Diagnosing polycythemia vera: conventional tools amid molecular options—case report and brief review

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April 2016—Case. The patient presented in May 2013 at age 42 with a two-year history of fatigue and pruritus of his legs. He smoked one pack of cigarettes per day as he had for 25 years and had about five to six alcoholic drinks daily. Physical exam was unremarkable with no rash or palpable splenomegaly. Height was 74 inches and weight 189 pounds. Complete blood count: hemoglobin 21.9 g/dL (14.0–18.0); RBC 6.96×10^6/µL (4.50–6.00); MCV fL 90.1 (80.0–99.0); WBC 10.1×10^3/µL (4.5–10.8) with 71 percent neutrophils, 18 percent lymphocytes, eight percent monocytes, two percent eosinophils, and one percent basophils; platelets 154×10^3/µL. No significant poikilocytosis was reported. Ferritin was 9 mg/mL (26–388), iron 55 µg/dL (65–175), total iron binding capacity 431 µg/dL (250–450), iron saturation 13 percent (22–55), and reticulocytes 1.12 percent (0.20–2.44).

Bone marrow evaluation was performed. The aspirate showed trilineage hematopoiesis without overt dysplastic features. The M:E ratio was slightly low at 1.0. The biopsy had 70 percent cellularity with areas of relative erythroid hyperplasia and megakaryocytes were slightly increased. Focal-equivocal stainable iron was seen on the aspirate while no convincing stainable iron was seen on either the clot or biopsy sections. Flow cytometry showed no diagnostic abnormalities, and cytogenetics were 46,XY.

Further workup showed a low erythropoietin level of 1 mU/mL (4–24), and an allele-specific polymerase chain reaction study was negative for the *JAK2* V617F mutation. What is the next step in making a definitive diagnosis?

Discussion. Polycythemia vera, or PV, needs to be differentiated from other myeloproliferative neoplasms for optimal patient management that often includes therapeutic phlebotomy.[1] Although not included in current diagnostic criteria, an elevated red blood cell count is a very helpful but underused clue (e.g. above 5.2 × 106/µL for women or 5.8 × 106/µL for men), but each laboratory needs to establish its own cutoffs. Excessive erythropoiesis in PV consumes iron stores, and most patients with PV are at least borderline iron deficient, often with a low mean corpuscular volume. For this reason, hemoglobin levels, albeit generally elevated, often do not achieve conventional diagnostic cutoffs—16.5 g/dL for women and 18.5 g/dL for men.[2,3] In other words, these hemoglobin criteria have reasonably good specificity for identifying increased red cell mass, but they lack sensitivity.[4]

Correlated with the CBC data, review of the peripheral blood smear helps guide further evaluation. In PV the smear may be rather unremarkable; perhaps hypochromic ovalocytes will suggest iron deficiency. Nucleated red cells, often accompanied by increased poikilocytosis (most specifically teardrop forms), would raise consideration of myelofibrosis. Basophilic stippling would tend to exclude PV; it may be accompanied by target cells (suggestive of thalassemia) or a dimorphic red population (subpopulation of cells with decreased central pallor), which is a clue to possible ring sideroblasts. Micromegakaryocytes and, to a lesser extent, giant platelets point to dysplasia in that lineage. Circulating blasts at two percent or higher raise concern for disease progression and possibly even acute myeloid leukemia.

An elevated white cell count with a left shift, basophilia, and sometimes thrombocytosis characterize chronic myelogenous leukemia. For genetic confirmation, conventional cytogenetics is the preferred modality and can be performed on a peripheral blood sample if there is sufficient immaturity; greater than 1,000 neutrophils at the myelocyte stage or earlier is a criterion we use in our laboratory. A shotgun approach in which molecular testing is ordered for both the *BCR/ABL1* fusion product and *JAK2* V617F mutation is not cost-effective and is generally discouraged.

Although thalassemia can mimic PV with high RBC counts and low MCV values, in practice it rarely poses much difficulty in being distinguished from PV. In thalassemia, the hemoglobin is normal or even slightly low. Previous CBC data, if available, document the presence of these abnormalities throughout life in patients with thalassemia whereas they are acquired during adulthood in PV. Iron studies, the peripheral smear, ethnic background, and family history can also be useful in this regard.

Reactive erythrocytosis is most often due to chronic hypoxemia. Chronic obstructive pulmonary disease from cigarette smoking is the most common cause. Incidentally, smoking often causes modest increases in the neutrophil count (without a significant left shift) that may engender an unnecessary workup for myeloproliferative neoplasms. Morbid obesity resulting in restrictive lung disease, sometimes accompanied by sleep apnea with nocturnal desaturation, is another cause. Reduced oxygen saturation on room air would support a diagnosis of reactive erythrocytosis. Erythropoietin-secreting tumors are rare. High-affinity hemoglobinopathies are also rare; history, if available, would show this is a lifelong finding.

The normal physiologic response to chronic hypoxemia is an increase in erythropoietin production by specialized peritubular cells in the kidney. In PV, increased red cell production occurs independent of these regulatory mechanisms driving down EPO levels. Thus, in the appropriate clinicopathologic setting, a low EPO is strongly suggestive of PV. However, only about half of patients with PV have levels below usual reference ranges; often they are near the low end of the reference range. The author has rarely encountered molecularly confirmed post-polycythemic myelofibrosis with slightly elevated EPO levels when hemoglobin levels have dropped (due to blood loss or superimposed myelofibrosis, for example) and no longer suppress EPO production. Markedly or moderately elevated EPO levels (say 50 percent above the upper limit of normal) essentially exclude PV, while normal EPO levels are not particularly helpful. Despite these caveats, EPO testing is relatively inexpensive and recommended for all cases of suspected PV.

Although a diagnosis of PV can often be established without bone marrow evaluation, this procedure is helpful for a number of reasons. Generally in PV a variably hypercellular marrow is encountered due to a panmyelosis (increase in all three cell lines) with notably increased and somewhat large megakaryocytes. The recognition of so-called cloud-like or staghorn megakaryocytes has poor interobserver reproducibility and is not particularly helpful in differentiating myeloproliferative neoplasms, except chronic myelogenous leukemia, which virtually always has relatively small megakaryocytes. A “dry tap,” increased megakaryocytes, or a “streaming” appearance on the biopsy raise concern for myelofibrosis. However, these morphologic clues may be absent, and reticulin staining should be done in all myeloproliferative neoplasm cases.

Myelofibrosis invokes a differential diagnosis of primary myelofibrosis versus secondary MF in a setting of preexisting PV or, rarely, essential thrombocythemia. Previous CBC data with attention to RBC and hemoglobin values may suggest preexisting PV. Iron stores are low or absent in PV. Increased iron stores would raise a cautionary flag before making a diagnosis of PV. Conversely, essential thrombocytopenia or primary myelofibrosis should rarely be diagnosed in the setting of iron deficiency; if the diagnosis is unclear, repletion of iron stores and reevaluation is suggested. Cytogenetics should be done for all myeloproliferative neoplasms and may provide prognostic, or occasionally somewhat diagnostically specific, information. For example, a complex karyotype (three or more abnormalities) is unfavorable, while 1q duplications are common in post-polycythemic myelofibrosis.[5]

Although some authors from the Polycythemia Vera Study Group era lament its demise, red cell mass measurement no longer has a practical role in the diagnosis of PV, and it is rarely performed in 2016. In the author’s experience, red cell mass measurements were often misleading. Potential sources of error included poorly validated reference ranges and inadequate correction for obesity. Corresponding to its effect on hemoglobin levels, iron deficiency may suppress the red cell mass. Measurement of endogenous erythroid colony formation, like red cell mass, is no longer needed for clinical diagnosis and is now relegated to a research tool.

The *JAK2* V617F mutation is seen in about 95 percent of patients with PV, and testing is recommended in all suspected cases. Peripheral blood or bone marrow are equally acceptable samples. The mutational burden (percentage of *JAK2* exon 14 copies mutated) does not currently guide therapy (ruxolitinib, for example) and serial

testing is not indicated. If the *JAK2* V617F study is negative but the presentation is highly suggestive of PV (often isolated erythrocytosis), and if the EPO level is low, then testing for an exon 12 mutation by sequencing is indicated.

Take-home points for diagnosing polycythemia vera

- Review CBC data, especially looking for trends in hemoglobin, RBC, and MCV.
- Review the peripheral smear to look for abnormal features in all three lineages.
- For suspected PV, check the EPO level and test for a JAK2 V617F mutation.
- Strongly consider bone marrow evaluation (with cytogenetics and flow cytometry), especially if uncertainty remains or features suggest myelofibrosis or progression toward acute myeloid leukemia.
- Consider testing for an exon 12 mutation (preferably sequencing exons 12–15) if all three of the following are present: JAK2 V617F is negative, the clinical presentation is highly suggestive of PV, and EPO level is low (or at least low-normal).

JAK2 V617F mutations are also seen in about 50 percent of cases of essential thrombocytopenia or primary myelofibrosis and in the majority of cases of refractory anemia with ring sideroblasts associated with marked thrombocytosis (RARS-T). Calreticulin and MPL mutations are seen in about 25 percent of cases of essential thrombocytopenia or primary myelofibrosis, but only rarely in PV or RARS-T; the latter also usually has an *SF3B1* mutation.6 A number of laboratories now offer a myeloproliferative neoplasm diagnostic cascade starting with *JAK2* V617F, followed by *CALR*, and lastly *MPL*. Testing stops once one of these virtually mutually exclusive mutations is found.

Returning to the case study, follow-up sequencing of exons 12–15 surprisingly found the V617F mutation after all. Importantly, there was a second mutation in exon 14 (L607N) that likely disrupted primer binding by the allele-specific assay, resulting in a false-negative study initially. This case has a couple of other interesting points. The patient was iron deficient despite an MCV of 90.1 fL. Perhaps his alcohol consumption caused this; he was on no medications associated with macrocytosis. The smoking history certainly presented a compelling explanation for reactive (secondary) polycythemia. However, the very high hemoglobin and low EPO level appropriately prompted further evaluation, with PV ultimately confirmed by sequencing of exons 12–15. Had the reference laboratory sequenced only exon 12, the two mutations in exon 14 would have been missed.

In follow-up 2.5 years later, the patient receives therapeutic phlebotomy every other month and has not needed cytoreductive therapy. In December 2015, his hemoglobin was 15.9 g/dL, RBC 7.25×10^6/µL, MCV 70.3 fL, WBC 12.2×10^3/µL, and platelets 231×10^3/µL.

In summary, within the past decade a host of genetic abnormalities associated with myeloproliferative neoplasms

have been discovered that can refine our diagnoses and may lead to molecular-targeted therapies. Yet the hematopathologist's conventional tools—automated CBC, peripheral blood smear, and bone marrow morphology—continue to be the basis for formulating a differential diagnosis and directing the diagnostic evaluation in a reasonable and cost-effective manner.

References

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