Myeloproliferative Neoplasms: The Role of Molecular Markers

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LEARNING OBJECIVES

After reading this article, the reader will be able to:

1. Describe the fusion gene formed as a result of the translocation t(9;22)(q34;q11) in chronic myelogenous leukemia.
2. Describe common symptoms of Philadelphia chromosome negative myeloproliferative neoplasms.
3. State the frequency of the occurrence of the *JAK2* V617F mutation in PV, ET, and PMF.
4. Describe the significance of the *JAK2* mutation in the diagnosis of PV, ET, and PMF.
5. Describe the significance of tyrosine kinase inhibitors on the treatment of MPNs.
6. Define current treatment for Philadelphia chromosome negative MPNs.
7. Describe the significance of imatinib treatment for CML on treatment for the other MPNs.

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The myeloproliferative neoplasms (MPN), formerly known as the myeloproliferative disorders (MPD), are a group of clonal disorders involving hyperproliferation of one or more of the myeloid cell lines (erythrocytic, granulocytic, megakaryocytic). The most common disorders traditionally classified as MPNs are chronic myelogenous leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). Other, less common disorders in this group include chronic neutrophilic leukemia (CNL), chronic eosinophilic leukemia (CEL), mastocytosis, and myeloproliferative neoplasm, unclassifiable (MPN,U).[1] All of these neoplasms are most common in people with a median age of 65 years at diagnosis but have been diagnosed in all age groups. Common presenting signs are fatigue, splenomegaly, and elevated cell counts. Often, there are bleeding or thrombotic symptoms.

Chronic Myelogenous Leukemia (CML)—*BCR/ABL1*-Positive MPN

CML is a disease of "firsts" that has led the way for diagnostic, treatment, and prognostic factors for the other MPNs. It is the first disorder for which the term "leukemia" was used; the first malignancy associated with a recurring chromosomal abnormality (Philadelphia chromosome); the first disease in which the chromosomal abnormality formed a fusion gene (*BCR/ABL1*); the first disease in which a protein fundamental to the pathogenesis of the disease was identified; and, the first disorder in which a therapeutic agent specifically targeted the molecular defect to effectively treat the disorder.[1]

Historically, CML was the first MPN to have a known acquired chromosomal abnormality, a reciprocal translocation between the long arms of chromosomes 9 and 22 known as the Philadelphia chromosome (Ph^1) and designated as t(9;22)(q34;q11). This translocation was then found to be present in 95% of patients with CML and was detected by conventional karyotyping methods, making the finding of this chromosomal abnormality a hallmark of CML. Because it can also be found in 2–10% of pediatric cases of acute lymphoblastic leukemia (ALL) and 25–30% of adult cases of ALL,[2] Ph^1 is not specific to CML. The translocation forms a fusion gene, *BCR/ABL1*,

producing a protein that dysregulates tyrosine kinase function[3] causing a proliferation of granulocytes. In the late 1990s, imatinib mesylate (Gleevec), a tyrosine kinase inhibitor (TKI) was found to target the *BCR/ABL1* fusion gene and inhibit the proliferation of the abnormal cells. This changed the standard treatment for this disease. Even though bone marrow transplantation is still the only cure for CML, the use of imatinib and newer generations of TKIs has turned this leukemia into a manageable disease Since most patients are diagnosed in the chronic phase of the disorder and that is when TKI is most effective, these drugs have become first line therapy for CML. Some patients have become resistant to imatinib and second and third generation TKIs have been shown to be effective in these patients.[2] The disease is monitored using fluorescent *in situ* hybridization (FISH) techniques to identify the *BCR/ABL1* translocation.

BCR/ABL1 Negative Myeloproliferative Neoplasms

The most common *BCR/ABL1*-negative MPNs are polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). These disorders are indolent and usually present with fatigue. Other symptoms common to all three disease processes are splenomegaly and hemorrhagic or thrombotic tendency. Often, they are diagnosed as part of a routine physical when elevated cell counts are noted on CBC and a peripheral blood smear is examined. These are primarily diseases of patients over 70 years old and have an indolent course, but approximately 15% of cases develop into acute leukemia. These secondary leukemias are resistant to treatment. Diagnosis of the MPNs involves peripheral cell counts and morphology, bone marrow morphology, and molecular studies for gene mutations. Treatment has centered on cytoreduction by therapeutic phlebotomy and/or treatment with cytoreductive pharmaceuticals such as hydroxyurea or α-interferon. Aspirin therapy is also used in PV and ET due to the increased risk of thrombosis.

In 2005, several research groups found that the *JAK2* (Janus kinase) mutation was common to the Philadelphia chromosome negative MPNs PV, ET, and PMF. This mutation involves an acquired valine to phenylalanine change at codon 617 of the *JAK2* gene,[5] designated *JAK2* V617F. The *JAK2* V617F mutation is present in almost all patients with PV and approximately 50% of patients with ET and PF. It is also seen in 20% of patients with less common MPNs but not seen at all in patients with CML, reactive myeloproliferation (e.g. thrombocytosis, leukocytosis, and secondary erythrocytosis), lymphoid disorders, or solid tumors.[6] The effect of this mutation is disruption of the normal tyrosine kinase signaling pathways (JAK-STAT) resulting in proliferation of myeloid cell lines. Presence of *JAK2* V617F is associated with higher white cell counts, red cell counts, platelet counts, splenomegaly, and increased risk of thrombosis.[6] The discovery of the *JAK2* V617F mutation has changed the diagnostic criteria, prognosis, and treatment options for patients with the *BCR/ABL*-negative MPNs. In 2008, the World Health Organization (WHO) revised the diagnostic criteria for PV, ET, and PMF to reflect the significance of the *JAK2* mutation.[1]

Polycythemia vera usually presents with panmyelosis. Red blood cell precursors are hypersensitive to erythropoietin,[6] causing an increase in the RBC count, hematocrit, and hemoglobin. These patients will have decreased serum erythropoietin levels. The *JAK2* V617F mutation is present in >95% of patients.[1] Most of the remainder have another *JAK2* mutation in exon 12 that has the same effect as *JAK2* V617F, resulting in 98% of PV patients having a *JAK2* mutation.[6] Patients with the *JAK2* exon 12 mutation display only erythrocytosis while those with *JAK2* V617F also have leukocytosis and thrombocytosis. The 2008 WHO criteria for diagnosis of PV include:[1]

Major PV criteria

1. Hemoglobin >18 g/dL (males), >16.5 g/dL (females)

   or

   Hemoglobin or hematocrit >99th percentile of reference range for age, sex, and altitude of residence

   or

   Hemoglobin > 17 g/dL (males), 15 g/dL (females) if associated with a sustained increase of 2 g/dL from baseline that cannot be attributed to correction of iron deficiency

   or

elevated red blood cell mass >25% above normal predicted value

2. Presence of *JAK2* V617F or similar mutation

Minor PV criteria

1. Bone marrow trilineage myeloproliferation
2. Subnormal serum erythropoietin level
3. Endogenous erythroid colony formation

Diagnosis requires the presence of both major criteria and one minor criterion or the first major criterion plus two minor criteria.

Treatment for PV includes therapeutic phlebotomy with the goal of maintaining the hematocrit at below 45% in males and below 40% in females.[7] Aspirin therapy to reduce the risk of thrombosis is also part of treatment. In patients with a history of thrombosis who are >60 years of age, cytoreductive therapy with hydroxyurea is also used.[6] Using current treatments, median survival of greater than ten years is common.[1]

Essential thrombocythemia (ET) is a MPN that presents with an elevated platelet count. More than half of patients are asymptomatic at presentation; the remainder has either a thrombotic or hemorrhagic manifestation.[1] In addition to peripheral blood thrombocytosis, the bone marrow demonstrates megakaryocytic hyperplasia. Approximately 50% of patients with ET have the *JAK2* V617F mutation. An additional mutation, *MPL* W515K/L, is present in approximately 8% of ET patients. *MPL* is the receptor for thrombopoietin and interacts with *JAK2*.[3] The 2008 WHO criteria for diagnosis of ET requires the presence of all of the following:

1. Sustained platelet count >450 x 10^9/L
2. Bone marrow demonstrates megakaryocytic proliferation with large and mature morphology and little or no granulocytic or erythrocytic proliferation.
3. Patient does not meet WHO criteria for CML, PV, PMF, MDS, or other myeloid neoplasms.
4. Demonstration of *JAK2* V617F mutation or other clonal markers and no evidence of reactive thrombocytosis.

Treatment for ET depends on the patient's risk of thrombosis. Patients with low (no history of thrombosis, age <60 years, platelet count <1,000 X 10^9/L) or intermediate risk are maintained on aspirin therapy. Those at high risk (history of thrombosis or age >60 years) also receive cytoreductive therapy with hydroxyurea.[6] ET patients without the *JAK2* V617F mutation tend to present with only thrombocytosis and a higher platelet count and require increased dosage of hydroxyurea.[5] Using current treatment, median survival is 10–15 years.[1]

Primary myelofibrosis (PMF) is the most serious of the Ph[1]-negative MPNs and can be life threatening. In PMF, the bone marrow is replaced by fibrotic material (reticulin), limiting the ability to produce normal blood cells and causing extramedullary hematopoiesis. Splenomegaly can become severe enough to threaten rupture, necessitating splenectomy. Both ET (15%) and PV (20%) may develop marrow fibrosis.[6] This condition progresses from a prefibrotic stage in which there is minimal reticulin in the bone marrow, to the fibrotic stage in which the bone marrow is predominantly reticulin. In the fibrotic stage, extramedullary hematopoiesis takes place primarily in the spleen and the peripheral blood smear shows a leukoerythroblastic morphology with teardrop red cells.[1] The *JAK2* V617F mutation is present in approximately 50% of PMF patients. PMF patients with this mutation are more likely to have thrombosis, pruritis, higher WBC counts, and are less likely to require transfusions.[6] Patients with the *JAK2* V617F mutation have a 5.2 times greater risk of transforming into acute leukemia, making the presence of this mutation an important prognostic indicator.[6] The 2008 WHO diagnostic criteria for PMF includes:

Major PMF criteria

1. Megakaryocytic proliferation and atypia accompanied by reticulin and/or collagen fibrosis

   or

   In the absence of reticulin fibrosis, the megakaryocytic changes must be accompanied by increased marrow cellularity, granulocytic proliferation, and, often, decreased erythropoiesis.
2. Patient does not meet WHO criteria for CML, PV, MDS, or other myeloid neoplasm.

3. Presence of *JAK2* V617F mutation or other clonal markers

   or

   in the absence of a clonal marker, no evidence that the bone marrow fibrosis or other changes are secondary to infection, autoimmune disorder or other chronic inflammatory condition, hairy cell leukemia or other lymphoid neoplasm, metastatic malignancy, or toxic myelopathies[1]

Minor PMF criteria

1. Leukoerythroblastosis
2. Increased LDH
3. Anemia
4. Splenomegaly

Diagnosis requires all three major criteria and two minor criteria.

Diagnostic, Prognostic, and Treatment Significance of Molecular Markers in MPNs

The *JAK2* V617F mutation has quickly become an important diagnostic tool for Philadelphia chromosome-negative MPNs. Testing for this mutation has become a routine part of diagnostic testing for patients suspected of having one of these disorders. The most effective testing method is real time polymerase chain reaction (RT-PCR), which assesses the allele load of the mutation and tracks the effectiveness of treatment. While MPNs tend to have an indolent and prolonged course, living with their effects makes it beneficial to find approaches that alleviate the symptoms and prevent progression to either myelofibrosis or leukemia. Current treatment for Philadelphia chromosome-negative myeloproliferative neoplasms is directed toward preventing thrombosis using low-dose aspirin therapy, cytoreduction using phlebotomy, and cytoreduction using pharmaceuticals such as hydroxyurea or interferons.[3] The success of imatinib in the treatment of CML by inhibition of tyrosine kinase has led to attempts to develop drugs that target the *JAK2* mutations. Currently, there are drugs in phase II and III clinical trials for targeted *JAK2* positive mutations.[6]

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ERRATA: In the Spring 2011 Volume 24 of Clinical Laboratory Science the figure on Page 80 should have read "Arrows point to sickle cells. Note NRBC on right as well as Howell-Jolley Body and polychromasia."