Bone Marrow Morphologic Features in Polycythemia Vera With JAK2 Exon 12 Mutations

Meredith A. Lakey, MD,1 Animesh Pardanani, MBBS, PhD,2 James D. Hoyer, MD,1 Phuong L. Nguyen, MD,1 Terra L. Lasho,2 Ayalew Tefferi, MD,2 and Curtis A. Hanson, MD1

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Abstract

The diagnosis of polycythemia vera (PV) requires the integration of clinical and laboratory findings, bone marrow morphologic features, and JAK2 analysis. JAK2V617F (exon 14) mutation is found in 95% of PV cases. Functionally similar mutations in JAK2 exon 12 have also been described, but a thorough bone marrow study has not been done. We identified 7 PV cases with exon 12 mutations; all had hypercellular bone marrow with erythroid hyperplasia. Small, atypical megakaryocytes predominated; atypical megakaryocyte lobation and abnormal chromatin distribution was identified in all cases. Rare clusters of megakaryocytes could be found but were typically subtle. Because JAK2 exon 12–positive PV cases lack the classic myeloproliferative morphologic features, bone marrow samples from the patients may be difficult to classify as myeloproliferative neoplasms. Clinically suspected PV with low serum erythropoietin and absent JAK2V617F, together with the bone marrow findings of erythroid hyperplasia and subtle megakaryocytic atypia, should prompt an evaluation for an exon 12 mutation.

The myeloproliferative neoplasms (MPNs) are characterized by effective bone marrow hematopoiesis with potential for evolution into myelofibrosis or transformation to acute leukemia and are subclassified by the World Health Organization (WHO) based on a combination of clinical, laboratory, morphologic, and molecular features.1 The classic WHO-defined and most commonly encountered MPNs are divided into those that are BCR-ABL1–positive (chronic myelogenous leukemia) and those that are BCR-ABL1–negative, including polycythemia vera (PV), essential thrombocythemia, and primary myelofibrosis.1,3

PV typically manifests as a pan-myelosis with an erythroid-predominant proliferation secondary to erythropoietin-independent stimulatory mechanisms.3,4 The genetic paradigm of PV changed dramatically in 2005 when several groups described a somatic gain-of-function mutation in the Janus kinase 2 (JAK2) gene, JAK2V617F, located in the JH2 pseudokinase domain in exon 14 of chromosome 9 in patients with MPNs5-8; the mutation has been identified in more than 95% of cases of PV and approximately 50% of cases of essential thrombocythemia and primary myelofibrosis.9,11 JAK2 is one of several members of the Janus family of protein tyrosine kinases that help mediate cytokine receptor signaling. It is a cytoplasmic protein kinase with 2 homologous kinase domains, one that is catalytically active (JH1) and one that serves a negative autoregulatory function (JH2). JAK2 mutations have been shown to promote cytokine-independent and erythropoietin- or interleukin-3-hypersensitive growth via constitutive activation of JAK/STAT, PI3K/AKT, and MAPK/ERK.6-8

The identification of JAK2V617F has significantly changed how MPNs are evaluated in the clinical laboratory. The
absence of the \( JAK2^{V617F} \) has been used as a near exclusionary criterion of PV and the finding of a \( JAK2^{V617F} \) as confirmatory evidence of an MPN.\(^4\)\(^,\)\(^10\)

More recently, we and others have reported that other, non-\( JAK2^{V617F} \) mutations can be identified in patients with PV.\(^{12} \)\(^-\)\(^25\) The majority of these mutations are located in exon 12 of the \( JAK2 \) gene, which lies in the linker region between the SH2 domain and the JH2 pseudokinase domain; to date, a single exon 12 mutation has been identified at the beginning of the JH2 domain. Like \( JAK2^{V617F} \), exon 12 mutations induce erythropoietin hypersensitivity by interfering with the ability of JH2 to autoregulate the kinase activity of JAK2.\(^{25} \) Unlike PV-associated \( JAK2^{V617F} \), exon 12 \( JAK2 \) mutations are typically heterozygous but are thought to be associated with stronger abnormal \( JAK2 \) activation.\(^{12} \) Exon 12 mutations seem to account for the majority of mutations in patients with \( JAK2^{V617F} \)-negative PV.\(^{13} \)

The clinical features and the in vitro laboratory characteristics of patients with PV with exon 12 mutations have been described in multiple studies.\(^{11} \)\(^-\)\(^20\) The bone marrow aspirate and biopsy manifestations have, however, been only minimally described.\(^{12} \)\(^,\)\(^13\)\(^,\)\(^15\)\(^,\)\(^16\)\(^,\)\(^18\) Because bone marrow histopathologic findings remain critical in the new WHO-defined criteria for PV, it is important that the morphologic features be characterized and well understood for this group of MPNs.

**Materials and Methods**

**Patient Accrual**

Approval for this study was obtained from the Mayo Clinic Institutional Review Board (Rochester, MN). All patients provided informed consent, and research was carried out according to the principles of the Declaration of Helsinki. Seven consecutive patients from our institution with PV and \( JAK2 \) exon 12 mutations were identified through review of our research database. Clinical and laboratory information was obtained by review of the patients’ electronic medical records. Peripheral blood smears, bone marrow aspirates, and bone marrow biopsy sections were reviewed for histopathologic features; at least 2 hematopathologists (M.A.L. and C.A.H.) reviewed each case. Cases of PV were diagnosed according to WHO criteria.\(^{1,\)\(^2} \) Reticulin stains and immunohistochemical stains with antibodies against hemoglobin, myeloperoxidase (MPO), CD61, and CD34 were done using standard techniques.

**JAK2 Analysis**

DNA was extracted from peripheral blood mononuclear cells (cases 1 and 2), granulocytes (cases 1, 3, and 5), or bone marrow cells (cases 1, 2, 4, 6, and 7) as previously described.\(^{13} \) DNA was quantified by spectrophotometry (NanoDrop Technologies, Wilmington, DE) and stored in 25-ng/μL aliquots for polymerase chain reaction (PCR) analysis. \( JAK2^{V617F} \) quantitative PCR assay and analysis was performed as previously described.\(^{26} \) \( JAK2 \) exon 12 mutation screening was accomplished by using methods previously described.\(^{13} \) Oligonucleotide primers flanking exon 12 of the JH2 domain were used to amplify a 490-base-pair product: exon 12 forward, 5’-CTC CTC TTT GGA GCA ATT CA-3’; exon 12 reverse, 5’-GAG AAC TTG GGA GTT GCG ATA-3’. PCR products were purified (QiAquick PCR Purification Kit, Qiagen, Valencia, CA) and subjected to bidirectional sequence analysis on the ABI PRISM 3730 DNA Analyzer (Applied Biosystems, Foster City, CA) using a sequencing primer: exon 12 sequence, 5’-TCA GTG TAT GAG AAC TTG GGA GTT GCG ATA-3’.

**Results**

**Clinical and Laboratory Findings**

Seven cases from our institution with PV and \( JAK2 \) exon 12 mutations were identified; 2 of the 7 cases were newly diagnosed PV based on clinical, laboratory, and morphologic findings. At the time of diagnosis, the mean age was 46 years with a male/female ratio of 4:3. At diagnosis, signs and symptoms included aquagenic pruritus and/or erythromelalgia (n = 3) and microvascular complications such as headache, dizziness, blurred vision, and distal extremity numbness (n = 4); 1 patient had bilateral deep venous thromboses postoperatively after resection of an intrasphenoidal meningioma. None had documented bleeding complications. One patient had palpable splenomegaly; two had undergone splenectomy for nonhematologic reasons.

Laboratory findings at diagnosis are summarized in Table 1. Hemoglobin concentrations at diagnosis ranged from 18.3 to 22 g/dL (183-220 g/L; median, 19.5 g/dL [195 g/L]). Only 1 patient had mild leukocytosis and concurrent mild thrombocytosis. Serum erythropoietin levels were low in all patients tested. Conventional cytogenetic karyotyping was normal in all patients (data not shown).

**JAK2 Studies**

Exon 12 mutations in the \( JAK2 \) gene were readily identified by PCR sequencing in all 7 cases: 4 with an F537-K539delinsL, 1 with an N542-E543del, 1 with a K539L, and 1 with a novel F533IK540I (Table 1). All were heterozygous mutations. Allele-specific PCR analysis for the \( JAK2^{V617F} \) was negative in all cases. No \( JAK2^{V617F} \) mutation was present in any of the cases.
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**Bone Marrow Features**

The bone marrow aspirates and biopsy specimens were hypercellular for the patients’ ages, with biopsy cellularity ranging from 45% to 100% (median, 90%) [Image 1]. Erythroid hyperplasia was present in all cases. No definitive dyserythropoiesis was seen. A slight granulocytic hyperplasia was present in 5 of the marrow samples; no dysplastic changes were noted. No increase in blasts was identified. Sinusoidal dilatation was not a prominent feature in any of the cases.

Although prominent tight clusters of large and bizarre megakaryocytes that characterize classic cases of JAK2V617F-positive PV were not a typical finding, all 7 cases had increased numbers of megakaryocytes that exhibited atypical features in aspirate and biopsy specimens [Image 2]. There was a spectrum of megakaryocytic cell sizes, with smaller forms predominating over larger forms. Megakaryocyte nuclei ranged from small and monolobate to hyperlobated with complex nuclear folding. The nuclear chromatin pattern varied from dispersed with prominent eosinophilic nucleoli to markedly hyperchromatic. In 4 cases, there were subtle megakaryocytic clusters (defined as ≥3 megakaryocytes lying adjacent to each other without intervening cells) that could have been easily missed on a cursory review. Only 1 case had prominent megakaryocyte clusters. Focal areas of increased atypical megakaryocytes without clusters were identified in the remaining cases.

Bone marrow reticulin fibrosis was normal or slightly increased in the majority of the biopsy specimens. One case eventually evolved into postpolycythemic myelofibrosis with grade 3 (of 3) reticulin fibrosis and associated osteosclerosis.

**Follow-up**

The time from symptom onset until last follow-up ranged from 2 to 491 months (median, 51 months). Fifteen years after initial diagnosis, 1 case evolved into postpolycythemic myelofibrosis with grade 3+ (of 3) reticulin fibrosis and associated osteosclerosis.

**Discussion**

Knowledge regarding the basic biology of PV was relatively quiescent until 2005 when several groups reported the presence of JAK2V617F, a gain-of-function mutation in exon 14 of chromosome 9 responsible for erythropoietin-independent erythropoiesis in the majority of PV cases.5-7 Since that time, JAK2V617F testing has become a critical diagnostic tool in the

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**Table 1**

**Laboratory Findings at Diagnosis**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Hemoglobin Level, g/dL (g/L)</th>
<th>WBC Count, μL (× 10^9/L)</th>
<th>Platelet Count, μL (× 10^9/L)</th>
<th>Epo* (IU/L)</th>
<th>JAK2 Exon 12</th>
<th>JAK2V617F</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19.4 (194)</td>
<td>7,700 (7.7)</td>
<td>292 (292)</td>
<td>&lt;2.5</td>
<td>N542-E543del</td>
<td>Negative</td>
</tr>
<tr>
<td>2</td>
<td>18.7 (187)</td>
<td>9,800 (9.8)</td>
<td>152 (152)</td>
<td>NA</td>
<td>F537-K539delinsL</td>
<td>Negative</td>
</tr>
<tr>
<td>3</td>
<td>22.2 (220)</td>
<td>11,500 (11.5)</td>
<td>157 (157)</td>
<td>&lt;1.0†</td>
<td>F533I-K540I</td>
<td>Negative</td>
</tr>
<tr>
<td>4</td>
<td>18.3 (183)</td>
<td>8,400 (8.4)</td>
<td>257 (257)</td>
<td>2.9†</td>
<td>F537-K539delinsL</td>
<td>Negative</td>
</tr>
<tr>
<td>5</td>
<td>19.4 (194)</td>
<td>23,300 (23.3)</td>
<td>790 (790)</td>
<td>1.2†</td>
<td>F537-K539delinsL</td>
<td>Negative</td>
</tr>
<tr>
<td>6</td>
<td>20.2 (202)</td>
<td>5,100 (5.1)</td>
<td>232 (232)</td>
<td>2.8</td>
<td>K539L</td>
<td>Negative</td>
</tr>
<tr>
<td>7</td>
<td>20.4 (204)</td>
<td>10,300 (10.3)</td>
<td>476 (476)</td>
<td>1.1</td>
<td>F537-K539delinsL</td>
<td>Negative</td>
</tr>
</tbody>
</table>

* NA, not available.
† Measured during treatment.

**Image 1** Hypercellular bone marrow sample showing prominent erythroid hyperplasia and scattered, increased megakaryocytes of varying sizes and shapes (H&E, ×100).

**Image 2** Megakaryocytes showing atypical features (H&E, ×400).

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**Image 3** Table of laboratory findings at diagnosis.
evaluation of patients with suspected PV; a positive test result in peripheral blood or bone marrow indicative of a clonal process together with a decreased serum erythropoietin result eliminates the need for extensive testing to exclude secondary causes of erythrocytosis.\textsuperscript{3,4,9} In 2007, investigators identified a second rarer group of mutations seen in \textit{JAK2}\textsuperscript{V617F}-negative PV cases.\textsuperscript{12} These mutations involve the \textit{JAK2} exon 12 region. Together, the \textit{JAK2}\textsuperscript{V617F} and exon 12 mutations account for nearly all PV cases. To date, at least 10 different \textit{JAK2} exon 12 mutations have been identified.\textsuperscript{12-25}

The quintessential laboratory feature in the evaluation of PV is a low or low-normal serum erythropoietin level, indicating erythropoiesis independent of erythropoietin-sensing mechanisms.\textsuperscript{30} Bone marrow samples often show pan-myelosis with a relative predominance of the erythroid and megakaryocytic lineages. In classic \textit{JAK2}\textsuperscript{V617F}-positive cases, there is usually prominent megakaryocytic clustering composed of large, bizarre megakaryocytes with complex nuclear features.\textsuperscript{27-29}

In patients with \textit{JAK2} exon 12–positive PV, clinical features are similar to those in patients with classic PV, including low serum erythropoietin levels and prominent erythrocytosis.\textsuperscript{12-25} Unlike the well-established bone marrow morphologic findings in \textit{JAK2}\textsuperscript{V617F}-positive PV cases, little has been published on the specific bone marrow morphologic findings in \textit{JAK2} exon 12–positive PV cases, and published descriptions, as summarized in the following paragraph, appear somewhat inconsistent, a likely consequence of low case numbers and the inherent subjectivity of morphologic descriptions, especially in regard to megakaryocytic atypia.

In the landmark article describing \textit{JAK2} exon 12 gain-of-functions mutations in 10 \textit{JAK2}\textsuperscript{V617F}-negative cases, Scott et al\textsuperscript{12} showed that the bone marrow biopsies in half of the patients at the time of diagnosis showed erythroid hyperplasia
without morphologic abnormalities of the megakaryocytic lineage. Two subsequent articles noted erythroid and megakaryocytic hyperplasia in a total of 6 exon 12 cases with or without megakaryocytic atypia; the atypical megakaryocytic features were not further described.\textsuperscript{15,16} Percy et al\textsuperscript{18} described bone marrow biopsies in 7 JAK2 exon 12–positive cases; they found all to be hypercellular with a prominent erythroid hyperplasia; a minority of their cases (2 of 7) had mild megakaryocytic hyperplasia and atypia with occasional large megakaryocytes with nuclear hypolobation. No megakaryocytic clusters were observed in any of the cases.\textsuperscript{18}

Our study clarifies and adds to these previous reports. Of the 7 cases reviewed in this study, 4 were described in brief in a prior manuscript.\textsuperscript{13} The biopsies in JAK2 exon 12–positive PV cases all showed prominent erythroid hyperplasia; megakaryocytic hyperplasia and atypia were also present. Although JAK2 exon 12 cases typically lack the prominent clusters of large, bizarre megakaryocytes that characterize classic JAK2\textsuperscript{V617F}-positive PV, subtle and small megakaryocytic clusters and/or aggregates, as well as significant megakaryocytic atypia, can be identified in all cases. A spectrum of megakaryocyte sizes can be seen with a predominance of smaller forms with atypical megakaryocytic lobation and abnormal chromatin distribution. Immunohistochemical stains against hemoglobin, MPO, CD61, and CD34 help to confirm the morphologic findings but do not prove essential in making this diagnosis. These findings further confirm the

\textbf{Image 2 (cont) D and E.} Megakaryocytes with nuclear chromatin pattern varying from dispersed with prominent eosinophilic nucleoli to markedly hyperchromatic (D, H&E, ×1,000; and E, H&E, ×500). \textbf{F.} Prominent megakaryocyte clusters seen in only 1 case (H&E, ×500). \textbf{G.} Subtle megakaryocytic clusters/aggregates seen in the majority of cases (H&E, ×500).
erythropoietin level and absent for the patient. Clinically suspected PV with a low serum erythropoietin results for pathologists to know the serum erythropoietin levels. It is imperative for the bone marrow evaluation of any clinically suspected PV in isolation without clinical and laboratory information. In PV or even as MPN if the bone marrow sample is looked at may be initially difficult for pathologists to recognize as morphologic features, bone marrow samples from the patients JAK2 exon 12–positive PV cases lack the classic MPN morphologic features, and molecular analysis have become the standard basis to the diagnostic criteria of these diseases. Based on the recent WHO revised guidelines, the integration of clini- copharmacologic correlates of JAK2 exon 12 mutations in JAK2V617F–negative polycythemia vera. Leukemia. 2007;21:1960-1963.


References

role of morphologic studies in the review and classification of the MPNs.

The JAK2 story has significantly contributed to our basic understanding of the MPNs and has brought a molecular basis to the diagnostic criteria of these diseases. Based on the recent WHO revised guidelines, the integration of clinical features, laboratory findings, bone marrow morphologic features, and molecular analysis have become the standard approach to the MPN diagnostic evaluation. JAK2 exon 12–positive PV cases lack the classic MPN morphologic features, bone marrow samples from the patients may be initially difficult for pathologists to recognize as PV or even as MPN if the bone marrow sample is looked at in isolation without clinical and laboratory information. In the bone marrow evaluation of any clinically suspected PV case or in any patient with erythrocytosis, it is imperative for pathologists to know the serum erythropoietin results for the patient. Clinically suspected PV with a low serum erythropoietin level and absent JAK2V617F, together with the bone marrow findings of erythroid hyperplasia and subtle megakaryocytic atypia and/or clustering, should prompt an evaluation for a JAK2 exon 12 mutation.

From the Divisions of 1Hematopathology and 2Hematology, Mayo Clinic, College of Medicine, Rochester, MN.

Address reprint requests to Dr Hanson: Mayo Clinic, College of Medicine, 200 1st St SW, Rochester, MN 55905.

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