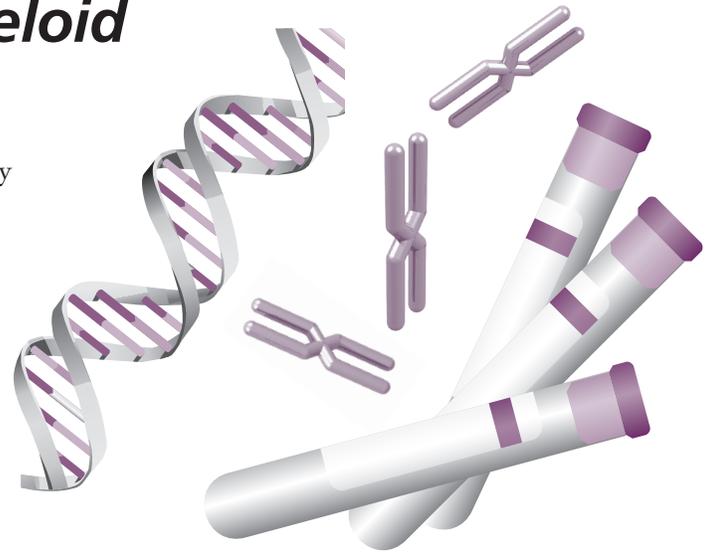


The JAK2 — Associated Myeloid Neoplasms: A Brief Review

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Introduction

The myeloproliferative neoplasms (MPN) are cancerous proliferations of cells of the bone marrow and blood involving, to varying degrees, progenitors of white blood cells, red blood cells, and platelets. The MPNs span eight disease categories in the 2008 World Health Organization (WHO) classification of hematopoietic neoplasms. MPNs are generally distinguished from acute myeloid leukemia by the presence of fewer than 20%



myeloblasts in the blood and bone marrow, and they are generally distinguished from other non-acute myeloid neoplasms by a lack of morphologic dysplasia, and by a pattern of “effective” hematopoiesis, either in the form of increased peripheral blood cell counts, splenomegaly, or both. This is as opposed to the pattern of “ineffective” hematopoiesis (cellular bone marrow but decreased peripheral cell counts) and morphologic dysplasia more typical of the myelodysplastic syndromes (MDS). Three of the major MPN categories—polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF)—are associated to varying degrees with a mutation in the Janus Kinase 2 (JAK2) gene. As a result, assessment for JAK2 mutation

status has become routine in the workup of patients with persistently elevated peripheral blood counts, organomegaly, unexplained thrombosis, or other signs suggestive of MPN. In addition, a minor subset of disorders classified by WHO in the category of myeloproliferative/myelodysplastic neoplasms (neoplasms that show overlap features of both MPNs and myelodysplastic syndromes) appear to be associated with JAK2 mutation.

The Janus kinases comprise a family of proteins named for the Roman god of transitions—a two-faced entity that was said to simultaneously face the past and the future—so named because the JAK proteins contain two principal binding domains, one that enhances and one that inhibits kinase activity. The JAK proteins are major components of the JAK-STAT pathway of cellular proliferation, and abnormalities in the JAK2 gene are thought to drive the proliferative process in JAK2-associated myeloid neoplasms. A substantial majority of JAK2-associated neoplasms harbor the JAK2 V617F mutation, in which a point mutation in the

Three of the major MPN categories are associated with JAK2 gene mutation.

of “effective” hematopoiesis, either in the form of increased peripheral blood cell counts, splenomegaly, or both. This is as opposed to the pattern of “ineffective” hematopoiesis (cellular bone marrow but decreased peripheral cell counts) and morphologic dysplasia more typical of the myelodysplastic syndromes (MDS). Three of the major MPN categories—polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF)—are associated to varying degrees with a mutation in the Janus Kinase 2 (JAK2) gene. As a result, assessment for JAK2 mutation

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DNA code at exon 14 results in the substitution of a phenylalanine residue (F) for the normal valine (V) residue, resulting in gain of JAK2 function and enhancement of proliferative activity. A small percentage of JAK2-associated neoplasms harbor mutations other than the V617F; the most common of these alternative mutations occurs in exon 12 of the JAK2 gene.

All MPN are thought to represent hematopoietic stem cell neoplasms. In other words, neoplastic proliferation is thought to

take place at the level of a pluripotent stem cell that subsequently differentiates into multiple specific hematopoietic lineages (erythroid, megakaryocytic, granulocytic, monocytic). Before the discovery of associated gene fusions and mutations, the classification of a given MPN historically was based on the predominant pattern of differentiation of the neoplastic stem cell. Predominantly neutrophilic differentiation characterized chronic myelogenous leukemia (CML) (now known to be specifically linked to the Philadelphia chromosome / BCR-ABL gene fusion and not generally included in the JAK2 associated neoplasms); predominantly erythroid differentiation characterized polycythemia vera (PV); and predominantly megakaryocytic/ platelet differentiation characterized essential thrombocythemia (ET). Primary myelofibrosis (PMF) (historically termed myelofibrosis with myeloid metaplasia, agnogenic myeloid metaplasia, or chronic idiopathic myelofibrosis) was marked by an expansion of multiple cell lineages, often most prominently megakaryocytic, in which the megakaryocytic component stimulated marked marrow fibrosis through secretion of platelet-derived growth factors, and resulted in prominent foci of extramedullary neoplastic hematopoiesis (most notably in the spleen).

Although some hematologic malignancies are defined categorically by the presence of a specific molecular genetic abnormality (such as the PML-RARA fusion in acute promyelocytic leukemia), the current consensus is that JAK2 mutation-associated MPN should still be classified according to clinicopathologic features and not considered variants of a single disorder. A brief discussion of the four major JAK2 mutation-associated disease categories follows.

Polycythemia Vera

The hallmark of polycythemia vera (PV) is the neoplastic overproduction of red blood cells, resulting in polycythemia (by WHO criteria a persistent whole blood hemoglobin in excess of 18.5 g/dL in men, or 16.5 g/dL in women). However, bone marrow examination in PV patients typically shows a

Table 1. World Health Organization Criteria for a Diagnosis of Polycythemia Vera (Reproduced from Thiele et al, WHO, 2008). Diagnosis requires both major criteria and one minor criterion, OR the first major criterion combined with two minor criteria.

Major Criteria	Hemoglobin >18.5 g/dL in men, 16.5 g/dL in women, or other evidence of increased red cell volume*
	Presence of JAK2 V617F or other functionally similar mutation such as JAK2 exon 12 mutation.
Minor Criteria	Bone marrow biopsy showing hypercellularity for age with trilineage growth (panmyelosis) with prominent erythroid, granulocytic, and megakaryocytic proliferation
	Serum erythropoietin level below the reference range for normal
	Endogenous erythroid colony formation <i>in vitro</i>

*Hemoglobin or hematocrit >99th percentile of method-specific reference range for age, sex, altitude of residence, or hemoglobin >17 g/dL in men, 15 g/dL in women if associated with a documented and sustained increase of at least 2 g/dL: from an individual's baseline value that cannot be attributed to correction of iron deficiency, or elevated red cell mass >25% above mean normal predicted value.

neoplastic expansion of all hematopoietic lineages (panmyelosis), rendering some morphologic similarities with other MPNs, particularly primary myelofibrosis (PMF).

Current WHO diagnostic criteria for a diagnosis of PV are summarized in Table 1. The findings of a persistently elevated whole blood hemoglobin level (greater than 18.5 g/dL in men, or greater than 16.5 g/dL in women), a documented JAK2 mutation, and serum erythropoietin below reference range are sufficient to diagnose PV by WHO criteria. However, a diagnosis can still be made in the absence of some of these features if other combinations of criteria are met (see Table 1).

Among the different MPNs, PV has the strongest association with JAK2 mutation. Approximately ninety-five percent of cases

PV has the strongest association with JAK2 mutation.

of PV are said to harbor a JAK2 V617F mutation. However, when variant mutations (particularly the exon 12 mutation) are included, the prevalence of

JAK2 mutation in PV likely exceeds 99%. Therefore, a diagnosis of PV in the absence of some form of JAK2 mutation should generally be approached with great caution.

There are many non-neoplastic (secondary) causes for polycythemia, and if a JAK2 mutation cannot be documented in a patient with persistent polycythemia, then these secondary causes should be considered. A list of potential causes of secondary polycythemia is provided in Table 2.

Table 2. Potential Causes of Secondary Polycythemia

Chronic hypoxia (cyanotic heart disease, hypoxic lung disease, sleep apnea, etc)
High oxygen-affinity hemoglobinopathy
Anabolic steroids
Ectopic erythropoietin production (e.g. from a neoplasm)
Familial abnormalities in erythropoietin feedback mechanism (e.g. "Chuvash" polycythemia)

In general, PV is an indolent disorder, with median survival typically reported as exceeding 10 years. There is some risk of transformation to acute leukemia, but in the absence of previous cytotoxic chemotherapy this risk is small (2 to 3% according to WHO). There is a risk for development of post-polycythemic myelofibrosis, and this risk increases with time, occurring in up to 50% of patients who survive at least 20 years with the disease. The onset of post-polycythemic myelofibrosis is often heralded by the development of peripheral cytopenias, and in some cases post-polycythemic myelofibrosis may be essentially identical to primary myelofibrosis clinically and histologically.

Essential Thrombocythemia

Essential thrombocythemia is marked by a persistent increase in peripheral blood platelet count, generally with normal leukocyte and red cell counts. The bone marrow in essential thrombocythemia is generally either normocellular or only slightly hypercellular, and morphologic abnormalities are generally limited to subtle increases and subtle clustering of megakaryocytes. Megakaryocytes may also show complex morphologic features including hyperlobation. Marked marrow hypercellularity, or extensive or cohesive clustering of megakaryocytes in the bone marrow should raise suspicion for a different MPN, particularly the cellular phase of PMF (see page 4).

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Table 3. Summary of World Health Organization Diagnostic Criteria for Essential Thrombocythemia (Reproduced from Thiele et al, WHO, 2008). Please see text for additional explanatory notes.

Sustained platelet count $\geq 450 \times 10^9/L$ (Sustained during work-up process)
Bone marrow biopsy specimen showing proliferation mainly of the megakaryocytic lineage with increased numbers of enlarged, mature megakaryocytes. No significant increase or left-shift of neutrophil granulopoiesis or erythropoiesis
Not meeting WHO criteria for polycythemia vera, primary myelofibrosis, BCR-ABL1 positive chronic myelogenous leukemia, or myelodysplastic syndrome or other myeloid neoplasm
Demonstration of JAK2 V617F or other clonal marker, or in the absence of JAK2 V617F, no evidence for reactive thrombocytosis

In contrast to PV, JAK2 mutation is generally documented in only about 50% of cases of ET. Since the peripheral blood platelet count may be elevated in a number of reactive or inflammatory conditions, and since there is substantial overlap in the magnitude of the platelet count between reactive thrombocytosis and ET, the diagnosis in JAK2-negative cases often involves the exclusion of potential secondary causes for thrombocytosis. In addition, since many MPNs may present initially with thrombocytosis, diagnostic criteria for ET include the exclusion of other WHO-defined MPNs. Finally, since PV may present initially with thrombocytosis, and since secondary iron deficiency may mask polycythemia, the diagnosis of ET in patients with evidence of iron deficiency (such as

decreased serum ferritin levels) requires the failure of iron therapy to return hemoglobin level to PV range. The WHO criteria for the diagnosis of ET are summarized in Table 3.

When strictly defined by WHO criteria, ET is an indolent disease, with some studies showing comparable survival to age-matched controls. Transformation to acute leukemia is rare, and while the WHO includes a category of post-ET myelofibrosis, the development of marrow fibrosis in a patient with an established diagnosis of ET should also raise the possibility of primary myelofibrosis presenting initially as isolated thrombocytosis (see below).

Primary Myelofibrosis

Primary myelofibrosis (PMF) historically has gone by several different names, including myelofibrosis with myeloid metaplasia (MMM), agnogenic myeloid metaplasia (AMM), and chronic idiopathic myelofibrosis (CIMF). Typical fibrotic-phase PMF is associated with a distinct array of peripheral blood and bone marrow findings. The peripheral blood typically shows leukoerythroblastic changes—that is, the combined presence of nucleated red blood cells and immature neutrophil precursors in the blood. Teardrop-shaped red blood cells (dacryocytes) are also typically seen. These changes have been described as correlating with marrow fibrosis, but may actually be a reflection of the extramedullary hematopoiesis (particularly splenic hematopoiesis) typical of fibrotic phase PMF. Fibrotic phase PMF is marked by the development of reticulin fibrosis, or in more advanced disease a more mature collagen fibrosis displacing the hematopoietic marrow space. The fibrosis is thought to be caused by the secretion of growth factors from the neoplastic megakaryocytes of this disorder. In its prefibrotic phase, PMF is marked by marrow hypercellularity due to proliferation of granulocytic precursors and megakaryocytes, often with very prominent and cohesive megakaryocyte clustering. The presence of megakaryocytes or other marrow precursor cells in bone marrow sinuses (intrasinusoidal hematopoiesis) also may be seen in PMF, and may provide a clue to diagnosis. Splenomegaly is a common clinical sign at presentation.

As is the case with ET, only about 50% of cases of PMF are associated with JAK2 mutation. Another 10% of cases are associated with activating mutations of the thrombopoietin receptor gene MPL. In addition, a number of other, much less prevalent, gene mutations have been described in associated with JAK2-negative PMF.

True fibrotic phase PMF carries a median survival duration of approximately five years. However, a number of patients

present in prefibrotic phase with more prolonged survival duration. The clinical and histopathologic findings of prefibrotic PMF may be subtle and nonspecific. In some cases, patients present with isolated thrombocytosis mimicking ET, but with certain findings atypical for ET such as marked splenomegaly, marked marrow hypercellularity, extensive megakaryocyte proliferation or cohesive or extensive megakaryocyte clustering in the marrow. There has been increased focus in recent years on the distinction between ET and prefibrotic PMF based on these types of findings. A summary of WHO criteria for a diagnosis of PMF is provided in [table 4](#).

PMF may be mimicked by other conditions as well. As noted above, post polycythemic myelofibrosis in patients with established PV may be indistinguishable from PMF except for the known history of antecedent PV. A number of conditions may lead to secondary fibrosis of the bone marrow, not driven by a primary myeloid neoplasm. Such causes include metastatic carcinoma to bone with extensive fibrotic reaction, various bone disorders including hyperparathyroidism, and autoimmune myelofibrosis. Autoimmune-associated fibrosis may be extensive in the marrow, but by definition should be accompanied by the detection of some form of autoantibody on serologic evaluation, and should not be accompanied by the other typical morphologic features of PMF such as intrasinusoidal hematopoiesis or abnormal megakaryocyte clustering in the marrow, or teardrop poikilocytosis in the blood.

Other JAK2-Associated Myeloid Neoplasms

Occasionally, myeloid neoplasms not classifiable as PV, ET, or PMF by WHO criteria may harbor the JAK2 V617F mutation. Chronic myelomonocytic leukemia (CMML), a disorder with features that overlap between myelodysplastic syndromes and myeloproliferative neoplasms, has been reported to harbor JAK2 mutation in a small subset of cases. There have been sporadic reports of JAK2 mutations in certain myelodysplastic syndromes and even in acute myeloid leukemia. A provisional WHO diagnostic entity known as

Table 4. Summary of World Health Organization Diagnostic Criteria for Primary Myelofibrosis (Reproduced from Thiele et al, WHO, 2008). Diagnosis requires all three major criteria and two minor criteria.

Major Criteria	Presence of megakaryocyte proliferation and atypia, usually accompanied by either reticulin and/or collagen fibrosis, OR, in the absence of significant reticulin fibrosis, the megakaryocyte changes must be accompanied by an increased bone marrow cellularity characterized by granulocyte proliferation and often decreased erythropoiesis (i.e. prefibrotic or cellular phase disease).
	Not meeting WHO criteria for polycythemia vera, BCR-ABL1 positive chronic myelogenous leukemia, myelodysplastic syndrome, or other myeloid neoplasms.
	Demonstration of JAK2 V617F or other clonal marker (e.g. MPL W515K/L)
Minor Criteria	Leukoerythroblastosis
	Increased serum lactate dehydrogenase (LDH) level
	Anemia
	Splenomegaly

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“refractory anemia with ring sideroblasts and thrombocytosis (RARS-T)” also has been associated with JAK2 mutation in roughly half of cases (a similar JAK2 mutation prevalence as seen in ET and PMF). RARS-T shows features similar either to ET or cellular phase PMF, but with the distinct finding of ring sideroblasts in the bone marrow. Ring sideroblasts are a light microscopic manifestation of abnormal

JAK2 mutation is generally documented in only about 50% of cases of ET.

iron sequestration within mitochondria of red blood cell precursors, and are often associated with certain myelodysplastic syndromes or toxic/metabolic abnormalities of bone marrow. RARS-T is now considered among the categories of JAK2 mutation-associated myeloid neoplasms.

JAK2 As a Target for Therapy

In 2011, the JAK2 inhibitor ruxolitinib was approved by the FDA for the treatment of intermediate or high risk PMF, as well as post PV myelofibrosis and post ET myelofibrosis. Approval was based upon clinical trials data that showed significant reductions in symptoms and significant reduction in splenomegaly in patients treated with this drug. Since initial approval, a large, double-blind, placebo-controlled clinical trial also demonstrated a statistically significant improvement in median survival for patients treated with ruxolitinib relative to patients treated with placebo. JAK2 inhibitor therapy will no doubt play a key role in the treatment of MPNs going forward.

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Revised Criteria for the Diagnosis of Hepatitis C Infection

In May of this year, the Centers for Disease Control and Prevention (CDC) issued revised guidelines for the diagnosis of hepatitis C virus (HCV) infection, largely in response to the discontinuation of the CHIRON RIBA test, currently the only commercially available recombinant immunoblot assay (RIBA) for supplemental testing following detection of HCV antibodies on initial ELISA screen. The lack of availability of this key supplemental test prompted changes to the CDC's diagnostic algorithm that may affect clients of Warde Medical Laboratory.

If there is clinical suspicion for HCV exposure, repeat testing should be considered since antibodies may not be detected for 12 weeks after exposure.

The previous approach was based on the enhanced specificity of RIBA in determining the presence of antibodies to proteins unique to HCV for those samples that yielded relatively low signal intensity on initial reactive ELISA screens. The new algorithm utilizes nucleic acid testing (NAT) for all positive screens in lieu of a supplemental antibody detection method for low-positive screens. There are advantages and disadvantages to the new approach. An advantage is the “gold standard” status of NAT for the determination of current HCV infection — NAT is extremely sensitive and a positive HCV NAT has extremely high predictive value for current HCV infection in the setting of an initial positive ELISA screen.

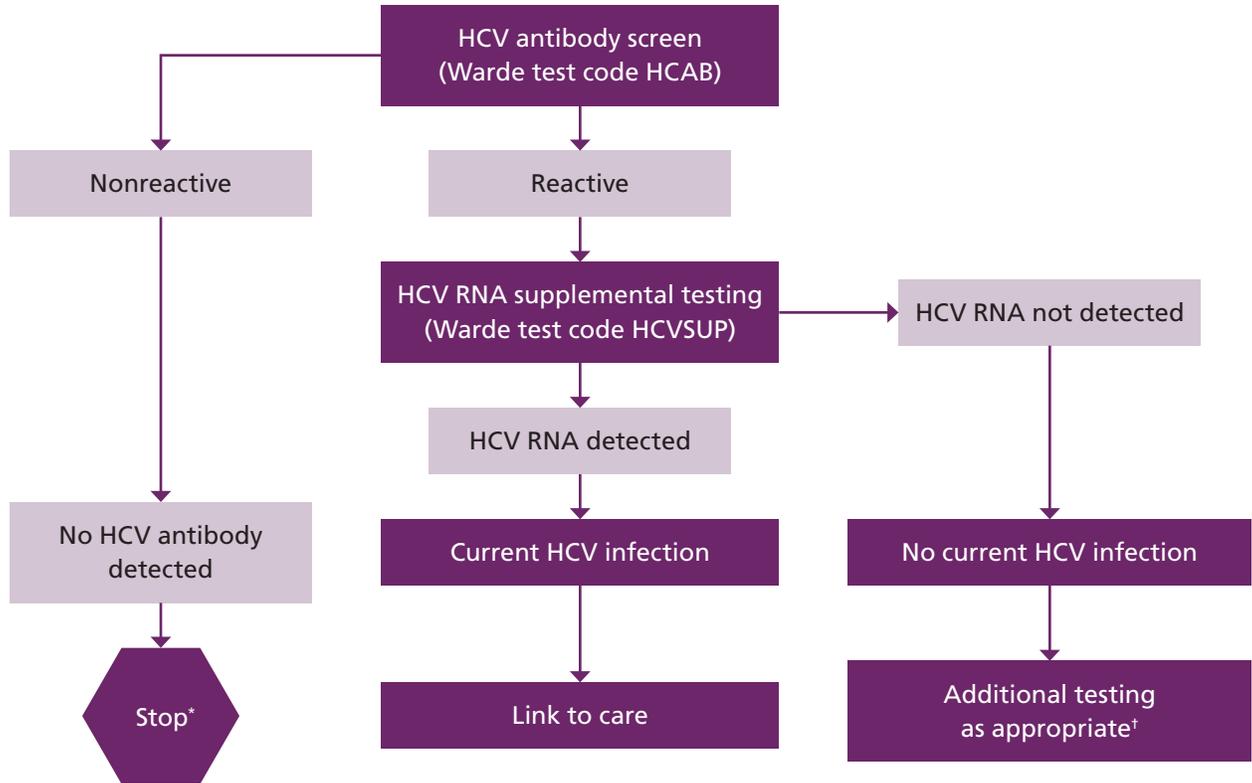
One potential disadvantage is the need to submit a separate sample for NAT, since the extreme sensitivity of NAT (and hence the prevention of sample contamination) precludes the use of a sample that has been tested previously by other methods.

Another potential disadvantage is that antibody screening (by either ELISA or RIBA) and NAT are fundamentally different tests that measure different things. The goal of ELISA and RIBA is to detect host immune response to the infection, whether the infection is active or not. The goal of NAT is to detect the virus itself. In the case of certain other infectious agents (for instance, HIV) these goals are essentially similar, since spontaneous clearance of HIV does not occur, and an untreated patient with active infection will harbor both anti-HIV antibodies and HIV nucleic acid. In the case of HCV, however, approximately 15 to 25% of individuals who are initially infected with the virus will clear it spontaneously (through normal immune mechanisms), but will still harbor anti-HCV antibodies. Hence, it remains difficult to distinguish a false-positive ELISA screen from a situation in which an individual contracted, and then cleared, an HCV infection.

According to the CDC, if there is clinical importance to distinguishing past resolved HCV infection from a biologic false positive HCV antibody screen (i.e. cross-reactivity of naturally occurring patient antibodies to the anti-HCV reagent), then testing with an alternative HCV antibody screening platform may be considered. As always, if there is clinical suspicion for exposure to HCV, whether in the setting of a positive or negative ELISA screen, repeat testing should be considered since antibodies may not be detected for approximately 12 weeks after initial exposure.

The revised algorithm, including Warde test codes, is provided on the next page.

Revised Criteria for the Diagnosis of Hepatitis C Infection



* For persons who might have been exposed to HCV within the past 6 months, testing for HCV RNA or follow-up testing for HCV antibody is recommended. For persons who are immunocompromised, testing for HCV RNA can be considered.

† To differentiate past, resolved HCV infection from biologic false positivity for HCV antibody, testing with another HCV antibody assay can be considered. Repeat HCV RNA testing if the person tested is suspected to have had HCV exposure within the past 6 months or has clinical evidence of HCV disease, or if there is concern regarding the handling or storage of the test specimen.

The Warde Report

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