Genetic Testing in Myeloproliferative Disease

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DESCRIPTION

Myelofibrosis (MF), polycythemia vera (PV), and essential thrombocythemia (ET) are a group of heterogenous disorders of the hematopoietic system collectively knowns as Philadelphia chromosome-negative (BCR-ABL negative) myeloproliferative neoplasms (MPN). The prevalence of MF, ET and PV in the United States is estimated to be approximately 13,000, 134,000 and 148,000, respectively.

MPN are characterized by a complicated symptom profile; the symptoms profile varies within and between each MPN subtype, but often includes constitutional symptoms, fatigue, pruritis, weight loss, symptoms from splenomegaly, and variable lab abnormalities, including erythrocytosis, thrombocytosis, and leukocytosis.

The diagnosis and management of patients with MPN has evolved since the identification of "driver" mutations (JAK2, CALR, and MPL mutations), and the development of

targeted therapies has resulted in significant improvements in disease-related symptoms and quality of life. However, certain aspects of clinical management regarding the diagnosis, assessment of symptom burden, and selection of appropriate symptom-directed therapies continue to present challenges of hematologists and oncologists.

The 2017 WHO diagnostic criteria include molecular testing for JAK2, CALR, and MPL mutations for PMF and ET and molecular testing for JAK2 V617F or JAK2 exon mutations for PV. In the absence of JAK2, CALR, and MPL mutations, the presence of another clonal marker is included as one of the major diagnostic criteria for PMF. Additional mutations in ASXL1, EZH2, TET2, IDH1, IDH2, SRSF2 and SF3B1 genes are noted to be use in determining the clonal nature of the disease.

The diagnosis of myeloproliferative neoplasms (MPN) should be based on the 2017 WHO diagnostic criteria and requires a combination of clinical, laboratory, cytogenetic, and molecular testing. The diagnosis of PMF (primary myelofibrosis) requires meeting all three major criteria and at least one minor criterion as outlined in the revised 2017 WHO criteria. The diagnosis PV (polycythemia vera) requires meeting either all three major criteria or the first two major criteria and the minor criterion, whereas the diagnosis of ET requires meeting all four major criteria or the first three major criteria and the minor criterion as outlined in the revised 2017 WHO criteria. The diagnosis of post PV MF or post-ET MF is based on the 2008 IWG-MRT diagnostic criteria, requiring the documentation of previous diagnosis of PV or ET as defined by the WHO criteria and the development of bone marrow fibrosis of grade 2 to 3 (or 3-4 depending on the scale) and at least two minor criteria.

Janus kinase 2 (JAK2) V617F mutation analysis is laboratory test used to assist in the diagnosis of the following myeloproliferative neoplasms (MPNs): polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF). JAK2 V617F mutations account for the majority of patients with PV (greater than 90%), ET or PMF (60%). Most of the mutations occur in exon 14 with rare insertions/deletions in exon 12.

While the detection of the JAK2 mutation helps support a diagnosis of these MPNs, it does not distinguish between them. Because the mutation is not always present, a negative result does not exclude the diagnosis. The JAK2 exon 12, exon 13, exon 14 and exon 15 tests may be ordered to help diagnose cases in which PV is suspected but are negative for the JAK2 V617F mutation. The JAK2 exon 12, exon 13, exon 14 and exon 15 tests are often automatically initiated in the lab after a negative JAK2 V617F mutation test, if ordered by a physician. This process is referred to as reflex testing. JAK2 exon 12 mutations have been seen in approximately 2-3% of patients with PV

CALR and MPL mutations have been detected in individuals diagnosed with ET or PMF but not in individuals with PV. Typically, these mutations are tested following a negative JAK2 V617F mutation result. CALR mutations are reported in approximately 20-35% of patients with ET and PMF, accounting for approximately 60-80% of patients with JAK2/MPL-negative ET and PMF. CALR deletion mutations are more commonly seen in

patients with PMF and are associated with a significantly higher risk of myelofibrosis transformation in ET. CALR insertion mutations are associated with ET, low risk of thrombosis and an indolent course. CALR mutations are associated with a lower hemoglobin level, lower WBC count, higher platelet count and lower incidence of thrombosis than the JAK2 V617F mutation. MPL mutations have been reported in 5-8% of patients with PMF and 1-4% of patients with ET. MPL mutations are associated with lower hemoglobin levels at diagnosis and increased risk of transfusion dependence in patients with PMF.

Qualitative Versus Quantitative Testing
Some laboratories offer both qualitative and quantitative JAK2 V617F tests:

- A qualitative test expresses the result in terms of the presence (positive) or absence (negative) of a property or condition.
- A quantitative test expresses the results as a number. A quantitative test has been proposed for use in monitoring changes in the number of cells with the JAK2 V617F mutation over time, which is purportedly useful in monitoring treatment effectiveness.

There are various methods to test for the cytogenetics and molecular abnormalities associated with MPN. Tests for the cytogenetic and molecular abnormalities include:

- Bone marrow (BM) cytogenetics: karyotype with or without FISH
- Single gene mutation analysis for JAK2, MPL and CALR
- Panel testing using next generation sequencing for somatic mutations in genes associated with MPN

Clinical Context and Test Purpose
The purpose of JAK2, CALR and MPL testing of individuals with suspected myelofibrosis (MF), polycythemia vera (PV), and essential thrombocythemia (ET) which are a group of heterogenous disorders of the hematopoietic system collectively knowns as Philadelphia chromosome-negative (BCR-ABL negative) myeloproliferative neoplasms (MPN) to establish a molecular genetic diagnosis to inform management decisions.

Populations
The relevant population of interest includes individuals with suspected myeloproliferative neoplasms (MPN).

Interventions
The testing being considered is genetic testing for JAK2, CALR and MPL.

Comparators
The following practice is currently being used to make decisions about treating individuals with a suspected MPN: standard clinical management without genetic testing.

Outcomes

The potential beneficial outcomes of primary interest include establishing a molecular genetic diagnosis of polycythemia vera (PV), essential thrombocythemia (ET), or primary myelofibrosis (PMF) to inform management decisions when test results are provided.

The time frame for outcomes measures varies from several months for the improvement of symptoms to long-term survival as a result of disease-related complications.

Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

The World Health Organization (WHO) 2017 criteria recommends testing for the following when additional criteria are met:

- Pre-primary myelofibrosis (pre-PMF): JAK2, CALR, or MPL mutations
- Overt primary myelofibrosis (PMF): JAK2, CALR, or MPL mutations
- Polycythemia vera (PV): JAK2 V617F or JAK2 exon 12 mutations
- Essential Thrombocythemia (ET): JAK2, CALR, or MPL mutation

Testing for these genetic mutations have potential clinical utility in several different clinical scenarios:

- Diagnosis of patients with clinical, laboratory, or pathologic findings suggesting classic MPNs (PV, ET, or PMF);
- Phenotyping of disease subtypes in patients with MPNs to establish disease prognosis;
- Identification, selection, and monitoring of treatment.

Due to the strong epidemiologic and biologic literature linking JAK2 pathway variants to the occurrence of MPNs, there has been considerable recent attention on using JAK2 as a molecular target for drug discovery. In preclinical and early clinical studies, a number of promising JAK2 inhibitors have been identified, and reports have suggested that some are useful in symptom relief. Many patients with these diseases have good responses to cytotoxic drugs, and the natural course of the disease, particularly for PV and ET, can be quite indolent. Considerable study will be required to sort through the safety and efficacy of these new treatments before they enter routine clinical use. Several early-phase and preliminary treatment trials evaluating the safety and efficacy of tyrosine kinase inhibitors in patients with JAK2 V617F-positive MPNs have been reported. It also has been noted that benefits from tyrosine kinase therapy may not be specific for JAK2 V617F-positive MPNs but may be observed in wild-type disease as well.

In the absence of any of the 3 major genetic variants (JAK2, CALR and MPL) the search for the most frequent accompanying mutations will help determine the clonal nature of the disease. Examples of the most frequent accompanying mutations include:

- ASXL1
- EZH2
- TET2
- IDH1
- IDH2
- SRSF2
- SF3B1

Summary of Evidence

JAK2 Testing for a Suspected Myeloproliferative Neoplasm

Testing for JAK2 V617F or JAK2 exon 12 variants have potential clinical utility in several different clinical scenarios:

- Diagnosis of patients with clinical, laboratory, or pathologic findings suggesting classic MPNs (PV, ET, or PMF).
- Phenotyping of disease subtypes in patients with MPNs to establish disease prognosis.
- Identification, selection, and monitoring of treatment.

For individuals with a suspected MPN who receive genetic testing for JAK2, the evidence includes case series, retrospective studies, meta-analyses, and randomized controlled trials. For patients with suspected Philadelphia chromosome-negative (Ph-negative) MPN, JAK2 variants are found in nearly 100% of those with polycythemia vera, 60% to 65% of those with essential thrombocythemia (ET), and 60% to 65% of those with primary myelofibrosis (PMF). In individuals with suspected MPN, a positive genetic test for JAK2 satisfies a major criterion for the World Health Organization (2017) classification for Ph-negative MPNs and eliminates secondary or reactive causes of erythrocytosis and thrombocythemia from the differential diagnosis. The presence of a documented JAK2 variant may aid in the selection of ruxolitinib, a JAK2 inhibitor; ruxolitinib, however, is classified as second-line therapy. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

MPL Testing for a Suspected Myeloproliferative Neoplasm

Testing for MPL exon 10 variants has potential clinical utility in several different clinical scenarios:

- Diagnosis of patients with clinical, laboratory, or pathologic findings suggesting classic ET or PMF.
- Phenotyping of disease subtypes in patients with ET and PMF to establish disease prognosis.

For individuals with a suspected MPN who receive genetic testing for MPL, the evidence includes case series and retrospective studies. For patients with suspected Ph-negative MPN, MPL variants are found in approximately 5% of those with ET and PMF. In individuals with suspected MPN, a positive genetic test for MPL satisfies a major criterion for the World Health Organization (2017) classification for ET and PMF and eliminates secondary or reactive causes of thrombocythemia from the differential diagnosis. The goal of ET treatment is to alleviate symptoms and minimize thrombotic events and bleeding. While clinical utility may not be established the current WHO classification (2017) for ET and PMF and the current NCCN guideline recommends genetic testing for MPL and is considered medically necessary when criteria is met.

CALR Testing for a Suspected Myeloproliferative Neoplasm
Testing for CALR exon 9 variants has potential clinical utility in several different clinical scenarios:

- Diagnosis of patients with clinical, laboratory, or pathologic findings suggesting classic ET or PMF;
- Phenotyping of disease subtypes in patients with ET and PMF to establish disease prognosis.

The goals of treatment and management for ET are to alleviate symptoms and minimize complications of the disease such as thrombotic events and bleeding.

For individuals with a suspected MPN who receive genetic testing for CALR, the evidence includes case series and retrospective studies. For patients with suspected Ph-negative MPN, CALR variants are found in approximately 20% to 25% of those with ET and PMF. For individuals with suspected MPN, a positive genetic test for CALR satisfies a major criterion for the World Health Organization classification (2017) for ET and PMF and eliminates secondary or reactive causes of thrombocythemia from the differential diagnosis. While clinical utility may not be established the current WHO classification (2017) for ET and PMF and the current NCCN guideline recommends genetic testing for CALR and is considered medically necessary when criteria is met.

Practice Guidelines and Position Statements

National Comprehensive Cancer Network (NCCN)

- **Myeloproliferative Neoplasms Version 3.2022**
  - Suspicion of myeloproliferative neoplasms (MPN): Workup
    - Molecular testing (blood or bone marrow) for JAK2 V617F mutation; if negative, test for CALR and MPL mutations (for patients with ET and MF) and JAK2 exon 12 mutations (for patients with PV) or molecular testing using multigene NGS panel that includes JAK2, CALR, and MPL

2017 WHO Diagnostic Criteria for Primary Myelofibrosis
WHO pre-PMF Criteria:
(Diagnosis of prePMF requires meeting all 3 major criteria, and at least 1 minor criterion)

- **Major criteria**
  - Megakaryocytic proliferation and atypia, without reticulin fibrosis > grade 1, accompanied by increased age-adjusted BM cellularity, granulocytic proliferation, and often decreased erythropoiesis
  - Not meeting WHO criteria for BCR-ABL1 + CML, PV, ET, myelodysplastic syndromes, or other myeloid neoplasms
  - Presence of JAK2, CALR, or MPL mutation or in the absence of these mutations, presence of another clonal marker, or absence of minor reactive BM reticulin fibrosis
- **Minor Criteria**
  - Presence of at least one of the following, confirmed in 2 consecutive determinations:
    - Anemia not attributed to a comorbid condition
    - Leukocytosis $\geq$ 11 x10^9/L
    - Palpable splenomegaly
    - LDH increased to above upper normal limit of institutional reference range

WHO Overt PMF Criteria:
(Diagnosis of overt PMF requires meeting all 3 major criteria, and at least 1 minor criterion)

- **Major Criteria**
  - Presence of megakaryocytic proliferation and atypia, accompanied by either reticulin and/or collagen fibrosis grades 2 or 3
  - Not meeting WHO criteria for ET, PV, BCR-ABL1 + CML, myelodysplastic syndromes, or other myeloid neoplasms
  - Presence of JAK2, CALR, or MPL mutation or in the absence of these mutations, presence of another clonal maker, or absence of reactive myelofibrosis
- **Minor Criteria**
  - Presence of at least one of the following, confirmed in 2 consecutive determinations:
    - Anemia not attributed to a comorbid condition
    - Leukocytosis $\geq$ 11 x10^9/L
    - Palpable splenomegaly
    - LDH increased to above upper normal limit of institutional reference range
    - Leukoerythoblastosis

Grading of Myelofibrosis

- MF-0

- Scattered linear reticulin with no intersections (crossovers) corresponding to normal BM
- MF-1
  - Loose network of reticulin with many intersections, especially in perivascular areas
- MF-2
  - Diffuse and dense increase in reticulin with extensive intersections, occasionally with focal bundles of thick fibers mostly consistent with collagen, and/or focal osteosclerosis*
- MF-3
  - Diffuse and dense increase in reticulin with extensive intersections and course bundles of thick fibers consistent with collagen, usually associated with osteosclerosis*

*In grades MF-2 or MF-3 an additional trichrome stain is recommended

2017 WHO Diagnostic Criteria for Polycythemia Vera and Essential Thromobocytopenia

Polycythemia Vera (PV)
(Diagnosis requires meeting either all 3 major criteria, or the first 2 major criteria and the minor criterion)

- **Major criteria**
  - Hemoglobin > 16.5 g/dL in men, >16.0 g/dL in women; OR
    Hematocrit > 49% in men, > 48% women; OR
    Increased red cell mass (RCM)[1]
  - Bone marrow biopsy showing hypercellularity for age with trilineage growth (panmyelosis) including prominent erythroid, granulocytic, and megakaryocytic proliferation with pleomorphic, mature megakaryocytes (difference in size)
  - Presence of JAK2 V617F or JAK2 exon 12 mutations

- **Minor criteria**
  - Subnormal serum EPO level

  More than 25% above mean normal predicted value.
  Criterion number 2 (BM biopsy) may not be required in cases with sustained absolute erythrocytosis; hemoglobin levels > 18.5 g/dL in men (hematocrit 55.5%) or > 16.5 g/dL in women (hematocrit 49.5%) if major criterion 3 and the minor criterion are present. However, initial myelofibrosis (present in up to 20% of patients) can only be detected by performing a BM biopsy; this finding may predict a more rapid progression to overt myelofibrosis (post-PV MF).

Essential Thrombocythemia (ET)
(Diagnosis requires meeting all 4 major criteria or the first 3 major criteria and the minor criterion)

- **Major criteria**
  - Platelet count $\geq$ 450 x 10^9/L
  - Bone marrow biopsy showing proliferation mainly of the megakaryocyte lineage with increased numbers of enlarged, mature megakaryocytes with hyperlobulated nuclei. No significant increase or left shift in neutrophil granulopoiesis or erythropoiesis and very rarely minor (grade 1) increase in reticulin fibers
  - Not meeting WHO criteria for CML, PV, PMF, myelodysplastic syndromes, or other myeloid neoplasms
  - Presence of JAK2, CALR, or MPL mutation

- **Minor criterion**
  - Presence of a clonal marker or absence of evidence for reactive thrombocytosis

Suspicion of myeloproliferative neoplasms (MPN)
Molecular testing (blood) for JAK2 V617F mutation: if negative, test for CALR and MPL mutations (for patients with ET and MF) and JAK2 exon 12 mutations (for patients with PV) or molecular testing using multigene NGS panel that includes JAK2, CALR and MPL.

Prognostic Significance of Mutations in MPN

Mutated Gene	Essential Thrombocytopenia (ET)
CALR	Lower risk of thrombosis compared to JAK2-mutated ET No difference in overall survival or myelofibrotic or leukemic transformation compared to JAK2-mutated with ET CALR mutation does not modify the IPSET score for predicting thrombosis in patients with ET
TP53	Associated with inferior leukemia-free survival in multivariate analysis
SH2B3/IDH2/ U2AF1/SRSF2/ SF3B1/EZH2/ TP53/RUMX1	The presence of at least 1 of these “adverse variants/mutations” is associated with inferior overall survival (compared to other sequence variants/mutations, or none) independent of age and karyotype

	Adverse variants/mutations also affect myelofibrosis – free survival (U2AF1 and SF3B1) and leukemia-free survival (EZH2 and RUNX1)

Mutated Gene	Polycythemia Vera (PV)
ASXL1/SRSF2/ IDH1/2/RUNX1	The presence of at least 1 of these "adverse variants/mutations" is associated with inferior overall survival (compared to other sequence variants/mutations, or none) independent of age, IWG prognostic model for PV, and karyotype. Adverse variants/mutations also affected myelofibrosis-free survival (ASXL1) and leukemia-free survival (IDH2 and RUNX1
JAK2 exon 12 mutation	Patients with JAK2 exon 12-mutated PV exhibit younger age, increased mean hemoglobin/hematocrit, and lower mean white blood cell and platelet counts at diagnosis compared to those with JAK2 V617F-mutated PV. However, both JAK2 mutations are associated with similar rates of thrombosis, evolution to myelofibrosis or leukemia and death

Mutated Gene	Primary Myelofibrosis (PMF)
Jak2 V617F	Intermediate prognosis and higher risk of thrombosis compared to patients with CALR mutation
MPL W515L/K	Intermediate prognosis and higher risk of thrombosis compared to patients with CALR mutation
CALR	Improved survival compared to JAK2 mutation and "triple-negative" PMF
CALR Type 1/Type 1-like	Improved overall survival (OS) compared to CALR type 2/type 2-like and JAK2 V617F mutation
Triple Negative (non-mutated JAK2, MPL and CALR)	Inferior leukemia-free survival compared to patients with JAK2 and/or CALR-mutated PMF Inferior OS compared to patients with CALR-mutated PMF
ASXL1	Independently associated with inferior OS and leukemia-free Survival as well as lower progression – free survival (PFS) following HCT
EZH2	Independently associated with inferior OS
RAS	Associated with decreased OS
IDH1/2	Independently associated with inferior leukemia-free survival as well as lower PFS following HCT

SRSF2	Independently associated with inferior OS and leukemia-free survival
Combined CALR and ASKL1 status	Survival longest for CALR(+) ASXl1(-) patients (medial 10.4 years) and shortest in CALR(-) ASXL1(+) patients (median 2.3 years) Intermediate survival (median 5.8 years) for CALR(+) ASXL1(+) Or CALR(-) ASXL1(-) patients
TP53	Associated with leukemia transformation
U2AF1 Q157	Inferior OS compared to patients with U2AF1 S34 mutated or U2AF1 unmutated PMF. The effect was most evidence in younger patients
U2AF1 or DNMT3A or CBL	Associated with worse OS in patients with MF undergoing allogeneic HCT

SRSF2 and SF3B1 mutations remain significant predictors in the context of the clinical outcomes

Molecular Abnormalities in MPN
JAK2 V1617F mutations account for the majority of patients with PV (more than 90%) and 60% of patients with ET or MF. The V617F mutation occurs in exon 14; however, rare insertions and deletions have been found in exon 12. JAK2 exon 12 mutations have been described in 2% to 3% of patients with PV.

Activating mutations in the thrombopoietin receptor gene (MPL W515L/K) are reported in approximately 5% to 8% of all patients with MF and 1% to 4% of all patients with ET.

Mutations in exon 9 of the calrecticulin gene (CALR) are reported in approximately 20% to 35% of all patients with ET and MF (accounting for about 60%-80% of patients with JAK2/MPL-negative ET and MF). Type 1 (52 base pair deletions) and type 2 (5 base pair insertions) mutations are the most frequent variants. CALR type 1 mutations are more frequent in patients with MF and CARL type 2 mutations are preferentially associated with ET.

Mutations in several other genes that are involved in signal transduction (CBL and LNK/SH2B3), chromatin modification (TET2, EZh2, IDh1/2, ASXL1, and DNM3TA), RNA splicing (SF3B1, SRSF2, and U2AF1), and tumor suppressor function (TP53) have also been reported in patients with MPN.

Myelofibrosis (MF)
The CALR mutation is associated with better overall survival (OS) than the JAK2 V1617F or MPL W515 mutations and the survival advantage is significant in patients with a type1/type-1 like mutation.

MPL mutations are associated with lower hemoglobin levels at diagnosis and increased risk of transfusion dependence in patients with MF. The "triple-negative" mutation status (lack of all three "driver" mutations JAK2, CALR, or MPL), which occurs in approximately 10% of patients, is associated with a worse prognosis in patients with MF.

ASXL1, EZH2, SRSF2, TP53, IDH1, or IDH2 mutations are considered as "high-molecular-risk" (HMR) mutations are associated with significantly shorter OS and leukemia-free survival (LFS) in patients with PMF. ASXL1, EZH2, SRSF2, and RAS mutations are predictive of OS, while ASXL2, SRSF2, and IDH1 or IDH2 are predictive of leukemic transformation in patients with PMF. TET2 or TP53 mutations have also been associated with a worsened overall prognosis and an increased rate of leukemia transformation. U2AF1 mutations have also been associated with inferior survival in patients with PMF. OS was significantly shorter for patients with PMF. OS was significantly shorter for patients with U2AF1 Q157 mutations, compared to those with U2AF1 S34 mutations or unmutated U2AF1 and the survival effect was most evident in younger patients.

Polycythemia Vera and Essential Thrombocythemia
JAK2 exon 12-mutated PV is characterized by significantly higher hemoglobin level and lower platelet and leukocyte counts at diagnosis compared to JAK2 V617F-mutated PV. However, both JAK2 V617F and JAK2 exon 12 mutations are associated with similar rates of thrombosis, transformation to MF or leukemia, and death. The number of deaths was also significantly higher in patients with JAK2 V617V mutation.

CALR-mutated ET is characterized by younger age, male sex, higher platelet count, lower hemoglobin, lower leukocyte count, and lower risk of thrombosis than JAK2-mutated ET, whereas the presence of MPL mutations might be associated with a higher risk of fibrotic transformation.

CALR mutations have no impact on OS or myelofibrotic or leukemic transformation. CALR mutation status also did not have a significant impact on the International Prognostic Score for ET (IPSET) – thrombosis prognostic score for predicting the risk of thrombosis.

Next-generation sequencing (NGS) has identified adverse variants/mutations in several other genes and may be useful to identify a minority of patients with PV and ET with increased risk of leukemic transformation. In one report, the presence of a least one of the three non-driver mutations (ASXL1, SRSF2. and IDH2) was associated with inferior OS and MF-free survival but it did not significantly affect the LFS in patients with PV. In the multivariable analysis, ASXl1 and SRSF2 retained the prognostic significance for OS and ASXL1 and SRSF2 retained the prognostic significance for OS and ASXL1 was prognostic of MF-free survival. SH2B3, IDH2, U2AF1, SF3B1, EZH2 and TP53 mutations were identified as significant risk factors for inferior OS, for MF-free survival, and in patients with ET. Multivariable analysis confirmed the individual prognostic significance of U2AF1 mutation for OS and MF-free survival and TP53 mutations for

LFS. In a more recent report myelofibrotic transformation was more frequent in patients with SF3B1 and IDH1/2 mutations, although a persistently high or a progressive increase of the JAK2 V617F allele burden while receiving cytoreductive therapy was the strongest predictor of myelofibrotic transformation.

Work-up of Suspected MPN
Molecular testing for JAK2 V617F mutations is recommended as part of initial workup for all patients. If JAK2 V617F mutation testing is negative, molecular testing for MPL and CALR mutations should be performed for patients with MF and ET; molecular testing for the JAK2 exon 12 mutation should be done for those with suspected PV and negative for JAK2 V617F mutation. Alternatively, molecular testing using the multi-gene NGS panel that includes JAK2, CALR, and MPL can be uses a part of initial workup for all patients. The application of an NGS-based 28-gene panel in patients with MPN identified significantly more mutated splicing genes (SF3B1, SRSF2, and U2AF1) in patients with PMF compared to those with ET, and no mutations in splicing genes were found in patients with PV. NGS may also be useful to establish the clonality in selected circumstances (e.g., triple negative MPN with non-mutated JAK2, MPL, CALR). It can also identify second, third and fourth mutations that hold prognostic relevance.

Regulatory Status
Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests must meet the general regulatory standards of the Clinical Laboratory Improvement Amendments. More than a dozen commercial laboratories currently offer a wide variety of diagnostic procedures for JAK2, CALR, and MPL testing under the auspices of the Clinical Laboratory Improvement Amendments. Laboratories that offer laboratory-developed tests must be licensed by the Clinical Laboratory Improvement Amendments for high-complexity testing. To date, the U.S. Food and Drug Administration has chosen not to require any regulatory review of this test.

PRIOR APPROVAL

Not applicable.

POLICY

JAK2 V617F Mutation Analysis
Analysis of JAK2 V617F may be considered **medically necessary** when the following criteria are met:

- The individual meets at least **one** of the following diagnosis criteria for Philadelphia chromosome-negative (BCR-ABL negative) myeloproliferative neoplasms (MPN) (myelofibrosis [pre-primary myelofibrosis (pre-PMF); overt primary myelofibrosis (PMF)], polycythemia vera [PV] or essential thrombocythemia [ET]):
    - Bone marrow biopsy results that are consistent with WHO 2017 diagnostic criteria for pre-PMF, overt PMF, ET, or PV (See Policy Guidelines); **or**

- Platelet count ≥ 450 x 10^9/L; **or**
- Hemoglobin > 16.5 g/dL in men, > 16.0 g/dL in women; **or**
- Hematocrit >49% in men, >48% in women; **or**
- Increased red cell mass (RCM), defined as >25% above the mean normal predicted value; **OR**

- A combination of two of the following:
  - Anemia not attributed to a comorbid condition; **or**
  - Leukocytosis ≥ 11 x 10^9/L; **or**
  - Palpable splenomegaly; **or**
  - LDH increased to above upper normal limit of institutional reference range; **or**
  - Leukoerythoblastosis; **OR**

- Philadelphia chromosome-negative (BCR-ABL negative) myeloproliferative neoplasms (MPN) (myelofibrosis [pre-primary myelofibrosis (pre-PMF); overt primary myelofibrosis (PMF)], polycythemia vera [PV] or essential thrombocythemia [ET]) is being considered in a differential diagnosis with the individual meeting **ALL** of the following:
  - Variable lab abnormalities, including erythrocytosis, **and** thrombocytosis and leukocytosis, which are not otherwise assigned an etiology; **and**
  - Symptoms that include fatigue, pruritis, weight loss and symptoms of splenomegaly.

JAK2 Exon 12 Analysis
Analysis of JAK2 Exon 12 may be considered **medically necessary** when the following criteria are met:

- JAK2 V617F mutation analysis is negative; **and**
- The individual meets at least one of the following diagnostic criteria polycythemia vera (PV):
  - Bone marrow biopsy results that are consistent with WHO 2017 diagnostic criteria for PV (See Policy Guidelines); **or**
  - Hemoglobin > 16.5 g/dL in men, > 16.0 g/dL in women; **or**
  - Hematocrit >49% in men, >48% in women; **or**
  - Increased red cell mass (RCM), defined as >25% above the mean normal predicted value.

CALR Exon 9 and MPL Mutation Analysis
Analysis of CALR Exon 9 and MPL may be considered **medically necessary** when the following criteria are met:

- JAK2 V617F mutation analysis is negative; **and**
- The individual meets at least one of the following diagnostic criteria for essential thrombocythemia (ET) or primary myelofibrosis (PMF):

- Bone marrow biopsy results that are consistent with WHO 2017 diagnostic criteria for pre-primary myelofibrosis (pre-PMF), overt primary myelofibrosis (PMF) or essential thrombocythemia (ET) (See Policy Guidelines); **or**
- Platelet count ≥ 450 x 10^9/L; **or**
- A combination of two of the following:
    - Anemia not attributed to a comorbid condition; **or**
    - Leukocytosis ≥ 11 x 10^9/L; **or**
    - Palpable splenomegaly; **or**
    - LDH increased to above upper normal limit of institutional reference range; **or**
    - Leukoerythoblastosis.

Analysis of ASXL1, EZH2, TET2, IDH1/IDH2, SRSF2, and SF3B1 may be considered **medically necessary** for the diagnosis of primary myelofibrosis (PMF) when **ALL** of the following criteria are met:

- JAK2, CALR and MPL mutation analysis was previously completed and all negative; **and**
- The individual meets at least **one** of the following diagnoses for primary myelofibrosis (PMF):
    - Bone marrow biopsy results that are consistent with WHO 2017 diagnostic criteria for pre-primary myelofibrosis (prePMF) or overt primary myelofibrosis (PMF) (*See Policy Guidelines*); **or**
    - A combination of two of the following symptoms:
        - Anemia not attributed to a comorbid condition; **or**
        - Leukocytosis ≥ 11 x 10^9/L; **or**
        - Palpable splenomegaly; **or**
        - LDH increased to above upper normal limit of institutional reference range; **or**
        - Leukoerythoblastosis.

Analysis of JAK2 V617F, JAK2 Exon 12, CALR Exon 9, MPL ASXL1, EZH2, TET2, IDH1/IDH2, SRSF2, and SF3B1 are considered **investigational** when the above criteria is not met because the evidence is insufficient to determine the effects of the technology on net health outcomes.

Policy Guidelines

2017 World Health Organization (WHO) Diagnostic Criteria

- **Essential Thrombocythemia (ET)**

Essential Thrombocythemia (ET)
Diagnosis requires meeting all 4 major criteria or the first 3 major criteria and the minor criterion
Major criteria:

• Platelet count $\geq$ 450 x 10^9/L • Bone marrow biopsy showing proliferation mainly of the megakaryocyte lineage with increased numbers of enlarged, mature megakaryocytes with hyperlobulated nuclei. No significant increase or left shift in neutrophil granulopoiesis or erythropoiesis and very rarely minor (grade 1) increase in reticulin fibers • Not meeting WHO criteria for CML, PV, PMF, myelodysplastic syndromes, or other myeloid neoplasms • Presence of JAK2, CALR, or MPL mutation
Minor criteria: • Presence of a clonal marker or absence of evidence for reactive thrombocytosis

- **Polycythemia Vera**

Polycythemia Vera
Diagnosis requires meeting either all 3 major criteria, or the first 2 major criteria and the minor criterion[2]
Major criteria: • Hemoglobin > 16.5 g/dL in men, >16.0 g/dL in women; **OR** Hematocrit > 49% in men, > 48% women; **OR** Increased red cell mass (RCM)[1] • Bone marrow biopsy showing hypercellularity for age with trilineage growth (panmyelosis) including prominent erythroid, granulocytic, and megakaryocytic proliferation with pleomorphic, mature megakaryocytes (difference in size) • Presence of JAK2 V617F or JAK2 exon 12 mutations More than 25% above mean normal predicted value. Criterion number 2 (BM biopsy) may not be required in cases with sustained absolute erythrocytosis; hemoglobin levels > 18.5 g/dL in men (hematocrit 55.5%) or > 16.5 g/dL in women (hematocrit 49.5%) if major criterion 3 and the minor criterion are present. However, initial myelofibrosis (present in up to 20% of patients) can only be detected by performing a BM biopsy; this finding may predict a more rapid progression to overt myelofibrosis (post-PV MF).
Minor criteria: • Subnormal serum EPO level

- **Primary Myelofibrosis**

Pre-Primary Myelofibrosis (prePMF)	**Overt Primary Myelofibrosis**
Diagnosis of prePMF requires meeting all 3 major criteria, and at least 1 minor criterion	**Diagnosis of overt PMF requires meeting all 3 major criteria, and at least 1 minor criterion**

Major criteria: • Megakaryocytic proliferation and atypia, without reticulin fibrosis > grade 1, accompanied by increased age-adjusted BM cellularity, granulocytic proliferation, and often decreased erythropoiesis • Not meeting WHO criteria for BCR-ABL1 + CML, PV, ET, myelodysplastic syndromes, or other myeloid neoplasms • Presence of JAK2, CALR, or MPL mutation of in the absence of these mutations, presence of another clonal marker, or absence of minor reactive BM reticulin fibrosis	Major criteria: • Presence of megakaryocytic proliferation and atypia, accompanied by either reticulin and/or collagen fibrosis grades 2 or 3 • Not meeting WHO criteria for ET, PV, BCR-ABL1 + CML, myelodysplastic syndromes, or other myeloid neoplasms • Presence of JAK2, CALR, or MPL mutation or in the absence of these mutations, presence of another clonal maker, or absence of reactive myelofibrosis
Minor criteria: • Presence of at least one of the following, confirmed in 2 consecutive determinations: ▪ Anemia not attributed to a comorbid condition ▪ Leukocytosis $\geq$ 11 x10^9/L ▪ Palpable splenomegaly ▪ LDH increased to above upper normal limit of institutional reference range	**Minor criteria:** • Presence of at least one of the following, confirmed in 2 consecutive determinations: ▪ Anemia not attributed to a comorbid condition ▪ Leukocytosis $\geq$ 11 x10^9/L ▪ Palpable splenomegaly ▪ LDH increased to above upper normal limit of institutional reference range ▪ Leukoerythoblastosis

Grading of Myelofibrosis

- MF-0
  - Scattered linear reticulin with no intersections (crossovers) corresponding to normal BM
- MF-1
  - Loose network of reticulin with many intersections, especially in perivascular areas
- MF-2

    - Diffuse and dense increase in reticulin with extensive intersections, occasionally with focal bundles of thick fibers mostly consistent with collagen, and/or focal osteosclerosis
- MF-3
    - Diffuse and dense increase in reticulin with extensive intersections and course bundles of thick fibers consistent with collagen, usually associated with osteosclerosis

PROCEDURE CODES AND BILLING GUIDELINES

To report provider services, use appropriate CPT* codes, Alpha Numeric (HCPCS level 2) codes, Revenue codes, and/or ICD diagnosis codes.

- 81120 IDH1 (isocitrate dehydrogenase 1 [NADP+], soluble) (e.g., glioma), common variants (e.g., R132H, R132C)
- 81121 IDH2 (isocitrate dehydrogenase 2 [NADP+], mitochondrial) (e.g., glioma), common variants (e.g., R140W, R172M)
- 81219 CALR (calreticulin) (e.g., myeloproliferative disorders), gene analysis, common variants in exon 9
- 81175 ASXL1 (additional sex combs like 1, transcriptional regulator) (e.g., myelodysplastic syndrome, myeloproliferative neoplasms, chronic myelomonocytic leukemia), gene analysis; full gene sequence
- 81176 ASXL1 (additional sex combs like 1, transcriptional regulator) (e.g., myelodysplastic syndrome, myeloproliferative neoplasms, chronic myelomonocytic leukemia), gene analysis; targeted sequence analysis (e.g., exon 12)
- 81236 EZH2 (enhancer of zeste 2 polycomb repressive complex 2 subunit) (e.g., myelodysplastic syndrome, myeloproliferative neoplasms) gene analysis, full gene sequence
- 81237 EZH2 (enhancer of zeste 2 polycomb repressive complex 2 subunit) (eg, diffuse large B-cell lymphoma) gene analysis, common variant(s) (e.g., codon 646)
- 81270 Jak2 (Janus Kinase 2) (e.g., myeloproliferative disorder) gene analysis, p.val617phe (v617f) variant
- 81279 JAK2 (Janus kinase 2)(e.g., myeloproliferative disorder) targeted sequence analysis (e.g., exons 12 and 13)
- 81338 MPL (MPL proto-oncogene, thrombopoietin receptor) (e.g., myeloproliferative disorder) gene analysis; common variants (e.g., W515A, W515K, W515L, W515R)
- 81339 MPL (MPL proto-oncogene, thrombopoietin receptor) (e.g., myeloproliferative disorder) gene analysis; sequence analysis, exon 100017U Oncology (hematolymphoid neoplasia), JAK2 mutation, DNA, PCR amplification of exons 12-14 and sequence analysis, blood or bone marrow, report of JAK2 mutation not detected or detected

- 81403 Molecular Pathology Procedure Level 4 (Used for JAK2 exons12, 14, and 15)
- 81479 Unlisted molecular pathology procedure
- 0017U Oncology (hematolymphoid neoplasia), JAK2 mutation, DNA, PCR amplification of exons 12-14 and sequence analysis, blood or bone marrow, report of JAK2 mutation not detected or detected
- 0027U Jak2 (Janus Kinase 2) gene analysis, targeted sequence analysis exons 12-15

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Add and updated references

POLICY HISTORY

Date	Reason	Action
August 2022	Annual Review	Policy Renewed
August 2021	Annual Review	Policy Revised
November 2020	Interim Review	Policy Revised
August 2020	Annual Review	Policy Revised
August 2019	Annual Review	Policy Revised
August 2018	Annual Review	Policy Revised
August 2017	Annual Review	Policy Revised
August 2016		New Policy

New information or technology that would be relevant for Wellmark to consider when this policy is next reviewed may be submitted to:

Wellmark Blue Cross and Blue Shield
Medical Policy Analyst
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