# Panel-Based Molecular Profiling Finds CALR Mutation in Patient with Persistent MPN and History of JAK2 Mutation

Case reviewed by Frank Bauer, MD Medical Director, Precipio Inc.


## Abstract

JAK2, CALR, and MPL genes are useful biomarkers for assessing Myeloproliferative neoplasms (MPN) as they can play a role in either disease diagnosis or provide information regarding disease prognosis.(1,2,3,4) Historically, testing for these MPN-related mutations has been conducted in a stage & reflex approach, whereby once a single mutation is found, labs halt further testing. Recent studies indicate that dual mutations exist in a subset of the MPN patient population.(5) Therefore, using a stage and reflex approach can lead to missed identification of additional mutations, which can adversely impact care. This case demonstrates the shortcomings of the clinically-outdated stage & reflex approach commonly utilized by other laboratories, and the benefits of Precipio's HemeScreen panel approach.


## Pathology & Disease Management Background:

### Myeloproliferative neoplasm (MPN):

Essential thrombocythemia (ET) is a myeloproliferative neoplasm characterized by an increased platelet count, megakaryocytic hyperplasia, and a hemorrhagic or microvascular vasospastic tendency. Symptoms and signs may include headache (ocular migraine), paresthesia, bleeding, erythromelalgia, or digital ischemia.

A JAK2 V617F mutation is estimated to occur in 50-60% of ET patients. This mutation can occur rarely in other hematologic malignancies such as MDS, acute myeloid leukemia (AML), MDS/MPN, and in half of the cases of refractory anemia with ringed sideroblasts associated with marked thrombocytosis(6,7).

### Non Janus Kinase 2 mutations:

Since 2016, WHO guidelines recommend that CALR assessments be performed for patients with a history of ET and primary myelofibrosis (PMF) who do not exhibit JAK2 mutations.(7)

Recently, indels in CALR have been described in about 70% of cases of JAK2/MPL wild-type (double-negative) ET and PMF, in a mutually exclusive pattern with JAK2 and MPL mutations. CALR mutations were not found in other myeloid malignancies, except for the myelodysplastic syndrome, refractory anemia with ring sideroblasts and thrombocytosis. Mutations of CALR have been reported primarily in wild-type JAK2 and MPL-related ET. (8,9,10) CALR mutant essential thrombocythemia is associated with relatively younger age, higher platelet counts, lower erythrocyte counts, leukocyte counts, hemoglobin, and hematocrit, and increased risk of progression to myelofibrosis in comparison with JAK2 V617F-positive essential thrombocythemia. (11)

Recent studies have shown that CALR-mutated cases are associated with younger age, higher platelet counts, lower erythrocyte counts, lower leukocyte counts, and lower hemoglobin levels compared with JAK2-mutated cases. In addition, in most of these studies CALR mutations were linked to male gender. (10,11,12,13)

The goal of treatment for ET is to reduce the risk of developing thrombohemorrhagic complications. Use of cytoreductive therapy is based on the risk status determined by patient age, history of thrombosis, and JAK2 V617F mutational status (in patients with ET). Regular monitoring of disease-related symptoms, assessment of need for cytoreductive therapy, and appropriate evaluation to rule out disease progression should be an integral part of management for patients with PV and ET.(14)

## Patient Background:

A 76-year-old African American female patient, known hypertensive, was presented for evaluation. The patient was previously diagnosed with ET, and had a history of JAK2 v617f mutation, but recent testing showed this mutation was no longer present. Prior CBCs revealed the same pattern of cell counts (Table 1), and a limited workup for anemia revealed normal B12, folate, and adequate amounts of iron. In previous assessments, the patient denied experiencing any headaches, abdominal pain, early satiety, easy bruising, arthralgias or arthritis. There were no significant findings in mammogram, Pap smear, or Colonoscopy work ups.

To manage the patient's ET, anagrelide 0.5 mg was prescribed and tolerated by the patient. Platelets were minimally improved during a 1 month follow up visit at 1646 ×109/L down from 1721 ×109/L. Her complete blood count one month later, showed an elevated platelet count of 1438 ×109/L, with mild decreased Hemoglobin level of 10.7 g/dl, and normal white blood cell count with abnormal differential count (Table 2). In the blood smear, scattered large platelets were noted. White blood cells were low-normal in number with essentially normal differential count. Increased (circulating) blasts were not noted.

## Case Work Up & Results:

The case was referred to Precipio's Omnia™ personalized work up for a comprehensive assessment of the patient's molecular status which included HemeScreen® MPN panel testing. Flow

 

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cytometry showed no evidence of T or B cell neoplasia, dysmyelopoiesis or increased blasts, where the granulocytes exhibit a normal pattern of expression of CD11b, CD13, and CD16. The lymphocytes (19%) include 13% polyclonal B-cells, 68% mature T-cells with a normal CD4/CD8 ratio, and 14% natural killer (NK) cells. Moreover, the Fluorescence in situ hybridization (FISH) showed no evidence of an acquired clonal abnormality.

The HemeScreen® MPN panel revealed negative results for JAK2 V617F point mutation, JAK2 exon 12 mutations, JAK2 exon 13 (G571S) mutation, and MPL W515L/K point mutations. However, the analysis showed a positive CALR exon 9 insertion/deletion mutation. The gene encoding calreticulin (CALR) is mutated in the majority (~70-85%) of essential thrombocythemia (ET) and primary myelofibrosis (PMF) cases with non-mutated JAK2. (10,15) CALR mutations have been found to be the second most frequent genetic mutation in myeloproliferative neoplasms (MPN) after Jak2. (10,12) Mutations in CALR have been reported to be mutually exclusive with mutations in both JAK2 and MPL and are not reported in polycythemia vera (PV). (10,13)

Based on the latest clinical guidelines, the hematopathology team utilized a molecular panel-based approach for this patient to go beyond reflexive JAK2 mutation testing and monitoring, thereby identifying a CALR mutation that may otherwise go undetected. The identification of CALR Exon 9 insertion/ deletion mutations in myeloproliferative neoplasms (MPN) may be useful to assist diagnosis, classification, and monitoring. (10,13) The presence of CALR mutations have been associated with more benign clinical courses in comparison to corresponding disorders associated with JAK2 or MPL mutations. (10,15)

**Tables:**

*Table 1 Past Blood Counts*

| | 2013 | 2013 | 2009 |
|---|---|---|---|
| **WBC (x10$^3$/µL)** | 5.6 | 3.8 | 5.1 |
| **HB (g/dL)** | 10.7 | 10.9 | 11.2 |
| **Platelets (x10$^3$/µL)** | 1300 | 1400 | 1300 |

*Table2 Complete Blood Count Results*

| | 2022 |
|---|---|
| **WBC (x10$^3$/µL)** | 4.75 |
| **RBC (x10$^6$/µL)** | 3.33 |
| **HGB (g/dL)** | 10.14 |
| **HCT** | 30.1% |
| **MCV (fL)** | 90.3 |
| **MCH (pg)** | 30.5 |
| **MCHC (g/dL)** | 33.7 |
| **RDW** | 13.8% |
| **PLT (x10$^3$/µL)** | 1433 |
| **MPV (fL)** | 7.82 |
| **LYM** | 27.58% |
| **MON** | 10.76% |
| **NEU** | 60.25% |
| **EOS** | 1.05% |
| **BAS** | 0.36% |