Test Specific Guidelines

BCR-ABL Negative Myeloproliferative Neoplasm Testing

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Introduction

BCR-ABL negative myeloproliferative neoplasm (MPN) testing is addressed by this guideline.

Procedures Addressed

The inclusion of any procedure code in this table is provided for informational purposes and is not a guarantee of coverage nor an indication that prior authorization is required.

Procedures addressed by this guideline	Procedure codes
ASXL1 Mutation Analysis	81175
CALR Exon 9 Mutation Analysis	81219
DNMT3A Targeted Mutation Analysis	81403
EZH2 Common Variant(s) (e.g. codon 646)	81237
EZH2 Full Gene Sequencing	81236
IDH1 Mutation Analysis	81120
IDH2 Mutation Analysis	81121
JAK2 Exons 12 to 15 Sequencing	0027U
JAK2 Mutation	0017U
JAK2 Targeted Mutation Analysis (e.g exons 12 and 13)	81279
JAK2 V617F Mutation Analysis	81270
MPL Common Variants (e.g. W515A, W515K, W515L, W515R)	81338
MPL Mutation Analysis, Exon 10	81339
SF3B1 Common Variants (e.g. A672T, E622D, L833F, R625C, R625L)	81347
SRSF2 Common Variants (e.g. P95H, P95L)	81348
TET2 Mutation Analysis	81479
U2AF1 Common Variants (e.g. S34F, S34Y, Q157R, Q157P)	81357

Procedures addressed by this guideline	Procedure codes
Targeted genomic sequence analysis panel, hematolymphoid neoplasm or disorder	81450

What Are BCR-ABL Negative Myeloproliferative Neoplasms?

Definition

Primary myelofibrosis (PMF), polycythemia vera (PV) and essential thrombocythemia (ET) are a group of heterogeneous disorders of the hematopoietic system collectively known as Philadelphia chromosome-negative MPN.

Prevalence

The following table describes the prevalence of Philadelphia chromosome-negative MPNs in the U.S.[1]

Disorder	Prevalence in the U.S.
PMF	13,000
ET	134,000
PV	148,000

Symptoms

Symptoms vary among the subtypes, but generally include

- constitutional symptoms
- fatigue
- pruritus
- weight loss
- symptoms of splenomegaly, and
- variable lab abnormalities, including
  - erythrocytosis
  - thrombocytosis, and
  - leukocytosis.[1]

Risks

Individuals with MPNs are at risk of the condition transforming into acute myeloid leukemia (AML), which is associated with a poor response to therapy and short

survival. These disorders are also associated with an increased risk of major bleeding and thrombosis or thromboembolism compared to the general population.[1]

Diagnosis

The diagnosis and management of patients with MPN has evolved since the identification of mutations that activate the JAK pathway, including JAK2, CALR, and MPL. The development of targeted therapies has resulted in significant improvements in disease-related symptoms and quality of life.[1] In a minority of patients, recurrent mutations in other genes contribute to initiation or progression of disease. These mutations may serve as markers of clonality in cases where mutations in JAK2, MPL or CALR are not detected.[2]

- JAK2 V617F mutations — 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.[1]
- JAK2 exon 12 mutations — JAK2 exon 12 mutations have been seen in approximately 2-3% of patients with PV.[1]
- MPL mutations — 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.[1]
- CALR mutations — 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.[1]

Test Information

Introduction

Testing for BCR-ABL negative MPN may include cytogenetic testing, single gene mutation analysis, or panel testing.

Types of Tests

There are various methods used to test for the cytogenetic and molecular abnormalities associated with MPN.[1,3] 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, and
- panel testing using next generation sequencing for somatic mutations in genes associated with MPN.

Guidelines and Evidence

Introduction

This section includes relevant guidelines and evidence pertaining to BCR-ABL negative MPN testing.

National Comprehensive Cancer Network

The National Comprehensive Cancer Network (NCCN, 2021) evidence and consensus-based guidelines recommended the following initial laboratory evaluations for individuals suspected to have MPN:[1]

- "Laboratory evaluations should include complete blood count (CBC), microscopic examination of the peripheral smear, comprehensive metabolic panel with serum uric acid, serum LDH, liver function tests, serum EPO level and serum iron studies."
- "Fluorescence in situ hybridization (FISH) or a multiplex reverse transcriptase polymerase chain reaction (RT-PCR) on a peripheral blood specimen to detect BCR-ABL1 transcripts and exclude the diagnosis of CML is especially recommended for patients with left-shifted leukocytosis and/or thrombocytosis with basophilia."
- "Molecular testing for JAK2 V617F mutations is recommended as part of the 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 exon12 mutation should be done for those with suspected PV and negative for the JAK2 V617F mutation."
- "Alternatively, molecular testing using the multi-gene NGS panel that includes JAK2, CALR, and MPL can be used as 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."
- "Bone marrow aspirate with iron stain and biopsy with trichrome and reticulin stain and bone marrow cytogenetics (karyotype, with or without FISH; blood, if bone marrow is inaspirable) are necessary to accurately distinguish the bone

marrow morphological features between the disease subtypes (early or prefibrotic PMF, ET and masked PV)."

World Health Organization: PMF

The World Health Organization (WHO, 2016) established diagnostic criteria for PMF.[3]

Pre Primary Myelofibrosis (prePMF) [Diagnosis requires meeting all 3 major criteria, and at least 1 minor criterion]	Overt Primary Myelofibrosis (overt PMF) [Diagnosis 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	Major criteria: • Megakaryocytic proliferation and atypia, accompanied by either reticulin and/or collagen fibrosis grades 2 or 3 • 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 reactive BM myelofibrosis

Pre Primary Myelofibrosis (prePMF)	Overt Primary Myelofibrosis (overt PMF)
[Diagnosis requires meeting all 3 major criteria, and at least 1 minor criterion]	[Diagnosis requires meeting all 3 major criteria, and at least 1 minor criterion]
Minor criteria: Presence of at least one of the following, confirmed in 2 consecutive determinations: • Anemia not attributed to a comorbid condition • Leukocytosis ≥ 11 x 10^9/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 ≥ 11 x 10^9/L • Palpable splenomegaly • LDH increased to above upper normal limit of institutional reference range • Leukoerythroblastosis

Absence of 3 major clonal mutations

In the absence of any of the 3 major clonal mutations, the search for the most frequent accompanying mutations help determine the clonal nature of the disease.[2] Examples of the most frequent accompanying mutations include:

- ASXL1
- DNMT3A
- EZH2
- TET2
- IDH1
- IDH2
- SRSF2
- SF3B1

World Health Organization: PV

The World Health Organization (WHO, 2016) established diagnostic criteria for PV.[3]

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% in women OR Increased red cell mass (RCM), defined as >25% above the mean normal predicted value • 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 mutation Minor criteria: • Subnormal serum EPO level

Bone marrow biopsy not required in some cases

A bone marrow 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 3 major criterion 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 PMF).

World Health Organization: ET

The World Health Organization (WHO, 2016) established diagnostic criteria for ET.[3]

Essential Thrombocythemia (ET)
[Diagnosis requires meeting all 4 major criteria or the first 3 major criteria and the minor criterion]
Major criteria: • Platelet count ≥ 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 BCR-ABL1+ 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

Criteria

Introduction

Requests for testing for BCR-ABL negative MPN are reviewed using these criteria.

JAK2 V617F Mutation Analysis

- Member does not meet WHO criteria for BCR-ABL1+ CML, myelodysplastic syndromes, or other myeloid neoplasms, AND
- Member meets at least ONE of the following diagnostic criteria for MPN:
  - Bone marrow biopsy results that are consistent with WHO diagnostic criteria for prePMF, overt PMF, ET, or PV, 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 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
- Leukoerythroblastosis, OR

- MPN is being considered in the differential diagnosis with the member meeting both of the following:
  - Variable lab abnormalities, including erythrocytosis, thrombocytosis and leukocytosis, which are not otherwise assigned an etiology, and
  - Constitutional symptoms, including fatigue, pruritus, weight loss and symptoms of splenomegaly, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

JAK2 Exon 12 Analysis

- Member does not meet WHO criteria for BCR-ABL1+ CML, myelodysplastic syndromes, or other myeloid neoplasms, AND
- JAK2 V617F mutation analysis is negative, AND
- Member meets at least ONE of the following diagnostic criteria for PV:
  - Bone marrow biopsy results that are consistent with WHO diagnostic criteria for PV, 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, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

CALR Exon 9 and MPL Mutation Analysis

- Member does not meet WHO criteria for BCR-ABL1+ CML, PV, myelodysplastic syndromes, or other myeloid neoplasms, AND
- JAK2 V617F mutation analysis is negative, AND
- Member meets at least ONE of the following diagnostic criteria for ET or PMF:
  - Bone marrow biopsy results that are consistent with WHO diagnostic criteria for prePMF, overt PMF, or ET, or

- Platelet count ≥ 450 x 10^9/L, 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
  - Leukoerythroblastosis, AND

- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Analysis of ASXL1, DNMT3A, EZH2, TET2, IDH1, IDH2, SRSF2, And/or SF3B1

- Member does not meet WHO criteria for BCR-ABL1+ CML, PV, ET, myelodysplastic syndromes, or other myeloid neoplasms, AND
- JAK2, CALR, and MPL mutation analyses are all negative, AND
- Member meets at least ONE of the following diagnostic criteria for PMF:
  - Bone marrow biopsy results that are consistent with WHO diagnostic criteria for prePMF or overt PMF, 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
    - Leukoerythroblastosis, AND

- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Billing and Reimbursement Considerations

If requested, gene panels that include the following genes will be eligible for reimbursement according to the criteria outlined in this guideline: ASXL1, DNMT3A, EZH2, TET2, IDH1, IDH2, SRSF2, and SF3B1. This sequencing panel will only be considered for reimbursement when billed with the appropriate panel CPT code: 81450.

References

1. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology: Myeloproliferative Neoplasms. V.1.2020. Available at: https://www.nccn.org/professionals/physician_gls/pdf/mpn.pdf
2. Vainchenker W, Kralovics R. Genetic basis and molecular pathophysiology of classical myeloproliferative neoplasms. *Blood.* 2017;Feb 9;129(6):667-679.