Polycythemia Vera
New Clinicopathologic Perspectives

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Polycythemia vera (PV), also termed polycythemia rubra vera, is a clonal myeloproliferative disorder (MPD) of hematopoietic stem cells. Although trilineage proliferation is observed, erythrocyte mass is most increased. The blood, bone marrow, spleen, and liver are variably involved during progression through the 2 disease phases: (1) a proliferative or polycythemic phase with increased red cells; and (2) a postpolycythemic or spent phase with marked cytopenias, extramedullary hematopoiesis, hypersplenism, and myelofibrosis. Hemorrhage and thrombosis represent the primary clinical manifestation, but a subset of patients will transform to acute leukemia. The differential diagnosis includes secondary polycythemia and the other chronic MPDs. The first recognized reports of PV were attributed to Vaquez and Osler who described erythremia and polycythemia, respectively. The concept of chronic MPDs later emerged during the 1950s when PV was categorized with chronic myeloid leukemia, essential thrombocytopenia, and chronic idiopathic myelofibrosis (CIMF) based on their common clinicopathologic features. The Polycythemia Vera Study Group (PVSG) was then created to define the diagnostic criteria for this entity, and these recommendations remained the gold standard for many years. As laboratory techniques improved and scientific perspectives on neoplasia evolved during the 1970s, the understanding of PV also progressed. Several studies evaluating various markers such as X-chromosome inactivation, glucose-6-phosphate dehydrogenase isoenzymes, and DNA methylation demonstrated PV to arise from the clonal transformation of a single hematopoietic stem cell. However, the PVSG consensus remained unchanged until 2001 when the World Health Organization (WHO) modernized the diagnostic criteria using a comprehensive clinicopathologic approach. Recently, several reports have described a specific mutation in the Janus kinase 2 (JAK2) gene in a significant proportion of PV patients.

EPIDEMIOLOGY

PV has been reported in patients from all ethnic backgrounds; however, it is much more common in those of European than Asian descent, having an annual incidence ranging from 2 to 10 cases per million population per year. Males have a slightly higher incidence with a male-female ratio of 1:1 to 2:1. PV is generally a disease of older individuals, occurring most frequently between 50 and 70 years of age; childhood and adolescent disease is exceptionally rare. Familial cases have been documented, but their significance remains uncertain.

CLINICAL MANIFESTATIONS

The symptoms of PV are insidious in onset, and they generally involve peripheral manifestations of the primary bone marrow disorder. The most common presenting complaints are thrombosis and hemorrhage. Thrombotic complications may include deep venous thrombosis, stroke, myocardial ischemia, Budd-Chiari syndrome, or mesenteric ischemia. Bleeding complications typically include epistaxis, oral mucosal hemorrhage, gastrointestinal hemorrhage, or nonspecific ecchymoses. Hyperviscosity syndrome because of sludging of blood flow and microthrombi formation may cause hypertension, headache, dizziness, visual disturbances, vertigo, tinnitus, claudication, and erythromelalgia. Pruritus, classically described fol-
The classification represented a modernized approach to the diagnosis of PV, with the World Health Organization (WHO) classification for PV published in 2001 (Table 2). The WHO classification for PV was based on the combination of clinical and laboratory findings, including the National Cancer Institute to evaluate treatment modalities.

The combination of their recommended major and minor criteria (Table 1) remained the gold standard for several decades. In 2001, the WHO classification for PV was published. The classification represented a modernized approach to diagnosis and added several new laboratory studies. These studies included in vitro bone marrow endogenous erythroid colony (EEC) formation, the presence of clonal genetic abnormalities other than the Philadelphia chromosome (BCR/ABL fusion gene and/or protein), and low serum erythropoietin (EPO) levels.

### DIAGNOSTIC CRITERIA

The PVSG was originally created with the support of the National Cancer Institute to evaluate treatment modalities. During this process, the eligibility criteria for entering PV clinical trials were defined, and these became known as the PVSG diagnostic criteria. The combination of their recommended major and minor criteria (Table 1) remained the gold standard for several decades. In 2001, the WHO classification for PV was published. The classification represented a modernized approach to diagnosis and added several new laboratory studies. These studies included in vitro bone marrow endogenous erythroid colony (EEC) formation, the presence of clonal genetic abnormalities other than the Philadelphia chromosome (BCR/ABL fusion gene and/or protein), and low serum erythropoietin (EPO) levels.

### Red Blood Cell Mass, Hemoglobin, and Hematocrit

Red blood cell (RBC) mass, one component of the PVSG diagnostic criteria, is determined by comparing total blood volume to plasma volume. It was originally thought to be a sensitive marker for PV, but subsequent studies reported a relatively low predictive value when several confirmed PV cases were demonstrated to have an RBC mass less than the reference limit. The specificity of RBC mass is also controversial; however, it remains a commonly utilized test in the United States. Hemoglobin measurements are also utilized, despite their equally debatable value. Of note, a hematocrit greater than 45% in a well-hydrated patient is generally considered the appropriate criteria for initiating phlebotomy treatment.

### White Blood Cell and Platelet Count

The white blood cell and platelet counts can be variably affected in PV. Although the white blood cell count is often within the normal reference range, it may be increased up to 25 000 cells/mm³; counts exceeding this level should prompt suspicion for leukemic transformation. Leukocytosis greater than 12 000 cells/mm³ is a minor diagnostic criterion for the PVSG and WHO classification (Tables 1 and 2). Granulocytes typically demonstrate immature circulating forms (left-shifted maturation) without significant cytologic atypia. Thrombocytosis with platelet counts greater than 400 000/mm³ is another minor diagnostic criterion (Tables 1 and 2). Observation of giant and/or hypogranular platelet forms is not uncommon.

### Cytogenetic Abnormalities

Although no specific mutations had been identified at the time of publication, the presence of clonal cytogenetic abnormalities was added as a new major PV diagnostic criterion in the WHO classification (Table 2). The absence of the Philadelphia chromosome or the BCR/ABL fusion product is essential for exclusion of chronic myeloid leukemia. Conventional cytogenetic and fluorescent in situ hybridization methods have detected various mutations in the hematopoietic progenitor cells of PV patients, and these abnormalities have been shown to accumulate over time. Fewer than 20% of cases have an identifiable clone at diagnosis, whereas more than 80% to 90% have one at 10 years. The most frequent genetic aberrations include deletion or translocation of chromosome 20, trisomy 8, and trisomy 9. Abnormalities of 13q, 5q, 7q, 1q, 5, and 7 are less common. Because similar karyotypes are also observed in patients with other MPDs and myelodysplastic syndromes, none convey specific data. In addition, a mutation in the JAK2 gene (JAK2V617F) possibly having diagnostic and prognostic value to PV has recently been described; its significance is discussed in greater detail later.

### In Vitro EEC Formaition

Red blood cell production is primarily regulated by EPO. Hypoxia-inducible factor, the major transcriptional activator of EPO, is stimulated by low oxygen tension. Normal erythroid progenitors are sensitive to EPO in vivo and require exogenous EPO to proliferate in vitro. In PV, the clonal erythroid progenitors become independent of EPO stimulation. Thus, the in vitro EEC formation assay was added as a new major diagnostic criterion in the WHO classification (Table 2). Because the bone marrow of PV patients contains both the aberrant clone and the normal.
Table 3. Bone Marrow Features of Polycythemia Vera (PV) and Secondary Polycythemia (SP)*

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>PV</th>
<th>SP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased cellularity</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>Megakaryocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increased number</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>Large to giant size</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>Pleomorphic aspect (different in size)</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>Increased nuclear lobulation</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>Loose clusters</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>Naked nuclei</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>Stroma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presence of cellular debris</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Perivascular plasmacytosis</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Iron-laden macrophages</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>Lymphoid nodules</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Increased reticulin fibers</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>Increased eosinophils</td>
<td>−</td>
<td>±</td>
</tr>
</tbody>
</table>

* Incidence of feature; + indicates ≥80%; −, ≤10%; and ±, 11% to 79%. Adapted from Thiele et al.77,78

Serum EPO Level

The vast majority of PV patients express very low levels of serum EPO,68,70–72 This is a key diagnostic feature that differentiates PV from other polycythemic conditions (Table 2).60 Compared to the in vitro EEC assay, analytical testing of EPO is considerably less expensive to perform, less subjective to interpret, and less demanding on personnel. Although the molecular mechanism responsible for low EPO levels in PV is not fully understood, it may be related to an associated decrease in hemoglobin oxygen affinity.73–75 Further studies are also needed to determine the contribution from primary bone marrow disease and increased RBC mass.

Bone Marrow Pathology

Bone marrow morphology was not originally included in the PVSG diagnostic criteria for PV; however, biopsy studies were later shown to provide important data capable of differentiating it from the other MPDs, and they were subsequently added to the WHO classification (Table 2).1,38–42,76–80 Several large histopathologic series examining patients who presented with mild to significant erythrocytosis demonstrated that PV can be differentiated from secondary polycythemia in approximately 96% of cases (Table 3).2,76–80 The most distinguishing features are found within the megakaryocyte and stromal lineages. Marrow from PV patients characteristically shows moderate to marked hypercellularity, erythroid hyperplasia, and increased clustering of enlarged pleomorphic megakaryocytes having multilobulated nuclei. Also, PV specimens typically lack a prominent inflammatory stromal reaction, including only sparse numbers of perivascular plasma cells, eosinophils, hemosiderin-laden macrophages, and cellular debris. However, because PV, essential thrombocythemia, and CIMF share many common morphologic features, caution must be used whenever evaluating the bone marrow from a suspected case.9,80 Studies using semiquantitative histologic grading parameters for the MPDs have shown the existence of subtle discriminatory features such as megakaryocyte morphology, reticulin fibrosis, granulocyte maturation pattern, erythroid hyperplasia, and marrow cellularity (Table 4).78–81

Two distinct histopathologic stages of PV are currently recognized, although a third latent stage may also exist. In the polycythemic phase, the bone marrow is hypercellular with trilineage proliferation (panmyelosis). Hyperplastic normoblastic erythrocytosis predominates, but maturing granulopoiesis and megakaryopoiesis without dysplasia remain intact (Figure, A through C). In comparison, in the postpolycythemic or spent phase, erythrocytosis is markedly decreased, granulocyte maturation is delayed, and atypical megakaryocytes are frequently observed. As the disease process advances, overlapping features are typically observed, and the degree of myelofibrosis progresses from a dense meshwork of reticulin with thickened sinuses (sinus wall-sclerosis) through the deposition of coarse collagen bundles to end-stage myeloid scarring (Figure, D).

Table 4. Bone Marrow Differential Diagnosis in Non-Chronic Myeloid Leukemia Myeloproliferative Disorder (CML MPD)*

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>PV</th>
<th>ET</th>
<th>CIMF-0</th>
<th>CIMF-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellularity</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Megakaryocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maturation defects</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Nuclear lobulation</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Naked nuclei</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Small forms</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Bulbous nuclei</td>
<td>−</td>
<td>±</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Erythropoiesis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left shift</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Quantity</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Granulopoiesis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left shift</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Reticulin fibrosis</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
</tbody>
</table>

* WHO indicates World Health Organization; PV, polycythemia vera; ET, essential thrombocythemia; CIMF-0, prefibrotic chronic idiopathic myelofibrosis; and CIMF-1, early fibrotic CIMF. Incidence of feature: ≥, ≥80%; ≤, ≤10%; and ±, 11% to 79%. Adapted from Thiele et al.77,78

MOLECULAR BIOLOGIC MARKERS

Decreased expression of the thrombopoietin receptor (Mpl) was the first molecular biomarker recognized in association with PV.82,83 Although Mpl immunohistochemistry may provide presumptive discrimination between PV (decreased staining) and benign conditions (normal staining); it is also negative in essential thrombocythemia and CIMF.29,85–80 In addition, a subset of PV cases demonstrates normal Mpl gene expression. Additional studies are needed to better understand the role of Mpl in PV.

Expression of polycythemia rubra vera-1 (PRV-1) mRNA in granulocytes was first thought to represent a selective...
Biomarker for PV, but subsequent studies also revealed PRV-1 in the other MPDs and various reactive conditions. Recent investigations have renewed interest in PV-1 after demonstrating gene overexpression to be highly correlated with in vitro EEC formation and the JAK2 gene mutation. Additional studies are pending.

Biologic studies of erythroid progenitor cells from PV patients have demonstrated that the erythroid precursor cells are hypersensitive to insulin-like growth factor-1, interleukin-3, granulocyte-macrophage colony-stimulating factor, and stem cell factor (CD117). Increased expression of the antiapoptotic BCL-XL and NF-E2 proteins and elevated telomerase activity are also observed. Similarly, the megakaryocytes of PV patients are capable of producing unusually high levels of lysozyme and various inflammatory cytokines. Expression profiling by cDNA microarray also showed up-regulation of several antiapoptotic genes in PV patients. The significance of these findings remains uncertain.

Molecular Pathogenesis and the JAK2V617F Mutation

Perhaps the most significant discovery in PV pathogenesis is the presence of a specific mutation in the JAK2 gene on chromosome 9p, a frequent site of heterozygosity loss in PV patients. Recent studies from several independent laboratories reproducibly demonstrated a guanine to cytosine mutation at position 1849 in the JH2 pseudokinase domain of exon 12, resulting in a valine to phenylalanine substitution (V617F). The acquired mutation is present only within the clonal hematopoietic progenitor cell population. JAK2V617F has also been observed in essential thrombocytopenia, CML, chronic myelomonocytic leukemia, juvenile myelomonocytic leukemia, hypereosinophilic syndrome, and systemic mastocytosis, whereas it is repeatedly absent in all secondary bone marrow conditions, unrelated leukemias, and healthy controls. Of note, PV shows a considerably higher carrier rate for the JAK2V617F mutation (65%-97%) than essential thrombocytopenia (30%), CML (50%), or the various other disorders. JAK2V617F appears to confer a slower rate of disease progression, a higher risk for thrombotic or hemorrhagic complication, and an increased degree of myelofibrosis. In addition, compared to the heterozygous condition, clones homozygous for JAK2V617F may confer a poorer overall prognosis.

The V617F mutation results in constitutive JAK2 tyrosine kinase activity that probably functions through mul-
tiple molecular pathways involved in cellular proliferation and apoptosis.\textsuperscript{157,130} Among these, the STAT family of transcription factors and the Fas/Apo-1/TRAIL ligands have been most thoroughly studied.\textsuperscript{157,130} Compared to wild-type cells, JAK2\textsuperscript{V617F} erythroblasts are hypersensitive to insulin-like growth factor-I, more proliferative in EEC assays, and resist apoptosis.\textsuperscript{151,152} In addition, the presence of JAK2\textsuperscript{V617F} was shown to enhance in vivo granulocyte activation and CD34\textsuperscript{+} positive cell mobilization.\textsuperscript{134,135} There is also an murine model of PV-like disease induced by retroviral transfection of JAK2\textsuperscript{V617F}.\textsuperscript{17,125}

The majority of research studies examining JAK2 in PV patient specimens have utilized various DNA sequencing platforms to identify the mutated allele in granulocyte fractions.\textsuperscript{16–24,117} Although this is an appropriate approach, nucleotide sequencing lacks analytical sensitivity and is not well suited for routine use in a clinical laboratory. In comparison, amplification based techniques such as real-time polymerase chain reaction represent the preferred diagnostic approach. Several polymerase chain reaction based assays have been recently published, enabling the rapid detection of JAK2\textsuperscript{V617F} from peripheral blood, bone marrow aspirate, and paraffin-embedded tissue specimens.\textsuperscript{126,133–146} The anticipated diagnostic value of JAK2\textsuperscript{V617F} in PV has led Tefferi and Spivak\textsuperscript{120} to suggest mutation screening in all patients having low erythropoietin and high hematocrit. As additional clinical data are accumulated, the potential diagnostic and/or prognostic value of JAK2\textsuperscript{V617F} in PV and the other MPDs will be better understood. Similarly, the JAK2 tyrosine kinase molecule may also represent an appropriate target for the rational development of new therapeutic agents.\textsuperscript{141}

**CLINICAL MANAGEMENT OF PV**

The primary causes of morbidity and mortality in PV patients are thrombosis, hemorrhage, marrow failure, and leukemic transformation.\textsuperscript{142,146} A personal or family history of thrombosis is the most important independent predictor of death. Thrombotic complications may be due to elevated hematocrit, increased RBC mass, high blood viscosity, persistent granulocyte or platelet activation, and increased acute phase reactants.\textsuperscript{149,150} Thus, management is generally focused on minimizing risk factors. Thrombosis prevention with low-dose aspirin or aspirin-like is recommended for all PV patients, and phlebotomy remains a key intervention. A target hematocrit less than 45% is usually achieved. Overall, few therapeutic advances have been achieved over time.\textsuperscript{52,155} Chemotherapy should be considered when splenomegaly, leukocytosis, or thrombocytosis persist. Older patients are treated with radioactive isotopes (\textsuperscript{32}P), busulfan, and pipobroman whereas younger patients are managed with hydroxyurea. Interferon-\alpha can be used to suppress hematopoietic proliferation, but its utility is limited by high cost and undesirable side effects. Recently, bone marrow transplantation has been considered for younger patients who rapidly progressed to myelofibrosis or leukemia.\textsuperscript{147,149,152}

**CONCLUSIONS**

In summary, PV has long been recognized as a distinct disease entity, but many recent discoveries have significantly improved our understanding of its molecular pathophysiology. The new WHO classification system has clarified the diagnostic criteria and improved clinical management. Also, identification of the JAK2\textsuperscript{V617F} mutation in a majority of PV patients has potentially provided a new tool having diagnostic, prognostic, and therapeutic utility.

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