Review

LABORATORY ASPECT OF PRIMARY POLYCYTHEMIA (PV)

Didik Agus Santoso[1,2*]

1) *Faculty of Medicine, University of Darussalam Gontor*
2) *Yasyfin Darussalam Gontor Hospital*

Article history:
Received March 9, 2025
Received in revised form April 19, 2025
Accepted May 5, 2025

KEYWORDS:
Polycythemia Vera,
Erythropoietin (Epo) levels,
JAK2 V617F mutation,

***Corresponding Author:**
da.santoso@yahoo.com

ABSTRACT

Polycythemia or erythrocytosis is an increase in red blood cell mass. There is an increase in the concentration of red blood cells and hemoglobin in the blood. Primary polycythemias are caused by acquired or inherited mutations that cause functional changes in hematopoietic stem cells or erythroid progenitors resulting in accumulation of red blood cells. Common primary polycythemias include: Polycythemia Vera (PV), which is a clonal disorder. The assessment steps for polycythemia require the patient's clinical history and complete blood count (CBC) results, Erythropoietin (Epo) levels to determine the cause of erythrocytosis, and molecular analysis of JAK2 mutations to evaluate Polycythemia vera. Patients with clinical suspicion of congenital erythrocytosis need mutational analysis as a confirmatory test. The development of molecular tests for mutations associated with polycythemia can establish a clearer classification and etiology. The JAK2 V617F mutation is most common in PV (>95%) and is present in the majority of PMF and ET (50% to 60%). Various methods have been advocated for the detection of JAK2 V617F, including high-resolution PCR product melting curve analysis, Sanger sequencing or pyrosequencing, and allele-specific PCR variants (eg, amplification refractory mutation system [ARMS] and RQ-PCR). Of these, the allele-specific PCR approach using DNA as a template reproducibly achieves the highest analytical sensitivity.

INTRODUCTION

Polycythemia or erythrocytosis is an increase in red blood cell mass.[1] There is an increase in the concentration of red blood cells and hemoglobin in the blood, can be caused by an absolute increase in red blood cell mass (absolute erythrocytosis or "polycythemia"), or as a result of a decrease in plasma volume (relative erythrocytosis).[2] Absolute erythrocytosis or polycythemia is either primary or secondary. Primary erythrocytosis is either congenital or acquired. Congenital primary polycythemia is a consequence of mutations in the erythropoietin receptor (Epo R) that confer hypersensitivity to erythropoietin.[2]

Common primary polycythemias include: polycythemia vera (PV), which is a clonal disorder. Other polycythemias are inherited from mutations in the erythropoietin receptor or congenital disorders of hypoxia sensing.[1] PV is most often diagnosed in middle-aged to older individuals but can occasionally be seen in children and young adults.[2] Male patients are more likely to have polycythemia vera.[3] Driver mutations in the JAK2, CALR, and MPL genes are present in 90% of PV, ET, and PMF. The remaining 10% of cases are negative for all three.[4]

Primary Familial and Congenital Polycythemia (PFCP) is a rare autosomal dominant disorder, but is more common than the others, because many patients are initially misdiagnosed as having polycythemia vera. Its prevalence is similar to that of congenital

polycythemia of high oxygen affinity mutant hemoglobin. The prevalence of PFCP is lower when compared with 2,3-bisphosphoglycerate (2,3-BPG) deficiency.[1]

This literature review will discuss the laboratory aspects of primary polycythemia (PV).

METHODS

The method of writing this article uses a literature review approach by analyzing and synthesizing information from various relevant scientific sources. Literature data were collected through searches in trusted databases, including PubMed, ScienceDirect, and NCBI, using the keywords "Polycythemia Vera," "erythrocytosis," "Erythropoietin (Epo) levels," and "JAK2 V617F mutation." The results of the literature were synthesized to identify key findings and research gaps that can serve as a basis for discussion.

LITERATURE REVIEW

Primary polycythemias are caused by acquired or inherited mutations that cause functional changes in hematopoietic stem cells or erythroid progenitors resulting in accumulation of red blood cells. Common primary polycythemias include: polycythemia vera (PV), which is a clonal disorder. Other polycythemias are inherited from mutations in the erythropoietin receptor or congenital disorders of hypoxia sensing.[1]

Etiology of Primary Polycythemia

The most common cause of primary polycythemia is polycythemia vera (PV), an acquired somatic mutation in hematopoietic stem cells that carries the risk of evolving to myelofibrosis and acute leukemia.[2] Acquired absolute erythrocytosis, called Polycythemia vera (PV) is a clonal disorder of stem cells belonging to the group of chronic myeloproliferative neoplasms (MPNs). PV is associated with an increase in all cells of the myeloid series, but its main clinical manifestations are related to erythrocytosis.[2]

It is important to differentiate PV from hereditary causes of polycythemia. Hereditary polycythemia may be inherited in progenitors, but does not carry the risk of clonal evolution associated with PV. The success of PV treatment strategies is better than that of hereditary causes because of a more complete understanding of PV risk and the natural history of the disorder. Clinicopathologic and molecular evaluation of PV should be performed early in the evaluation of polycythemia, especially if serum erythropoietin (EPO) levels are low, because PV is much more common than congenital polycythemia.[2]

Mutations Causing Polycythemia

Polycythemia can result from mutations that cause abnormalities in hemoglobin (high oxygen affinity hemoglobin variants), hemoglobin effector proteins (2,3-biphosphoglycerate [2,3 BPG] deficiency), erythropoietin receptors and oxygen sensing pathway proteins (hypoxia-induced mutations, prolyl hydroxylase domain, and von Hippel-Lindau).[2] Mutations that cause polycythemia are shown in Table 1.

Of the hereditary causes, high oxygen affinity hemoglobin variants are the most common. Most other conditions are much rarer, but their true prevalence remains to be determined by a wider investigation of patients previously classified as idiopathic.[2]

The inherited hemoglobin abnormalities of the high oxygen affinity hemoglobin variants and the increased carboxyhemoglobin in smokers, result in failure to deliver oxygen to the tissues effectively and result in compensatory increased erythropoietin production by the kidneys.[2]

Table 1. Mutations that cause Polycythemia.[2]

Involved Gene	Inheritance	Serum Erythropoietin	P50
JAK2 V617F	Acquired	Decresaed	NL
JAK2 exon 12	Acquired	Decresaed	NL
EPOR	Dominant	Decresaed	NL
PHD2	Dominant	NL or elevated	NL to slightly decreased
BPGM	Dominant	NL	Decreased
β globin	Dominant	NL or elevated	Decreased
α globin	Dominant or recessive	NL or elevated	Decreased
HIF2α	Dominant	NL or elevated	NL
VHL	Recessive	NL or elevated	NL

NL: normal range level

Some patients may have aberrant erythropoietin production by neoplasm. There is a rare form of secondary congenital polycythemia and abnormal HBs. Chuvash polycythemia is caused by germline mutations in the von Hippel–Lindau (VHL) gene (R200W), a gene involved in the regulation of cellular hypoxia-inducible factor alpha (HIF-α) subunits involved in the oxygen-sensing pathway.2 Mutations in HIF1-α result in increased erythropoietin and erythropoietin responsiveness.[2]

The JAK2 V617F kinase mutation plays a central role in acquired myeloproliferative neoplasms (MPNs).[5] Underlying mutations in the JAK2, MPL, and calreticulin (CALR) drivers in 90% of patients with myeloproliferative neoplasms (MPNs), a condition characterized by JAK-STAT (signal transducer and activator of transcription) pathway signaling are now clear.[6]

None of these mutations have been shown to be specific for a disease subtype, and therefore cannot be used in the molecular classification of MPNs. Furthermore, it is unclear whether the same mutation acquired in any of these genes can cause clinical entities with different phenotypes.[6]

Pathogenesis Polycythemia

Myeloproliferative neoplasms are a heterogeneous group of clonal disorders characterized by excess production of mature cells in the peripheral blood, along with an increased risk of thrombosis and progression to acute myeloid leukemia. The majority of patients with Philadelphia chromosome-negative myeloproliferative neoplasms have somatic mutations in Janus kinase 2, leading to its constitutive activation.[7] Myeloproliferative neoplasms (MPNs) are chronic, clonal hematopoietic disorders that include polycythemia vera (PV), essential thrombocytosis (ET), and primary myelofibrosis (PMF). Most transform to acute myeloid leukemia (AML) with a poor prognosis.[4]

Canonical JAK2 signaling relies on the binding of extracellular ligands to membrane receptors that jump to activate downstream pathways, including the phosphorylation of transcription factors Stat3 and Stat5, which trigger translocation from the nucleus.[8]

Binding of extracellular erythropoietin (EPO) to the erythropoietin receptor (Epo R) causes Epo

R dimerization. This in turn activates Janus kinase 2 (JAK2) to auto phosphorylate several sites in the cytoplasmic domain of Epo R. These phosphorylated sites allow binding of transcription factors, including Stat5, resulting in increased red blood cell production. This process is regulated by the binding of SHP1 to the cytoplasmic binding domain of Epo R.

Polycythemia vera is caused by a constitutively activated JAK-STAT pathway from somatic gain-of-function mutations in JAK2. Hereditary polycythemia has been associated with heterozygous germline Epo R mutations that disrupt SHP1 binding to Epo R. Most result in a truncated Epo R protein that results in the loss of the cytoplasmic domain of SHP1 and is located in exon 8.[6]

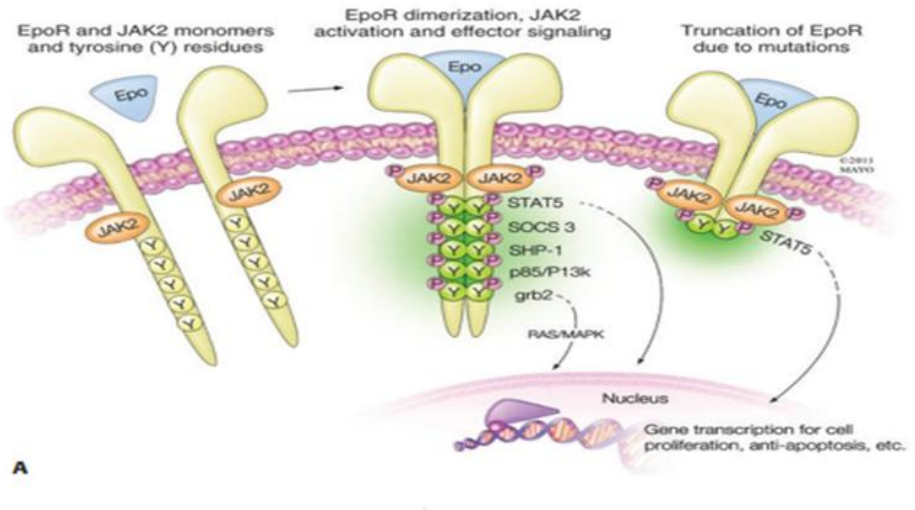

Figure 1. Erythropoietin receptor (EpoR) signaling in the regulation of erythroid cell production.[2]

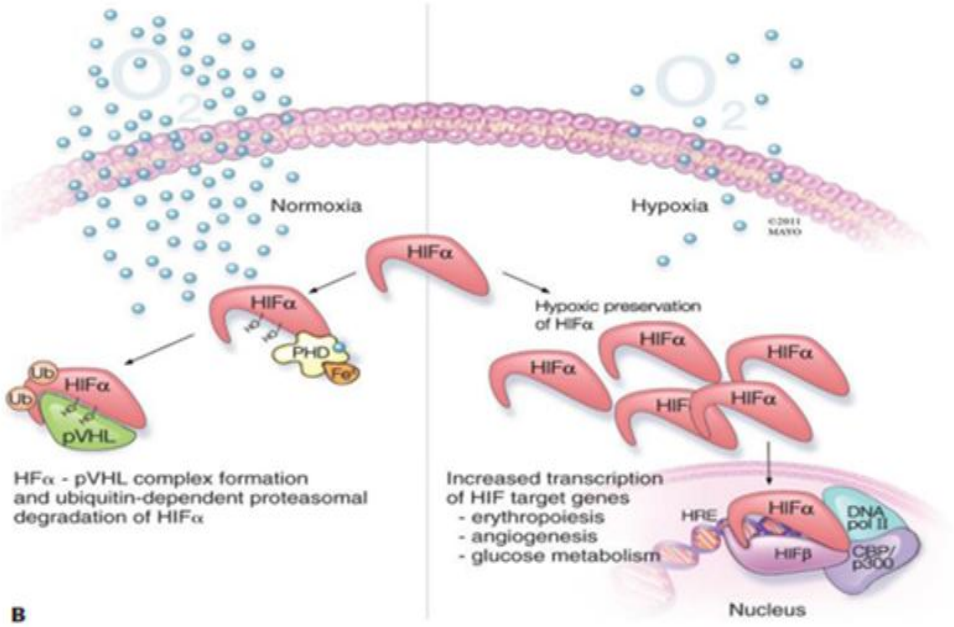

Figure 2. Oxygen-sensing pathway signaling in the regulation of erythroid cell production.[2]

Polycythemia vera (PV)

Philadelphia chromosome-negative myeloproliferative neoplasms (MPNs), including polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF), are a group of clonal disorders of the hematopoietic system characterized by excessive production of myeloid cells.[3,4] Philadelphia chromosome (Ph1)-negative chronic myeloproliferative neoplasms (MPNs) are clonal stem cell disorders characterized by hematopoietic stem/progenitor cell (HSPC) expansion and overproduction of mature blood cells.[5]

Polycythemia vera (PV) is a chronic myeloproliferative neoplasm (MPN) characterized by increased red blood cell (RBC) production, an independent production mechanism that regulates erythropoiesis.[9] Polycythemia vera (PV) is defined as a myeloproliferative neoplasm (MPN) with erythrocytosis and a JAK2 V617F mutation or equivalent clonality markers.[10]

Polycythemia vera (PV) is a myeloproliferative neoplasm (MPN) in which there is increased hematopoiesis is predominantly of erythroid lineage. The diagnosis of PV has been simplified by JAK2 mutations, affecting the canonical V617 in exon 14 in more than 95% of cases, or exon 12 in the majority of JAK2 V617F negative patients.

PV patients have elevated hemoglobin and hematocrit levels, and the 2008 WHO classification proposed additional minor criteria, low serum erythropoietin (Epo) levels, an endogenous (Epo-independent) growth of erythroid colonies, and specific histologic features of bone marrow MPN.[11] In the current classification, bone marrow biopsy has become a major criterion, while subnormal Epo levels are a minor criterion. JAK2 mutations are found in the majority of PV patients, namely mutations in exon 12 or 14 of JAK2.[11]

The World Health Organization (WHO) Classification of Myeloid Neoplasms was revised in 2016, and includes refined criteria for characterizing the disease, particularly at earlier stages.[12] Part of the criteria includes evaluation of 'phenotypic driver' mutations in MPN: JAK2 V617F, CALR and MPL W515L/K.[12]

These mutations arise in the hematopoietic stem and progenitor cell (HSPC) compartment, and confer cytokine hypersensitivity and constitutive Jak2 tyrosine kinase signaling in the presence of erythropoietin (EPO), thrombopoietin (TPO) or the interleukin-3 (IL-3) receptor scaffold.[8]

JAK2 is a tyrosine kinase gene that plays a critical role in normal hematopoiesis. JAK2 hyperactivation occurs in myeloproliferative neoplasms through different mechanisms.[13]

JAK2 associates with the cytoplasmic portion of various receptors, such as those for erythropoietin (EpoR), thrombopoietin (MPL), and granulocyte/macrophage colony-stimulating factor (G-CSFR). JAK2 is also activated in response to additional cytokines (eg, growth hormone and IL-5).[14]

Mutant CALR Complexes with MPL, both mutations in CALR and MPL result in receptor dimerization and activation of JAK2, MAPK/ERK, mitogen-activated protein kinase/extracellular signal-regulated kinase; PI3/AKT, phosphoinositide 3-kinase/serine/threonine kinase Akt; STAT, signal transducer and activator of transcription.[14]

PV and ET are modeled as a shared biological continuum disease spectrum in which different genetic lesions skew the clinical phenotype of polycythemia with additional thrombocytosis and leukocytosis.

CALR mutations result in excessive MPL signaling, in a manner similar to that resulting

from MPL mutations. JAK2 mutations signal downstream of several cell surface receptors, including MPL, and are thus associated with thrombocytosis but also erythrocytosis and leukocytosis. The exact nature of the phenotype depends on driver mutations, germline genetics, and the influence of additional somatic mutations on the disease phenotype.

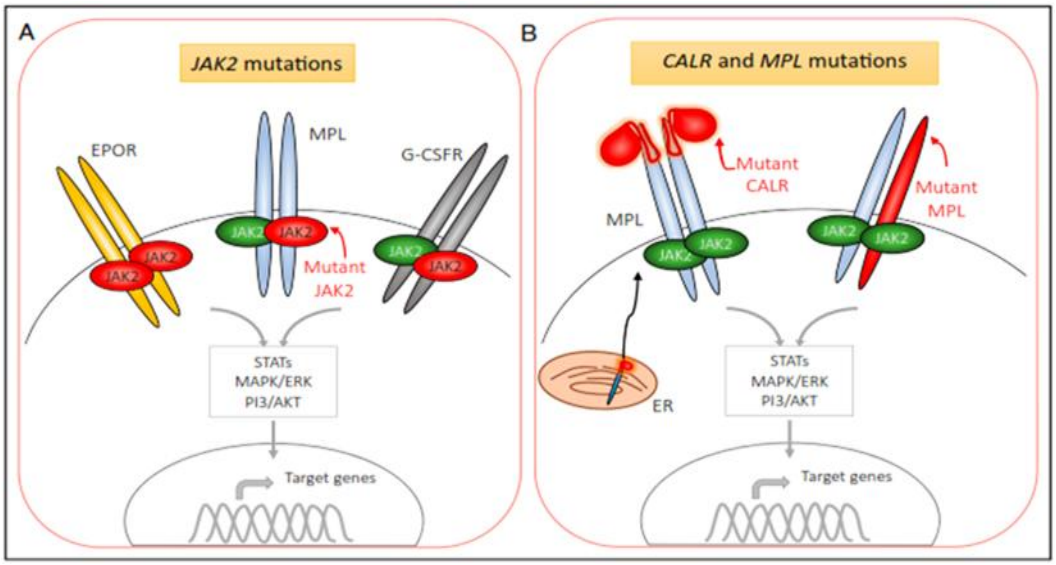

Figure 3. Mutations in JAK2, CALR, and MPL drive excessive myeloproliferation through downstream constitutively active JAK2 signaling. (A). JAK2 mutants, shown in red, are constitutively active and induce proliferation and differentiation at variable lower levels: erythroid, megakaryocyte, and granulocytic. It is unclear whether mutant JAK2 dimerizes with mutant or wild-type JAK2, with respect to individual receptors. (B). Mutations in CALR and MPL result in aberrant activation of downstream MPL receptor signaling.[14]

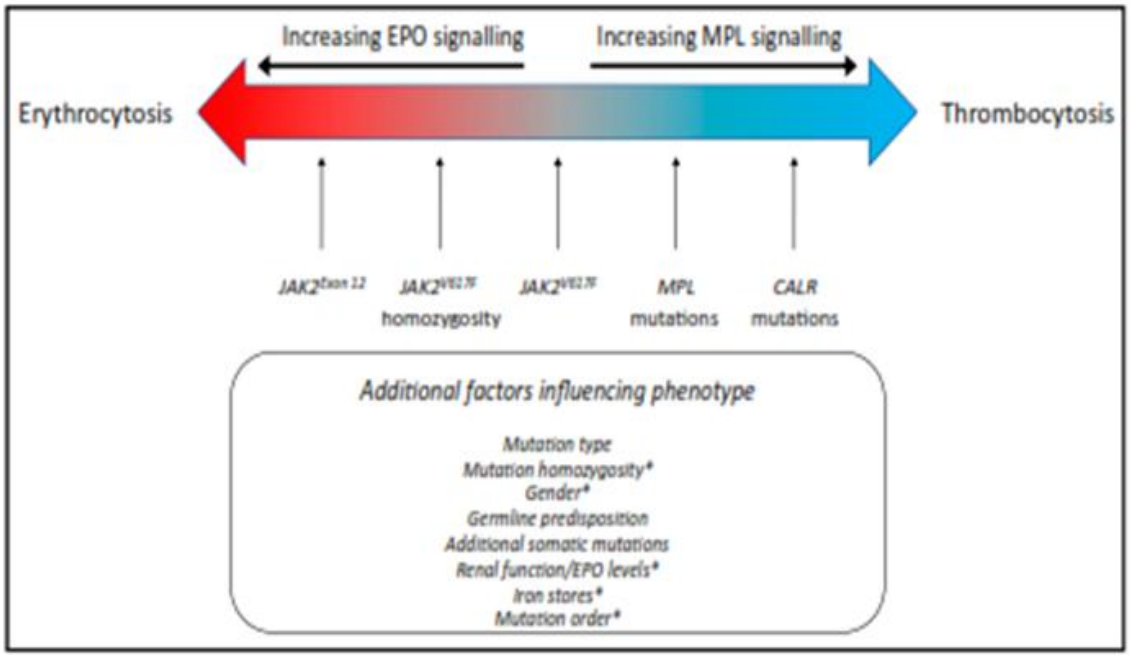

Figure 4. Clinical presentation in chronic phase and relationship to Myelof driver phenotype.[14]

Clinical Symptoms of Polycythemia Vera (PV)

Philadelphia chromosome (Ph) negative myeloproliferative neoplasms (MPNs) namely: polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) vary in their morphologic features, disease presentation, and clinical outcomes.[12]

The spectrum of clinical symptoms associated with MPNs is broad: constitutional symptoms (fever, night sweats, and weight loss), symptoms related to splenic enlargement (abdominal discomfort or pain and early satiety), symptoms related to microvascular disorders (vertigo, lightheadedness, dizziness, insomnia, sexual dysfunction, numbness, tingling, headache, and concentration problems), fatigue, cough, bone pain, inactivity, and pruritus.20 Thrombosis is the major cause of morbidity and mortality in JAK2-positive myeloproliferative patients.[15]

Myeloproliferative neoplasms (MPNs) are a heterogeneous phenotype, a group of blood cancers characterized by proliferation of progenitor cells and accumulation of mature myeloid cells, linked by a tendency to transform into myelofibrosis or acute myeloid leukemia. JAK2 V617 mutations can be found in patients with MPNs, in polycythemia vera (>95%), essential thrombocythemia and primary myelofibrosis (~50%).[8]

Myeloproliferative neoplasms (MPNs) originate from hematopoietic stem cells (HSCs) in the bone marrow (BM) that undergo clonal proliferation as a result of intrinsic and extrinsic factors of the cell.[16] MPNs are characterized by progressive marrow fibrosis with heterogeneous symptoms, extramedullary hematopoiesis, splenomegaly, a tendency to bleed and thrombose, and a risk of transformation into acute myeloid leukemia (AML).[16]

JAK2 exon 12 mutations can be found in PV patients with JAK2 V617F negative. JAK2 exon 12 mutations have never been reported in ET. Compared with PV patients with JAK2V617F, patients with JAK2 exon 12 have higher hemoglobin concentrations, lower white blood cell and platelet counts, and bone marrow erythroid hyperplasia.[17]

Laboratory Aspects of Primary Polycythemia (PV)

Polycythemia or erythrocytosis is an increase in red blood cell mass. The pathogenesis of polycythemia includes: Erythropoietin receptor pathway signaling, Oxygen sensing pathway signaling, Mutations in oxygen sensing pathway genes, and Mutations in JAK2. The most important laboratory aspects of primary and secondary polycythemia include: erythropoietin levels and JAK2 V617F mutation analysis.

Differentiating polycythemia vera from other polycythemia disorders used to be difficult, but the discovery of JAK2 mutations and JAK2 exon 12 mutations has made it more straightforward in many cases. Several clinical and laboratory features can be helpful in the differential diagnosis.[1]

The assessment steps for polycythemia require the patient's clinical history and complete blood count (CBC) results, Erythropoietin (Epo) levels to determine the cause of erythrocytosis, and molecular analysis of JAK2 mutations to evaluate polycythemia vera. Patients with clinical suspicion of congenital erythrocytosis need mutational analysis as a confirmatory test. The development of molecular tests for mutations associated with polycythemia can establish a clearer classification and etiology.

Polycythemia vera is distinguished by the fact that erythroid cells proliferate in the absence of high levels of erythropoietin; one would expect that at high hematocrit levels the production of

erythropoietin would be inhibited and serum Epo levels would be reduced. Erythropoietin tests used to be insensitive to detect subnormal erythropoietin levels, but with advances in technology, several studies have reported subnormal serum erythropoietin levels in patients with polycythemia vera. Erythropoietin levels remain low, even after phlebotomy, which can increase erythropoietin levels in normal individuals.

Proper subclassification requires clinical, laboratory, and histopathological findings, and the presence of JAK2 mutations provides evidence for the diagnosis of MPN and is an integral component of the WHO diagnostic criteria. Mutations result in a constitutively activated cytokine (Epo, TPO, GCSF) receptor/JAK2 complex that can recruit and phosphorylate any of several STAT (signal transducer and activator of transcription) proteins, leading to downstream effects that include increased cell division.[2]

A single point mutation (G1849T) in exon 14 replacing valine with phenylalanine at codon 617 (JAK2 V617F) is found in the majority of MPNs. This mutation involves the pseudokinase (JH2) region of JAK2, which normally negatively regulates and inhibits autophosphorylation of the adjacent kinase domain in the absence of ligand-associated receptor activity.[2]

Amino acid substitutions alter the conformation of inactive JAK2 and abrogate its intrinsic control, resulting in cell-autonomous signaling independent of cytokine-receptor involvement, with central constitutive activation of JAKSTAT as well as the ERK/MAPK and PI3K/AKT pathways.[2]

The JAK2 V617F mutation is most common in PV (>95%) and is present in the majority of PMF and ET (50% to 60%).[2,18,19] The relative number of mutated JAK2 V617F alleles to wild-type alleles varies widely between individuals with the same disease type. Patients with advanced PV and a subset of patients with PMF have biallelic homozygous mutations arising from copy-neutral loss-of-heterozygosity events (acquired uniparental disomy) of chromosome 9 (p24). The heterogeneous distribution of the JAK2 V617F allele in tumor cells from different patients also suggests that a relatively sensitive test is needed for detection.[2]

It has been recommended that molecular analysis tests should be able to detect at least 0.1% of DNA mutations in patient specimens. Various methods have been advocated for the detection of JAK2 V617F, including high-resolution PCR product melting curve analysis, Sanger sequencing or pyrosequencing, and allele-specific PCR variants (eg, amplification refractory mutation system [ARMS] and RQ-PCR). Of these, the allele-specific PCR approach using DNA as a template reproducibly achieves the highest analytical sensitivity.[2]

Quantitative reporting of mutational burden is currently not required for diagnostic or therapeutic purposes, but the development of newer generation JAK2 inhibitors may change this aspect as more data emerge.[2]

The JAK2 V617F mutation is also found in patients with MDS, CMML, atypical CML (BCR-ABL1 negative), and de novo AML (5% or less each), as well as in most cases of MDS/MPN-RS-T. Thus, the presence of the JAK2 V617F mutation does not necessarily determine the diagnosis of classic MPN and should not be used in the context of conclusively differentiating from other rare MPNs, atypical myeloid tumors with proliferative morphologic features.[2]

A negative JAK2 V617F analysis result does not exclude the diagnosis of classic MPN, if definitive clinical and morphologic findings are otherwise present. The use of high-sensitivity PCR methods can also identify the obvious V617F

mutation at very low levels (<0.1%), and the presence of rare mutated cells in healthy subjects. By extension, the finding of very low-level, JAK2 V617F abnormalities in patients with unexplained minimal thrombocytosis or leukocytosis may be misleading.

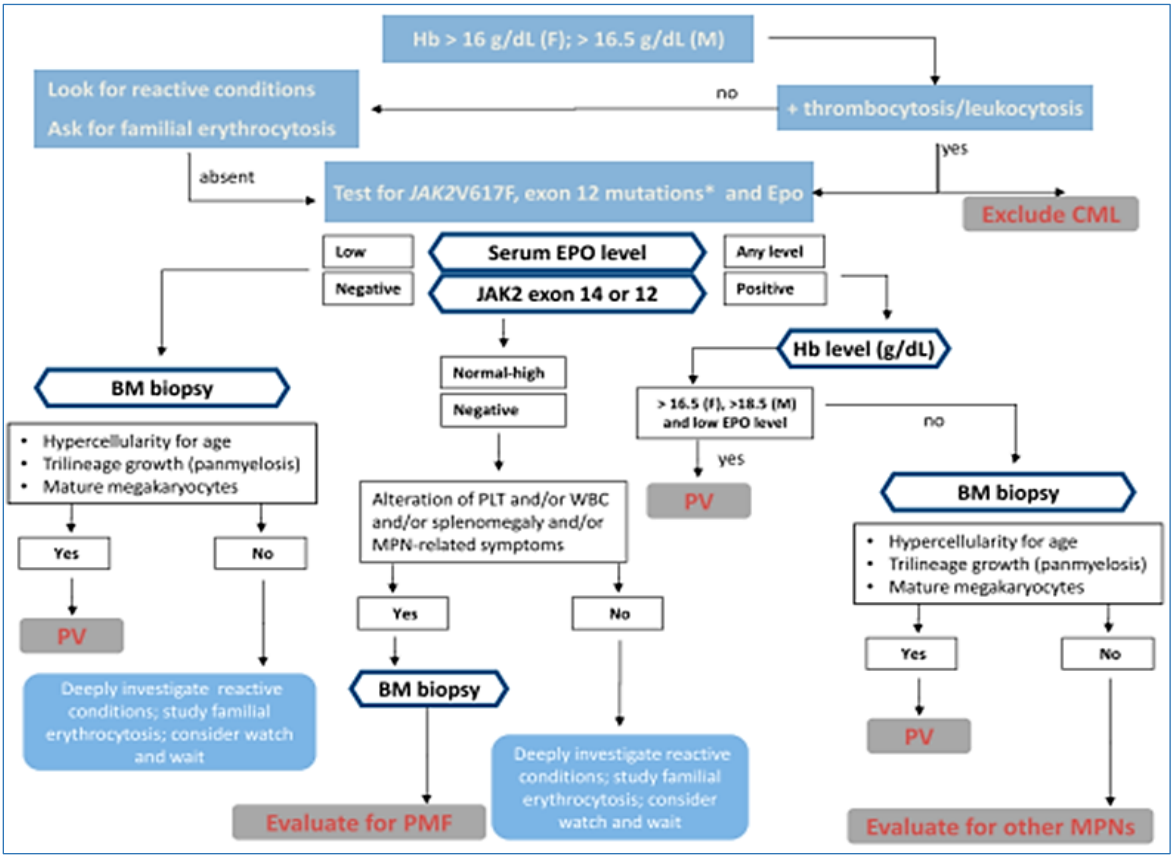

Figure 6. Algorithm for evaluation of MPN phenotype with Polycythemia. *Test for JAK2 V617F first and JAK2 exon 12 mutation if V617F is negative. CML, chronic myeloid leukemia; Epo, erythropoietin; F, female; M, male; MK, megakaryocytes; PLT, platelets; WBC, white blood cells.[20]

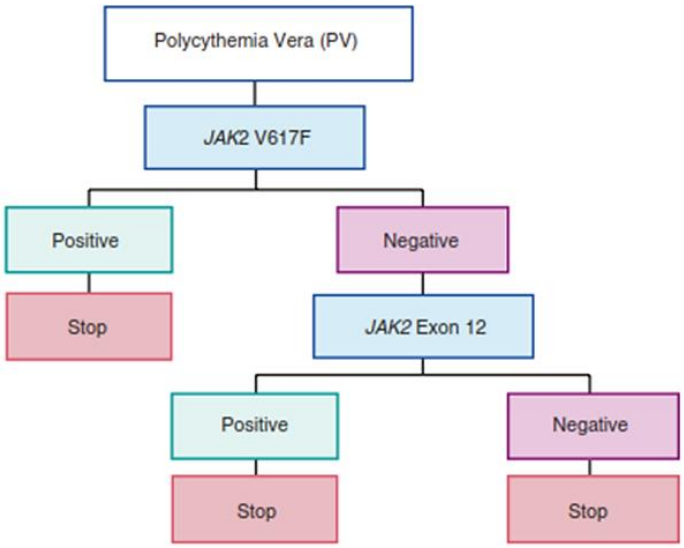

Figure 7. Algorithm of molecular diagnostic testing in polycythemia vera (PV).[2]

Molecular diagnostic laboratories should be aware of the wide dynamic detection range for JAK2 V617F and understand the relative clinical validity of positive values, especially at low levels (<1%), when reporting such results.

This algorithmic approach has been adopted in practice by several clinical laboratories and was recently incorporated into the 2017 NCCN guidelines for diagnostic workup of MPNs. Similarly, if the clinicopathological indication for evaluation is PV, a reflexive testing approach of JAK2 V617F assessment followed by JAK2 exon 12 sequencing is recommended.

This approach reduces the need for JAK2 exon 12 analysis given the very high prevalence of the V617F mutation in the majority of PV cases. JAK2 exon 12 analysis is not recommended for the evaluation of non-PV MPNs.[2]

Reflexive testing algorithm for JAK2 V617F and JAK2 exon 12 mutations during the diagnostic workup of MPN. When the clinicopathologic suspicion is PV, JAK2 V617F and JAK2 exon 12 mutations are tested sequentially until a positive result is found. A negative result almost excludes PV. The testing algorithm is based on the predominance of JAK2-marked V617F mutations seen in PV (>95%) with rare V617F-negative cases showing other mutually exclusive mutations in exon 12.

CONCLUSION

Polycythemia or erythrocytosis is an increase in red blood cell mass. The pathogenesis of polycythemia includes: Erythropoietin receptor pathway signaling, Oxygen sensing pathway signaling, Mutations in oxygen sensing pathway genes, and Mutations in JAK2. The most important laboratory aspects of primary and secondary polycythemia include: erythropoietin levels and JAK2 V617F mutation analysis.

The assessment steps for polycythemia require a patient's clinical history and complete blood count (CBC) results, Erythropoietin (Epo) levels to determine the cause of erythrocytosis, and molecular analysis of JAK2 mutations to evaluate polycythemia vera. Patients with clinical suspicion of congenital erythrocytosis need mutational analysis as a confirmatory test. The development of molecular tests for mutations associated with polycythemia can establish a clearer classification and etiology. The development of molecular tests for mutations associated with polycythemia can establish a clearer classification and etiology. The JAK2 V617F mutation is most common in PV (>95%) and is present in the majority of PMF and ET (50% to 60%).

Various methods have been advocated for the detection of JAK2 V617F, including high-resolution PCR product melting curve analysis, Sanger sequencing or pyrosequencing, and allele-specific PCR variants (eg, amplification refractory mutation system [ARMS] and RQ-PCR). Of these, the allele-specific PCR approach using DNA as a template reproducibly achieves the highest analytical sensitivity.

REFERENCES

1. Prchal JT. Chapter 57: Primary and Secondary Erythrocytoses. In: *Williams Hematology, 9e.* 9th ed. McGraw-Hill Education; 2015. https://accessmedicine.mhmedical.com/content.aspx?sectionid=94306075&bookid=1581&jumpsectionid=94306174&Resultclick=2
2. He R, Oliveira JL, Hoyer JD, Viswanatha DS. Molecular Hematopathology. In: *Hematopathology (Third Edition)*. 3rd ed. Elsevier; 2018:712-760.e18. doi:10.1016/B978-0-323-47913-4.00024-0
3. Geyer HL, Kosiorek H, Dueck AC, et al. Associations between gender, disease features and symptom burden in patients with myeloproliferative neoplasms: An analysis by the MPN QOL international

working group. *Haematologica*. 2017;102(1):85-93. doi:10.3324/HAEMATOL.2016.149559,

4. Courtier F, Carbuccia N, Garnier S, et al. Genomic analysis of myeloproliferative neoplasms in chronic and acute phases. *Haematologica*. 2017;102(1):e11-e14. doi:10.3324/HAEMATOL.2016.152363,
5. Lin CHS, Zhang Y, Kaushansky K, Zhan H. JAK2V617F-bearing vascular niche enhances malignant hematopoietic regeneration following radiation injury. *Haematologica*. 2018;103(7):1160-1168. doi:10.3324/HAEMATOL.2017.185736,
6. Chen CC, You JY, Lung J, et al. Aberrant let7a/HMGA2 signaling activity with unique clinical phenotype in JAK2-mutated myeloproliferative neoplasms. *Haematologica*. 2017;102(3):509-518. doi:10.3324/HAEMATOL.2016.154385,
7. Grinfeld J, Nangalia J, Green AR. Molecular determinants of pathogenesis and clinical phenotype in myeloproliferative neoplasms. *Haematologica*. 2017;102(1):7-17. doi:10.3324/HAEMATOL.2014.113845,
8. Vu T, Austin R, Kuhn CP, et al. Jak2V617F driven myeloproliferative neoplasm occurs independently of interleukin-3 receptor beta common signalling. *Haematologica*. 2016;101(3):e77-e80. doi:10.3324/HAEMATOL.2015.136705,
9. Swerdlow S, Campo E, Harris N, et al. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. IARC Publication; 2017. https://publications.iarc.fr/Book-And-Report-Series/Who-Classification-Of-Tumours/WHO-Classification-Of-Tumours-Of-Haematopoietic-And-Lymphoid-Tissues-2017
10. Tiong IS, Casolari DA, Nguyen T, et al. Masked polycythaemia vera is genetically intermediate between JAK2V617F mutated essential thrombocythaemia and overt polycythaemia vera. *Blood Cancer J*. 2016;6(8):e459. doi:10.1038/BCJ.2016.70
11. Chauveau A, Nibourel O, Tondeur S, et al. Absence of CALR mutations in JAK2-negative polycythemia. *Haematologica*. 2017;102(1):e15-e16. doi:10.3324/HAEMATOL.2016.154799,
12. Wong WJ, Hasserjian RP, Pinkus GS, Breyfogle LJ, Mullally A, Pozdnyakova O. JAK2, CALR, MPL and ASXL1 mutational status correlates with distinct histological features in philadelphia chromosome-negative myeloproliferative neoplasms. *Haematologica*. 2018;103(2):e63-e68. doi:10.3324/HAEMATOL.2017.178988,
13. Bellido M, Te Boekhorst PAW. JAK2 Inhibition: Reviewing a New Therapeutical Option in Myeloproliferative Neoplasms. *Adv Hematol*. 2012;2012:535709. doi:10.1155/2012/535709
14. Nangalia J, Green AR. Myeloproliferative neoplasms: From origins to outcomes. *Blood*. 2017;130(23):2475-2483. doi:10.1182/BLOOD-2017-06-782037,
15. Guy A, Gourdou-Latyszenok V, Le Lay N, et al. Vascular endothelial cell expression of JAK2 V617F is sufficient to promote a pro-thrombotic state due to increased P-selectin expression. *Haematologica*. 2019;104(1):70-81. doi:10.3324/HAEMATOL.2018.195321,
16. Herlihy N, Harrison CN, McLornan DP. Exploitation of the neural-hematopoietic stem cell niche axis to treat myeloproliferative neoplasms. *Haematologica*. 2019;104(4):639-641. doi:10.3324/HAEMATOL.2018.211896,
17. Godfrey AL, Chen E, Massie CE, et al. STAT1 activation in association with JAK2 exon 12 mutations. *Haematologica*. 2016;101(1):e15-e19. doi:10.3324/HAEMATOL.2015.128546,
18. Arulogun SO, Choong HL, Taylor D, et al. JAK1 somatic mutation in a myeloproliferative neoplasm. *Haematologica*. 2017;102(8):e324-e327. doi:10.3324/HAEMATOL.2017.170266,
19. Eder-Azanza L, Hurtado C, Navarro-Herrera D, Aranaz P, Novo FJ, Vizmanos JL. p.Y317H is a new JAK2 gain-of-

function mutation affecting the FERM domain in a myelofibrosis patient with CALR mutation. *Haematologica*. 2017;102(8):e328-e331. doi:10.3324/HAEMATOL.2017.166439,

20. Passamonti F, Maffioli M. Update from the latest WHO classification of MPNs: A user's manual. *Hematol (United States)*. 2016;2016(1):534-542. doi:10.1182/ASHEDUCATION-2016.1.534,