

Classic *BCR-ABL1*-negative Myeloproliferative Neoplasms

Indications for Ordering

Diagnose and manage classic *BCR-ABL1*-negative myeloproliferative neoplasms (MPNs)

- Polycythemia vera (PV)
- Essential thrombocythemia (ET)
- Primary myelofibrosis (PMF)
- Other myeloproliferative disorders

Test Descriptions

JAK2 Gene, V617F Mutation, Qualitative

- Polymerase chain reaction (PCR)

JAK2 Gene, V617F Mutation, Quantitative

- PCR
- Mutant-to-wild type allele ratio is reported

CALR (Calreticulin) Exon 9 Mutation Analysis by PCR

- PCR/capillary electrophoresis

Myeloid Malignancies Mutation Panel by Next Generation Sequencing

- Massively parallel sequencing

JAK2 Exon 12 Mutation Analysis by PCR

- Allele-specific PCR amplification with subsequent sequencing of positive products to detect single and multiple base substitutions within exon 12 of the *JAK2* gene

MPL codon 515 Mutation Detection by Pyrosequencing, Quantitative

- PCR/pyrosequencing
- Wild type-to-mutant allele ratio is reported

Myeloproliferative Disorders Panel by FISH

- Fluorescence in situ hybridization

Tests to Consider

[JAK2 Gene, V617F Mutation, Qualitative 0051245](#)

- Reasonable screening test for routine evaluation of
 - Unexplained erythrocytosis
 - Thrombocytosis
 - *BCR-ABL1*-negative granulocytosis
 - Bone marrow (BM) fibrosis
- Appropriate screen in patients with symptoms characteristic for *BCR-ABL1*-negative MPN
 - Unusual thrombotic events (eg, splanchnic vein thrombosis, Budd-Chiari)
 - Aquagenic pruritus
 - Unexplained splenomegaly

[JAK2 Gene, V617F Mutation, Quantitative 0040168](#)

- May be useful in assessment of residual disease after stem cell transplant
- Enhances diagnostic certainty in cases with low mutant allelic burden

[CALR \(Calreticulin\) Exon 9 Mutation Analysis by PCR 2010673](#)

- Use for diagnostic and prognostic information in patients with myeloproliferative neoplasms (MPNs) when *JAK2* testing is negative

[Myeloid Malignancies Mutation Panel by Next Generation Sequencing 2011117](#)

- Assess for single gene mutations, including substitutions and insertions and deletions that may have diagnostic, prognostic, and/or therapeutic significance

[JAK2 Exon 12 Mutation Analysis by PCR 2002357](#)

- Use when MPN suspected and *JAK2* V617F testing is negative

[MPL Mutation Detection by Capillary Electrophoresis 2005545](#)

- May be useful when essential thrombocythemia or idiopathic myelofibrosis is suspected in *JAK2* V617F-negative individuals

[JAK2 Gene, V617F Mutation, Qualitative with Reflex to JAK2 Exon 12 Mutation Analysis by PCR 2012085](#)

- Use when diagnosis of PV is suspected

[JAK2 Gene, V617F Mutation, Qualitative with Reflex to CALR \(Calreticulin\) Exon 9 Mutation Analysis by PCR with Reflex to MPL codon 515 Mutation Detection 2012084](#)

- Use when diagnosis of ET or PMF is suspected

[Myeloproliferative Disorders Panel by FISH 2002360](#)

- Detect specific recurrent genomic aberrations in suspected MPNs
 - *BCR/ABL1*
 - *PDGFRA*
 - *PDGFRB*
 - *FGFR1*

Disease Overview

Incidence – 5/100,000 worldwide for combined PV, ET, and PMF

PV

- Must exclude secondary causes of erythrocytosis
- **WHO diagnostic criteria** (2008) – must meet both major and 1 minor criteria or the first major and 2 minor criteria
- **Major criteria**
 - Evidence of increased RBC volume, including greater than or equal to one of the following
 - Hgb >18.5 g/dL (men), >16.5 g/dL (women)
 - Hgb or Hct >99th percentile of reference
 - Red cell mass >25% above mean predicted
 - Hgb >17 g/dL (men), Hgb >15 g/dL (women) if associated with sustained increase ≥ 2 g/dL not attributed to correction of iron deficiency anemia
 - Presence of *JAK2* (V617F) or *JAK2* exon 12 mutations
- **Minor criteria**
 - BM trilineage proliferation
 - Subnormal serum erythropoietin level
 - Endogenous erythroid colony (EEC) growth in vitro

ET

- Must exclude secondary causes of thrombocytosis
- **WHO diagnostic criteria** (2008) – must meet all four criteria
 - Sustained platelet count $\geq 450 \times 10^9/L$ before treatment
 - BM proliferation of enlarged, mature megakaryocytes
 - Does not meet WHO criteria for *BCR-ABL1*-positive CML, PV, PMF, MDS, or myeloid malignancy
 - Demonstration of *JAK2* (V617F), *MPL* gene mutations (eg, W515L and W515K), or no evidence of reactive thrombocytosis

PMF

- **WHO diagnostic criteria** (2008) – must meet all three major and two minor criteria
- **Major criteria**
 - Megakaryocyte proliferation with atypia in association with reticulin and/or collagen fibrosis **or**, in the absence of reticulin fibrosis, an increase in BM cellularity associated with granulocyte proliferation and often decreased erythrocyte proliferation
 - Does not meet the WHO criteria for *BCR-ABL1*-positive CML, PV, ET, MDS, or myeloid malignancy
 - Presence of *JAK2* V617F or other clonal marker **or** no evidence of reactive marrow fibrosis
- **Minor criteria**
 - Leukoerythroblastosis
 - Increased serum LDH
 - Anemia
 - Palpable splenomegaly

Genetics

Gene – *JAK2*

Structure/function

- Located on 9p24
- Nonreceptor tyrosine kinase involved in cytokine receptor signaling

Mutations

JAK2 V617F mutation

- Present in classic MPNs
 - PV – 90-95%
 - ET – ~50%
 - PMF – 50%
- Mutations rarely found in non-MPNs such as AML, MDS
- Mutation is common in the rare MDS/MPN subtype RARS-T (refractory anemia with ring sideroblasts and thrombocytosis)

JAK2 exon 12 mutation

- Present in the majority of patients with PV who are *JAK2* V617F negative
- Rarely occurs in PMF, ET

Gene – *CALR*

Structure/function

Chromosome 19p13.3-13.2

- *CALR* encodes a calcium-binding chaperone protein involved in glycoprotein folding and calcium homeostasis

Mutations

Exon 9 mutations

- A variety of insertions/deletions (indels) have been identified
- Net result of the exon 9 indels is a frame shift

Prevalence

- Essential thrombocythemia (ET) – 67% of *JAK2* and *MPL* negative cases (Nangalia, 2013)
- Primary myelofibrosis (PMF) – 88% of *JAK2* and *MPL* negative cases

Diagnostic issues

- *CALR* somatic mutations are seen in a subset of patients with MPNs who lack *JAK2* and *MPL* mutations (Nangalia, 2013)
- *CALR* mutations appear restricted to patients with ET and PMF and are not found in patients with PV (Klampfl, 2013; Nangalia, 2013)

Prognostic issues

- *CALR* mutated ET and PMF are associated with increased overall survival when compared with *JAK2* and *MPL* mutated ET/PMF (Rotunno, 2013)
- *CALR* mutated ET is associated with a decreased incidence of thrombosis when compared to *JAK2* mutated ET (Rotunno, 2013)

Gene – *MPL*

Structure/function

- Located on 1p34
- Thrombopoietin receptor; mutation causes downstream signaling and contributes to megakaryocytic myeloproliferation

Mutations

Several acquired mutations in exon 10 have been reported

- W515L and W515K – most common
 - ET – 3-4%
 - PMF – 8-10%
 - PV – rare
- W515A and S505N – rare

Test Interpretation

JAK2 Gene, V617F Mutation, Qualitative

Clinical sensitivity

- PV – 95%
- ET – 50%
- PMF – 50%

Results

- Positive – meets a diagnostic criterion for *JAK2* MPN
- Not detected – no evidence of the *JAK2* V617F point mutation

Limitations

- Only one point mutation is detected
- Limit of detection is 0.5% mutant allele

JAK2 Gene, V617F Mutation, Quantitative

Clinical sensitivity

- PV – 95%
- ET – 50%
- PMF – 50%

Results

- The percentage of *JAK2* alleles positive for the V617F mutation (allele burden) is reported
 - 0% – no evidence of the *JAK2* V617F mutation
- Lower *JAK2* V617F allele burden in PMF is associated with worse outcome

Limitations

- Only the one point mutation is detected
- Limit of detection is 0.2% mutant allele

CALR (Calreticulin) Exon 9 Mutation Analysis by PCR

Results

- Positive – a mutation in *CALR* exon 9 is detected
 - Highly suggestive of MPN in conjunction with appropriate clinical and histological findings
- Negative – a mutation in *CALR* exon 9 is not detected

Limitations

- Detects only exon 9 indel mutations and does not detect mutations in other regions of the *CALR* gene
- Results must be interpreted in the context of morphological and other relevant data
- Test should not be used alone to diagnose malignancy

JAK2 Exon 12 Mutation Analysis by PCR

Clinical sensitivity

- PV – 2%

Results

- Positive – meets a diagnostic criterion for *JAK2* MPN
- Not detected – no evidence of the *JAK2* exon mutation

Limitations

- Only exon 12 mutations are detected
- Limit of detection is 1/1,000 cells

MPL Mutation Detection by Capillary Electrophoresis

Clinical sensitivity

- ET – 3-4%
- PMF – 8-10%
- PV – very rare

Results

- Detected
 - Meets one diagnostic criterion for *JAK2* MPN
 - *MPL* mutations present (W515K, W515L, W515A, and S505N)
 - Mutant allele burden is reported
- Not detected
 - *MPL* codon 515 mutation not detected

Limitations

- Does not detect mutations in other locations within the *MPL* gene
- Limit of detection for this test is 5% mutant allele

References

- Nangalia J, Massie CE, et al. Somatic *CALR* mutations in myeloproliferative neoplasms with nonmutated *JAK2*. *N Engl J Med*. 2013 Dec 19;369(25):2391-2405
- Klampfl T, Gisslinger H, et al. Somatic mutations of calreticulin in myeloproliferative neoplasms. *N Engl J Med*. 2013 Dec 19;369(25):2379-2390
- Rotunno G, Mannarelli C, et al. Impact of calreticulin mutations on clinical and hematological phenotype and outcome in essential thrombocythemia. *Blood*. 2014 Mar 6;123(10):1552-5