma-based, Leumeta™††	Predict imatinib drug resistance prior to clinical relapse in patients with CML Identify individuals who may benefit from alternative therapy
17853X	<i>bcr/abl</i> Gene Rearrangement, Quantitative PCR, Plasma-based, Leumeta††	Diagnose CML Monitor CML patients for therapeutic response, MRD, and early relapse
15101X	<i>bcr/abl</i> Gene Rearrangement, Quantitative PCR with Reflex to Subtype†§	Diagnose CML Distinguish ALL (subtype e1a2) from CML (subtypes b2a2/b3a2)
17637X	BCR/ABL Protein Quantitation (Total and Phosphorylated), Leumeta†	Diagnose CML Assess prognosis and response to imatinib therapy <sup>9</sup> Monitor MRD
4420X	C-Reactive Protein	Rule out inflammatory conditions as a cause of reactive thrombocytosis, leukocytosis, or eosinophilia
16104X	<i>c-kit</i> Mutation Analysis, Plasma-based, Leumeta††	Diagnose SM Predict response to imatinib therapy
14600X	Chromosome Analysis, Hematological Malignancy†	Diagnose, assess prognosis, and select treatment for cMPDs Detect clonal evolution in cMPD disease progression
17734X	Comprehensive Hematopathology Report	Diagnose hematologic and hematopoietic disorders involving the bone marrow Graphic report integrates morphologic, flow cytometric, and genetic test results
427X	Erythropoietin (EPO)	Differentiate secondary from primary polycythemia
22764P	Ferritin	Rule out iron deficiency as cause of anemia Differentiate PV from secondary erythrocytosis or from ET
16099X	<i>FIP1L1-PDGFRα</i> Gene Rearrangement [del (4q12)], Real-time PCR†	Support the diagnosis of CEL and rule out HES Predict response to imatinib therapy Monitor therapeutic response in <i>FIP1L1-PDGFRα</i> -positive patients <sup>10,11</sup>
12070X	FISH, CML/ALL, <i>bcr/abl</i> Translocation 9,22†	Diagnose or rule out CML Assess prognosis and effectiveness of therapy in <i>bcr/abl</i> -positive patients
10055X	FISH, Chromosome 20q Deletion†	Assess prognosis in patients with PV, CIMF, CNL, and CEL <sup>1,12</sup>
17355X	FISH, Locus-specific Probe†	Assess prognosis when a chromosomal abnormality is present (specify probe to be used)
10709X	FISH, Myeloid Disorders Profile†	Diagnose myeloid malignancies Monitor therapeutic response in myeloid marker-positive patients
36743X	Hematopathology Consultation	Differential diagnosis of hematologic disorders
15684X	Immunohistochemistry (IHC) Marker†	Differential diagnosis of SM: identify CD2- and/or CD25-positive mast cells <sup>3</sup>
10248X	Intracellular Markers by Flow Cytometry†	Differentiate myeloid from lymphoid malignancy
16101X	<i>JAK2</i> Mutation (V617F) Analysis, Plasma-based, Leumeta††	Diagnose or confirm the diagnosis of PV, ET, or CIMF
35080X	Leukemia/Lymphoma Evaluation†	Diagnose leukemia or lymphoma Monitor therapeutic response and detect relapse
233X	Leukocyte Alkaline Phosphatase Stain	Differentiate CML from other causes of leukocytosis including CNL and PV
17862X	T-Cell Receptor (TCR) Gene Rearrangement, Qualitative PCR, Leumeta††	Differentiate HES due to T-cell clonality from CEL due to eosinophilic clonality Determine leukemia and lymphoma lineage
17861X	T-Cell Receptor (TCR) Gene Rearrangement, Quantitative PCR, Leumeta††	Differentiate HES due to T-cell clonality from CEL due to eosinophilic clonality Determine leukemia and lymphoma lineage Detect and monitor MRD in patients with T-cell neoplasm
34484X	Tryptase <sup>ll</sup>	Differentiate systemic from cutaneous mastocytosis in the absence of other myeloid disease <sup>9</sup> Monitor effectiveness of SM therapy

Table 3. Tests available (continued)

Test Code	Assay	Clinical Use
928X	Vitamin B12 Binding Capacity, Unsaturated (Transcobalamin)	Differentiate PV from secondary polycythemia

MRD, minimal residual disease; ALL, acute lymphoblastic leukemia. Other abbreviations are defined in the footnote of Table 1.

\*Refer to Directory of Services for CPT codes and specimen collection and handling requirements.

<sup>†</sup>This test was developed and its performance characteristics determined by Quest Diagnostics Nichols Institute. It has not been cleared or approved by the U.S. Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary. Performance characteristics refer to the analytical performance of the test.

<sup>‡</sup>Cell-based tests are also available. Plasma-based (ie, Leumeta) tests may replace cell-based tests that require bone marrow sample or tissue biopsy. Plasma-based tests may be more sensitive in some situations.

<sup>§</sup>Reflex tests are performed at an additional charge and are associated with an additional CPT code(s).

<sup>||</sup>This test was performed using a kit that has not been approved or cleared by the FDA. The analytical performance characteristics of this test have been determined by Quest Diagnostics Nichols Institute. This test should not be used for diagnosis without confirmation by other medically established means.

### JAK2 V617F Mutation

JAK2 V617F is the first acquired somatic mutation found to be associated with PV, CIMF, and ET; its detection supports the diagnosis of these cMPDs but does not distinguish between them. A negative result does not rule out the diagnosis, however, because the mutation is not always present (Table 5). Several clinical studies have indicated that the JAK2 V617F mutation is not present in patients with CML, secondary erythrocytosis, SM, or normal control subjects,<sup>16,17</sup> but is present in up to 2% of de novo AML cases.<sup>19</sup>

Studies using the V617F mutation to determine prognosis have yielded inconsistent results. In one study of 244 patients with cMPDs (128 with PV, 93 with ET, and 23 with CIMF), the V617F mutation was associated with a longer duration of disease and a higher frequency of complications including secondary fibrosis, hemorrhage, and thrombosis.<sup>16</sup> In another study of 110 patients with CIMF, V617F-positive patients had significantly worse survival than V617F-negative patients

(hazard ratio 3.30, 95% confidence interval 1.26–8.68,  $P=0.01$ ).<sup>19</sup> However, the presence of the V617F mutation was not predictive of inferior survival in 150 patients with ET followed for a median of 11.4 years.<sup>20</sup>

### FIP1L1-PDGFRα Fusion Gene

The presence of the FIP1L1/PDGFRα somatic mutation confirms the diagnosis of CEL and predicts a favorable response to imatinib treatment.<sup>7,10</sup> This mutation produces a PTK, which is the cause of CEL, and inhibition of the PTK activity with imatinib accounts for the effectiveness of this treatment.<sup>21</sup> Such responses are also seen in a small subset of SM patients with eosinophilia who are positive for FIP1L1/PDGFRα (Table 5). FIP1L1/PDGFRα mutation testing is used to monitor therapy; molecular remission is documented when positive results become negative.<sup>11</sup>

### Bone Marrow Histology

WHO diagnostic guidelines include bone marrow findings as criteria for identifying and distinguishing between the cMPDs (Table 2). The WHO recommends that bone marrow biopsy and peripheral blood specimens be evaluated together to reach a diagnosis.<sup>1</sup> Quest Diagnostics uses various stains and immunohistochemical markers to evaluate bone marrow. Cellularity, collagen and reticulin fibrosis, and proliferation of granulocytes, megakaryocytes, mast cells, and erythrocytes are routinely evaluated to aid in the diagnosis of a cMPD.

Table 4. Recurring Chromosomal Abnormalities Associated with cMPDs<sup>1,4,8,12,14</sup>

Disease	Abnormalities
CML-chronic, accelerated, and blast phase	t(9;22)(q34;q11), <i>bcr/abl</i>
CML-accelerated or blast phase	+8, +Ph, +19, inv(17q), t(3;21)(q26;q22) ( <i>evi1/aml1</i> )
ET	+8, del(13q), t(X;5), inv(3), t(13;14), t(2;3), del(11q21)
PV	+8, +9, del(20q), del(13q), del(1p11), +19, t(Y;1), t(3;17)
CIMF	+8, del(20q), del(12p), -7/del(7q), del(11q), del(13q), del(3q), t(1;20), t(1;7), 5q-, 7q-, t(4;13), der(1q9p), t(5;17)
CEL	+8, t(5;12)(q33;p13)( <i>tel/pdgfrb</i> ), dic(1;7), 8p11( <i>fgfr1</i> ), del(20q), -7
SM	+9, del 20(q12), t(8;21)
CNL	+8, +9, del(20q), del(11q14), t(2;2) (q32p24)
8p11 MPS	t(8;13), t(8;9), t(6;8), t(8;22), t(8;19), t(8;17), t(8;11), t(8;12), ins(12;8)

Abbreviations for diseases are defined in the footnote of Table 1.

Table 5. Somatic Gene Mutations and Their Frequencies in cMPDs<sup>4,7,15</sup>

Disease	Genetic Mutation	Frequency (%)
ET	JAK2 V617F	31
PV	JAK2 V617F	76
CIMF	JAK2 V617F	50
CEL	FIP1L1/PDGFRα	26
SM	<i>c-kit</i> D816V	>80
	<i>c-kit</i> D816Y	<5
	<i>c-kit</i> D816H	<5
	<i>c-kit</i> D820G	<5
	<i>c-kit</i> V560G	<5
	<i>c-kit</i> F522C	<5
	<i>c-kit</i> V530I	<5
SM with eosinophilia	FIP1L1/PDGFRα	<5

Abbreviations for diseases are defined in the footnote of Table 1.

cMPD progression is associated with increased bone marrow fibrosis and transformation to AML. Thus, once a cMPD has been diagnosed, disease progression may be monitored with periodic evaluation of bone marrow cellularity, degree of fibrosis, and cytogenetic changes.

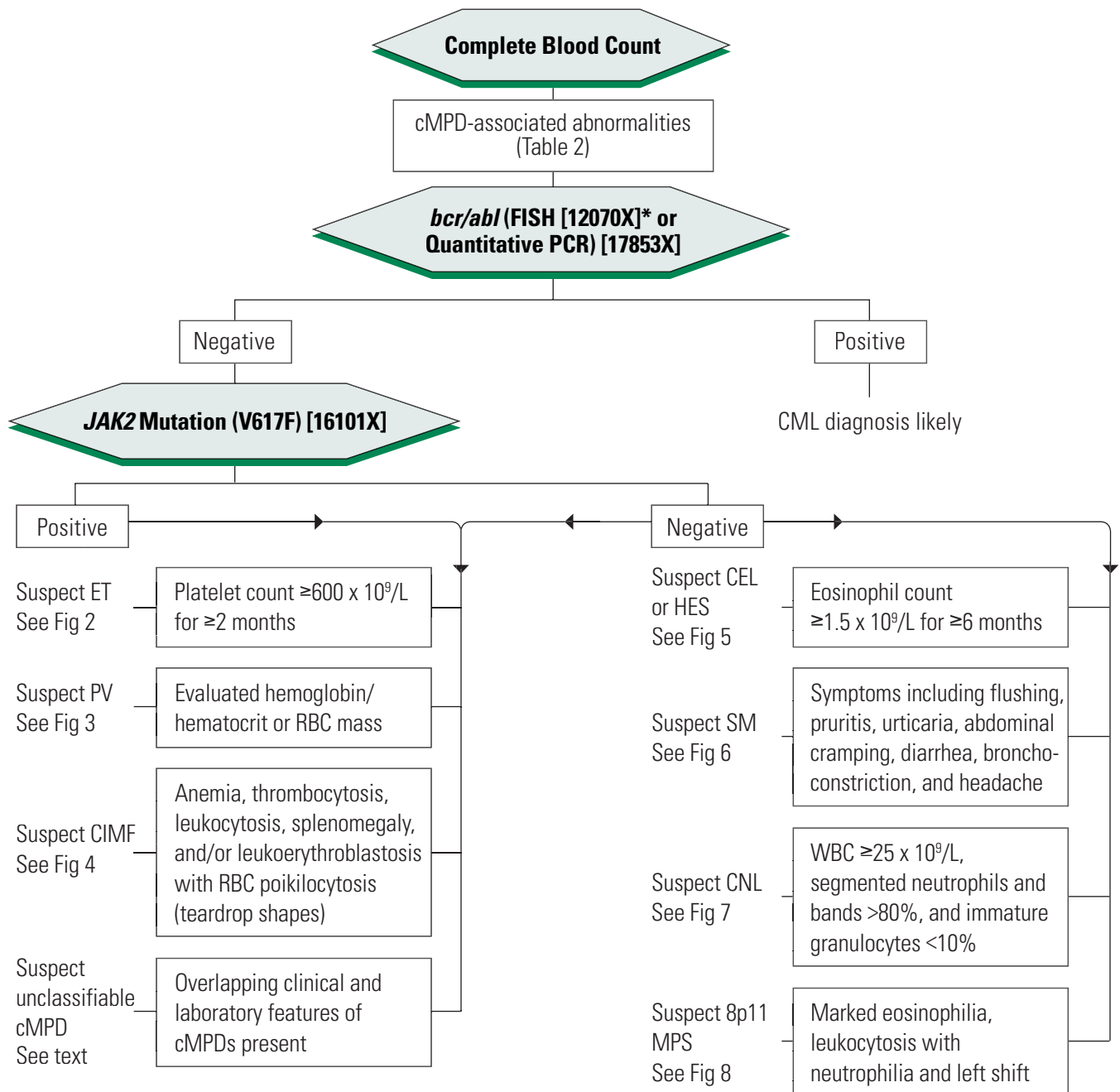
### Immunophenotyping

Flow cytometric analysis for cell surface markers (immunophenotyping) is used for differential diagnosis of leukemia and lymphoma, to assess response to therapy, and to detect relapse. Lineage-specific markers distinguish myeloid from lymphoid disorders and include myeloperoxidase found in myeloid disorders, cytoplasmic CD3 in T-lymphocytes, and cytoplasmic CD22 and IgM in B-lymphocytes.<sup>22</sup> Immunophenotyping helps guide therapeutic decisions when

a cMPD progresses as reflected by increased aberrant cells. On the other hand, decreases in abnormal marker-positive cells are associated with therapeutic success. Immunophenotype testing (ie, the Leukemia/Lymphoma Evaluation test) is particularly important when blast cells increase to >10% in blood or marrow for defining cell lineage associated with disease progression.

### Chronic Myelogenous Leukemia Testing

Differential diagnosis begins with the diagnosis or exclusion of CML in symptomatic patients or in those who have cMPD-related abnormalities in their CBC (Figure 1). Fluorescence in situ hybridization (FISH) testing provides qualitative results for *bcr/abl*, whereas reverse transcription-PCR (RT-PCR) results are quantitative. Positive results are diagnostic of



\*Test codes are included in brackets for all figures.

Figure 1. Differential diagnosis of cMPDs.<sup>1,3,4,8</sup>

CML, while negative results eliminate CML. Other cMPDs (*bcr/abl*-negative) should then be considered.

Approximately 95% of patients with CML are Ph+, whereas all are positive for *bcr/abl* by FISH and RT-PCR. This seeming discrepancy can be explained by the presence of a masked Ph, which is not observed by conventional karyotyping but is detected by the molecular tests.<sup>1</sup>

Following interferon or imatinib therapies, conventional bone marrow cytogenetic testing best predicts disease-free and overall survival. Once a complete cytogenetic response (CCR) is achieved (ie, the absence of Ph+ metaphase cells), molecular monitoring should be used.<sup>5,23</sup> RT-PCR is favored over FISH because *bcr/abl* mRNA correlates with CCR, can be used to monitor the kinetics of leukemia disease burden, and can be performed on peripheral blood.<sup>23</sup> Most studies have found a good correlation between blood and marrow PCR values.<sup>23</sup> At a recent consensus conference held by the National Institutes of Health (NIH), testing intervals of 3 to 6 months were recommended, although rising *bcr/abl* levels warrant more frequent testing.<sup>24</sup> In the International Randomized Study of Interferon versus STI571 (IRIS), a  $\geq 3$ -log reduction of *bcr/abl* transcript levels in peripheral blood was associated with a negligible risk of disease progression.<sup>25</sup>

An alternative to RT-PCR monitoring of imatinib therapy is serial measurement of BCR/ABL protein (total and phosphorylated forms). A preliminary study documented a statistically significant reduction in the proportion of phosphorylated BCR/ABL protein among patients who responded to imatinib therapy but not among those who were resistant to therapy.<sup>9</sup>

Treatment with imatinib can result in the acquired resistance that is most commonly associated with point mutation(s) in the PTK domain of *bcr/abl*.<sup>26</sup> Such mutations may precede or accompany progression to a more aggressive disease.<sup>26,27</sup> ABL kinase domain mutation testing is appropriate for patients presenting with advanced disease and for chronic-phase patients with inadequate initial response (failure to achieve a complete hematologic response at 3 months, minimal cytogenetic response at 6 months, or CCR at 12 months).<sup>24</sup>

Although risk of early mortality and chronic morbidity is present, allogeneic hematopoietic stem cell transplantation (AHSCT) provides another approach to CML treatment. As with drug treatments, AHSCT is most effective in the chronic phase (60% long-term remission) compared to either accelerated ( $\approx 30\%$ ) or blast phase ( $\approx 14\%$ ).<sup>28</sup> Following such treatment, detection of *bcr/abl* by RT-PCR is strongly associated with an increased risk of relapse. In a study of 346 patients, positive results at 6 to 12 months after transplant were associated with a 42% risk of relapse at a median of 200 days; negative results were associated with a 3% risk.<sup>29</sup> Transplant centers typically test for peripheral blood *bcr/abl* mRNA every 3 to 6 months for the first 2 years and yearly thereafter.<sup>23</sup>

Table 6 summarizes testing used in selecting and monitoring therapy and assessing prognosis for CML and other cMPDs.

## Essential Thrombocythemia

Although ET is diagnosed mainly by exclusion, positive criteria for the diagnosis are a sustained platelet count of  $\geq 600 \times 10^9/L$  and increased megakaryocyte proliferation in bone marrow studies (Table 2). Normal C-reactive protein levels rule out reactive causes of thrombocytosis, which are more common than clonal ET. However, elevated C-reactive protein levels are not helpful for diagnosis in this setting.<sup>30</sup>

Additional exclusion criteria include absence of underlying neoplasm and other cMPDs associated with increased megakaryocyte proliferation.<sup>1</sup> A positive result for the *JAK2* V617F mutation is likely to rule out CML, myelodysplastic syndrome (V617F-positive in 5% of patients), and non-hematologic cancer.<sup>16-18</sup> A negative result requires chromosome testing to rule out malignancies such as CML, 5q-syndrome, AML with inv(3), and myelodysplastic syndrome.<sup>1</sup> PV is ruled out with normal hemoglobin and/or RBC mass, and fibrotic stage CIMF is ruled out by the absence of reticulin and/or collagen fibrosis in bone marrow.<sup>1</sup>

A laboratory test guide for the differential diagnosis of ET is presented in Figure 2. Laboratory tests used to select and monitor therapy and to assess prognosis for ET are presented in Table 6. The use of cytoreductive therapy to reduce elevated platelet counts has been suggested for high-risk patients (ie, age  $\geq 60$  years or a history of thrombosis).<sup>31</sup>

## Polycythemia Vera

Increased RBC mass, measured using radioactive chromium, is the hallmark of PV diagnosis. This direct measurement is not necessary when the hemoglobin is  $>18.5$  g/dL in men or  $>16.5$  g/dL in women.

The WHO criteria for diagnosing PV include ruling out the more common inherited and secondary, acquired erythrocytosis.<sup>1</sup> Acquired erythrocytosis can be due to chronic hypoxia, treatment with erythropoietin (EPO) or androgens, or EPO-secreting tumors. Once elevated RBC mass or hemoglobin levels are observed (Table 2), a subnormal serum EPO level and a positive *JAK2* V617F mutation excludes secondary erythrocytosis and inherited polycythemia (see Figure 3). An elevated EPO level rules out PV, while a normal level is inconclusive. Negative *JAK2* V617F mutation makes the diagnosis of PV less likely but does not rule it out.

The WHO provides a second alternative to diagnose PV: hemoglobin or RBC mass is elevated, inherited and secondary erythrocytosis are ruled out, and 2 other criteria are met. These latter criteria include  $>400 \times 10^9/L$  platelet count,  $>12 \times 10^9/L$  WBC count, characteristic bone marrow cytology (Table 2), and low serum EPO level.

Laboratory tests to select and monitor therapy and to assess prognosis for PV are presented in Table 6. Phlebotomy targeting reduction in hematocrit is the cornerstone of PV therapy.

## Chronic Idiopathic Myelofibrosis

The classical presentation of CIMF is the appearance of a leukoerythroblastic blood smear with teardrop poikilocytosis, anemia, splenomegaly and possibly hepatomegaly due to EMH, and bone marrow fibrosis.<sup>1</sup> This picture is characteristic of the fibrotic, advanced stage of

Table 6. Tests Used in the Management of Patients with cMPD

Assay	Select Therapy	Monitor Therapy	Assess Prognosis
<b>CML</b>			
ABL Kinase Domain Mutation in CML	X	X	X
<i>bcr/abl</i> Gene Rearrangement. Quantitative PCR	X	X	X
BCR/ABL Protein Quantitation (Total and Phosphorylated)	X	X	X
Bone Marrow Histology		X	X
Bone Marrow Chromosome Analysis	X	X	X
% Blast Cells	X	X	X
FISH, Locus-specific Probe(s)	X	X	X
<b>ET</b>			
Bone Marrow Histology		X	X
Bone Marrow Chromosome Analysis		X	
Platelet Count	X	X	X
<b>PV</b>			
Bone Marrow Histology		X	X
Bone Marrow Chromosome Analysis		X	X
Hematocrit	X	X	
<b>CIMF</b>			
Bone Marrow Histology		X	X
Bone Marrow Chromosome Analysis		X	X
Platelet Count, Hemoglobin, and % Blast Cells		X	X
<b>CEL/HES</b>			
Bone Marrow Histology		X	X
Bone Marrow Chromosome Analysis		X	
Eosinophil Count		X	
<i>FIP1L1/PDGFRRA</i> Gene Rearrangement	X	X	X
% Blast Cells			X
<b>SM</b>			
Bone Marrow Histology		X	
<i>c-kit</i> Mutation Analysis	X	X	
<i>FIP1L1/PDGFRRA</i> Gene Rearrangement	X	X	X
Tryptase		X	
<b>CNL</b>			
Bone Marrow Histology		X	
Bone Marrow Chromosome Analysis		X	
% Blast Cells		X	X
<b>8p11 MPS</b>			
Bone Marrow Histology		X	X
Bone Marrow Chromosome Analysis		X	

Abbreviations for diseases are defined in the footnote of Table 1.

the disease. Diagnosis is more complicated in the 20% to 30% of patients who are at the prefibrotic stage, which can resemble PV or ET. The differential diagnosis is important since CIMF has worse survival than ET or PV.<sup>13</sup> Observation of prominent neutrophil proliferation, decreased numbers of erythroid precursors, and marked atypical forms of megakaryocytes in bone marrow confirms a diagnosis of prefibrotic CIMF.<sup>13</sup>

Detection of the *JAK2* V617F mutation supports the diagnosis of CIMF but does not distinguish between PV, ET, and CIMF. Absence of the mutation does not rule out the diagnosis. Detection of a clonal aberration, which is relatively common in CIMF (≈35%), also supports the diagnosis. A laboratory test guide for the differential diagnosis of CIMF is presented in Figure 4.

Table 6 summarizes tests used to monitor therapy and assess prognosis of CIMF. A combination of age >60 years, hemoglobin levels <10 g/dL, platelet counts <100 × 10<sup>9</sup>/L, and >3% circulating blast cells predict a median survival of <5 years as compared to ≈15 years in their absence.<sup>32</sup> The detection of certain chromosomal abnormalities also appears to be prognostic. In one study of 165 patients with CIMF, trisomy 8 or 12p deletion was associated with a poor prognosis while deletions of 13q or 20q were not.<sup>14</sup>

### Chronic Eosinophilic Leukemia/Hypereosinophilic Syndrome

Hypereosinophilia (≥1.5 × 10<sup>9</sup>/L) may be due to reactive eosinophilia, idiopathic HES, or CEL. The differential diagnosis of CEL and HES begins with the exclusion of all

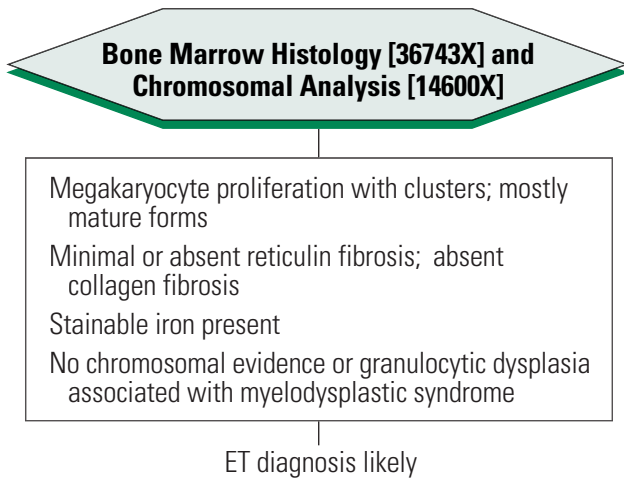


Figure 2. Differential diagnosis of ET. <sup>1</sup> (Continued from Fig 1)

causes of reactive eosinophilia, including parasitic infection, infectious disease, allergic reaction, pulmonary diseases such as hypersensitivity pneumonitis, collagen vascular diseases, and underlying neoplastic disease.<sup>1</sup> Neoplastic diseases to be excluded include T-cell lymphomas, Hodgkin lymphoma, acute lymphoblastic leukemia/lymphoma, other cMPDs, AML, and myelodysplastic syndromes. Once these diagnoses have been excluded, CEL is diagnosed if there is evidence of a clonal myeloid abnormality (eg, the presence of the *FIP1L1/PDGFR*A fusion gene); HES is diagnosed if no such evidence is found.<sup>1</sup>

Molecular testing can help distinguish the causes of hypereosinophilia. In a series of patients with persistent hypereosinophilia, T-cell receptor gene rearrangements were present in 32% leading to the diagnosis of T-cell associated HES and the *FIP1L1/PDGFR*A fusion gene was detected in

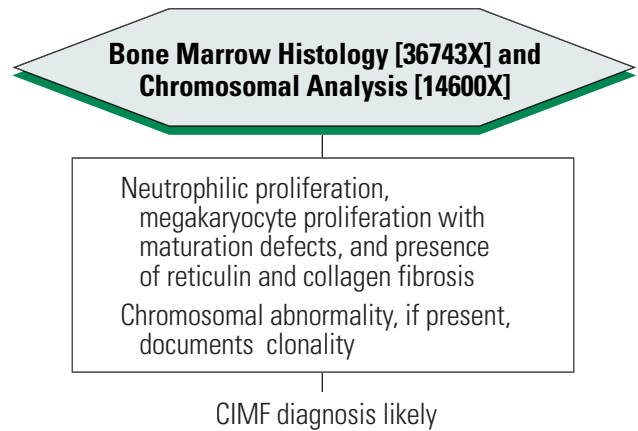


Figure 4. Differential diagnosis of CIMF. <sup>1</sup> (Continued from Fig 1)

17%, confirming the diagnosis of CEL.<sup>11</sup> Thus, detection of these 2 genetic mutations led to a specific diagnosis in nearly 50% of the patients and was useful in ruling out T-cell-associated HES in the remainder. Because the *FIP1L1/PDGFR*A fusion gene is not present in all patients with CEL, its absence does not rule out CEL. A laboratory test guide for CEL and HES diagnosis is presented in Figure 5.

Detection of the *FIP1L1/PDGFR*A fusion gene can also be used to predict response to imatinib.<sup>7,10</sup> Although the number of patients studied was limited, 57 of 57 (100%) positive for *FIP1L1/PDGFR*A achieved complete hematological response with imatinib.<sup>7</sup> Negative *FIP1L1/PDGFR*A results, however, did not rule out response to therapy; 12 of 53 (23%) achieved either a partial or a complete hematologic response.

In preliminary studies, MRD detection using *FIP1L1/PDGFR*A testing was useful for monitoring response to imatinib

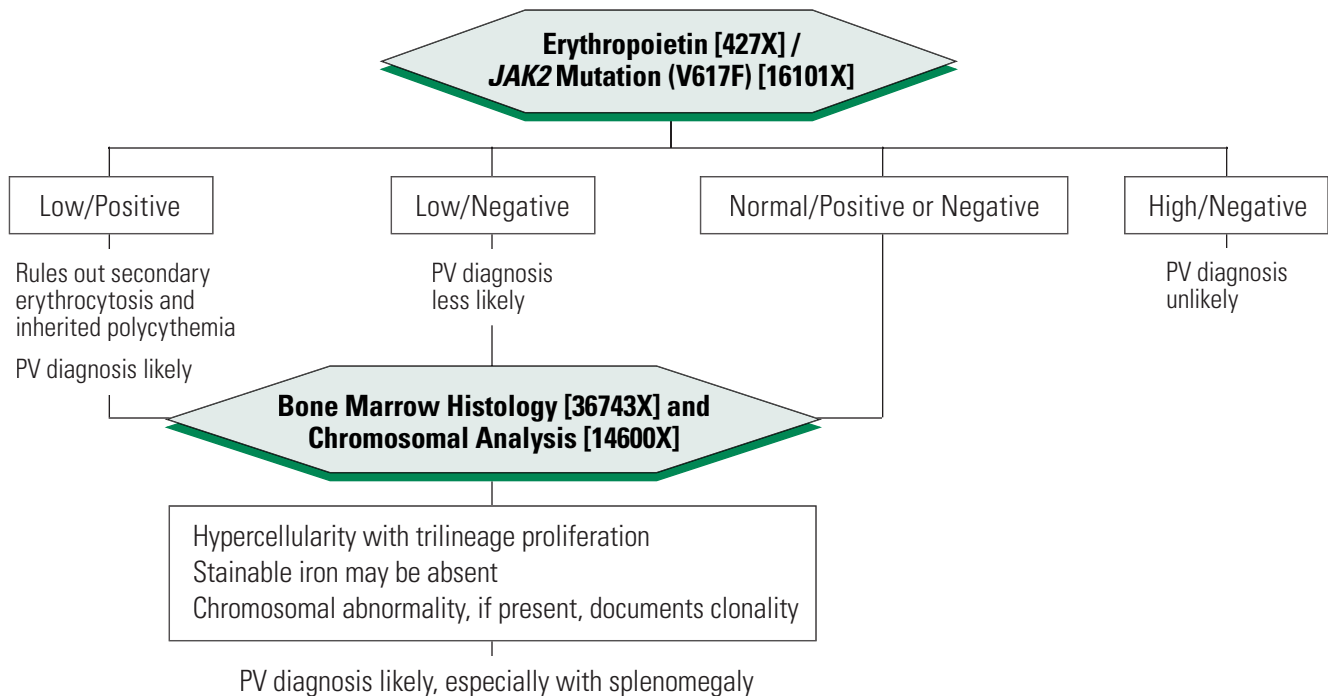


Figure 3. Differential diagnosis of PV. <sup>1</sup> (Continued from Fig 1)

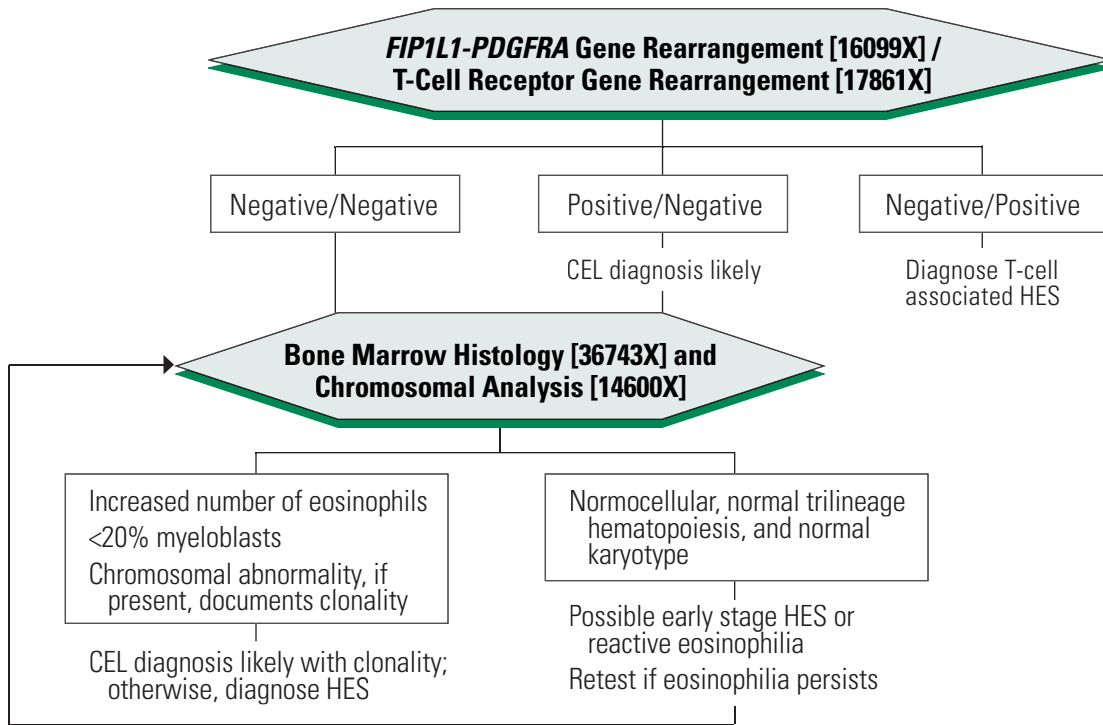


Figure 5. Differential diagnosis of CEL/HES.<sup>1,10</sup> (Continued from Fig 1)

therapy.<sup>10,11</sup> Table 6 summarizes tests used to select and monitor therapy and assess prognosis in patients with CEL or HES.

### Systemic Mastocytosis

The major diagnostic criterion for SM is the presence of dense multifocal clusters or aggregates of mast cells (>15 per aggregate) in a bone marrow biopsy specimen.<sup>3</sup> Minor criteria include: 1) abnormal morphology in >25% of mast cells; 2) *c-kit* mutation at codon 816; 3) mast cells coexpressing CD117 and CD2 and/or CD25; and 4) serum tryptase levels persistently >20 ng/mL in the absence of an associated hematologic clonal non-mast cell lineage disease. SM is diagnosed if at least the major criterion plus 1 minor criterion, or at least 3 minor criteria, are met. A laboratory test guide for SM diagnosis is presented in Figure 6.

An elevated tryptase level is an important marker of SM, but may also be seen in acute and chronic myeloid leukemias, other cMPDs, myelodysplastic syndromes, and myelomastocytic leukemia.<sup>4</sup>

In addition to being a criterion for diagnosis, the *c-kit* D816V mutation confers resistance to imatinib by interfering with the binding of the drug to the catalytic site of the KIT PTK. Conversely, the presence of wild-type or F522C *c-kit* or the *FIP1L1/PDGfra* fusion gene is associated with sensitivity to imatinib.<sup>4</sup>

Table 6 summarizes tests used to select and monitor therapy and assess prognosis in patients with SM.

### Chronic Neutrophilic Leukemia

CNL is diagnosed when the hematologic criteria are met (Table 2), hepatosplenomegaly is present, no evidence of a physiologic neutrophilia is found, and other cMPDs and

myelodysplastic disorders are ruled out.<sup>1</sup> A normal C-reactive protein level rules out inflammation and infection that could produce neutrophilia; however, an elevated level does not necessarily rule out CNL. Negative results for Ph or *bcr/abl* rule out CML, which also presents with neutrophilia. Other cMPDs are ruled out by the absence of their usual hematologic characteristics (Table 2). Furthermore, myelodysplastic disorders are ruled out by the absence of granulocytic dysplasia and myelodysplastic changes in other myeloid lineages. A laboratory test guide for CNL diagnosis is presented in Figure 7.

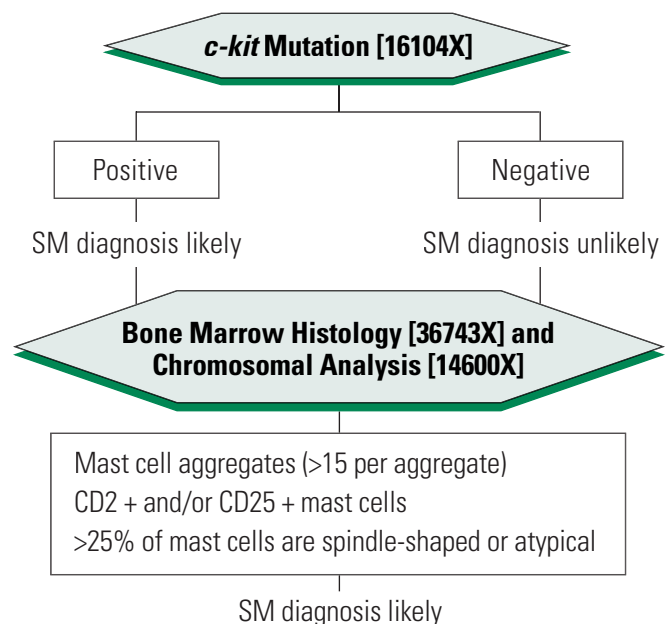


Figure 6. Differential diagnosis of SM.<sup>3</sup> (Continued from Fig 1)

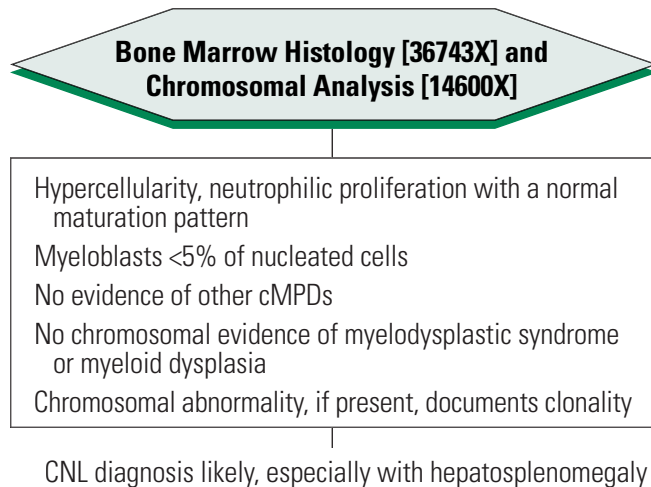


Figure 7. Differential diagnosis of CNL.<sup>1</sup> (Continued from Fig 1)

A summary of tests used to monitor therapy and assess prognosis of CNL is presented in Table 6.

### 8p11 Myeloproliferative Syndrome

Suspicion of the 8p11 MPS is triggered by eosinophil counts ranging from  $1.2 \times 10^9/L$  to  $40 \times 10^9/L$  (median  $4 \times 10^9/L$ ) as reported in a series of 14 patients.<sup>8</sup> Bone marrow studies show myeloid hyperplasia, and chromosome analysis identifies abnormalities associated with chromosome 8 (see Figure 8). Transformation to AML or lymphoma has been associated with +21 and this clonal evolution may be identified with karyotyping (see Table 6).<sup>8</sup>

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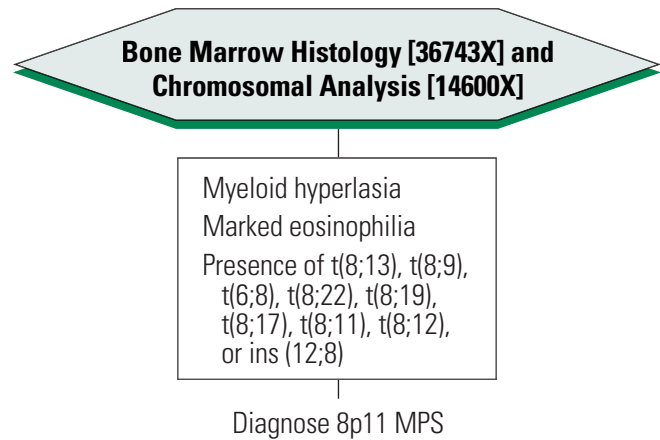


Figure 8. Differential diagnosis of 8p11 Myeloproliferative Syndrome.<sup>8</sup> (Continued from Fig 1)

### Unclassifiable cMPD

In approximately 15% of cMPD cases, clinical and laboratory features characteristic of a myeloproliferative disease are present but fail to meet the diagnostic criteria of any one cMPD.<sup>1</sup> Such patients are either in the early stages of the disease and characteristic features of a particular cMPD will develop with time or are in the advanced stages of the disease with marked marrow fibrosis or blastic infiltration. In the former case, reevaluation at intervals of 4 to 6 months is recommended.<sup>1</sup>

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