ADVANCES IN HEMATOLOGY

Current Developments in the Management of Hematologic Disorders

Section Editor: Craig M. Kessler, MD

Diagnosing or Ruling Out Polycythemia Vera in Patients With Erythrocytosis

Josef T. Prchal, MD
Professor
Division of Hematology and Hematologic Malignancies
University of Utah Huntsman Cancer Institute and George E. Wahlen Department of Veterans Affairs Medical Center
Salt Lake City, Utah

H&O How is erythrocytosis defined?

JP Erythrocytosis is the presence of too many red blood cells. Several different parameters are used to diagnose erythrocytosis in a blood sample: the number of red blood cells, the hematocrit, and the hemoglobin concentration. Because the production of red cells is determined by the amount of oxygen delivered to tissues, it makes sense to use first the most physiologically relevant parameter—that is, the hemoglobin concentration. The hemoglobin concentration in most US locations ranges from 12 to 16 g/dL in healthy women of European descent, and from 14 to 18 g/dL in healthy men of European descent. The range is slightly lower in healthy African Americans, partly because of a high prevalence of the α-thalassemia trait in this population, which approaches 30%.

H&O How is polycythemia defined?

JP *Polycythemia* is a general term for the presence of too many blood cells. Erythrocytes are far more numerous than leukocytes and platelets, so the term is actually synonymous with *erythrocytosis*. No consensus regarding usage has ever been reached, and in each instance, the term *erythrocytosis* or *polycythemia* is used as originally described—that is, polycythemia vera, post–renal transplant erythrocytosis, Chuvash polycythemia, etc.

A polycythemia can be classified as primary, wherein the erythroid progenitors are intrinsically hyperproliferative, or as in vitro, in which the progenitors can grow without erythropoietin, or at an erythropoietin concentration that is lower than normal.

The primary polycythemias include *polycythemia vera*, which is a chronic leukemia-like condition, and *primary familial and congenital polycythemia*, which is due to a germline mutation of the gain-of-function erythropoietin receptor. Polycythemia vera is acquired, whereas primary familial and congenital polycythemia is dominantly inherited.

In contrast, *secondary erythrocytosis* or polycythemia is caused by circulating erythropoiesis-stimulating factors, typically erythropoietin. Secondary erythrocytosis can result from smoking, heart or lung disease, high altitudes, or supplemental testosterone. Alternatively, it can be inherited, caused by mutations in genes of the hypoxia-sensing pathway or by hemoglobin variants with a high hemoglobin affinity for oxygen. In *spurious polycythemia*, the red cell mass in the body is normal but the plasma level is decreased. The accompanying high hemoglobin concentration and hematocrit create the false impression that too many red cells are present. This situation typically occurs when a person becomes dehydrated and the plasma volume decreases. One form of spurious polycythemia is Gaisböck syndrome, which occurs primarily in obese men. Theories abound regarding the causes of Gaisböck syndrome, but they have not been definitively established.

H&O How do hematologists go about determining the presence of erythrocytosis in a particular patient?

JP The measurements that we typically use to determine the presence of polycythemia are limited by the fact that we take only a small sample of blood. From this single

sample, we can measure the following: (1) the relative proportions of blood cells (mainly red cells) and plasma—that is, the hematocrit; (2) the hemoglobin concentration in the blood; and (3) the red blood cell count. We do not, however, learn from these measurements how much red cell mass is in the body overall. A technique for measuring the number of red blood cells in the body that was used routinely earlier in my career involved taking a blood sample and adding a radioactive marker to the red cells and a separate marker to the plasma (labeled albumin), thus labeling both of these blood components. Following the in vitro manipulation, the blood sample was injected back into the patient's body. From the degree of dilution of the 2 markers, we could calculate the red cell mass and plasma volume per kilogram of body weight, which allowed us to differentiate between true polycythemia and spurious polycythemia. The procedure also allowed us to unmask hidden polycythemia, in which an increased red cell mass is present but is diluted in the blood by a concomitant increase of plasma. The technique produced more accurate information than currently available tests do, but unfortunately it is no longer widely available in the United States.

When I see a patient with elevated hemoglobin, my next step is to take a medical and family history. Differentiating between acquired and congenital polycythemia and between sporadic and familial polycythemia requires a time-consuming evaluation. Complicating matters is that polycythemia vera is always acquired, arising from a somatic mutation, but well-documented clusters of cases of polycythemia vera in families do exist. In certain instances, a patient with polycythemia vera may have some relatives with the same condition and other relatives with related but different myeloproliferative disorders, such as essential thrombocythemia or primary myelofibrosis, yet the conditions are all acquired rather than congenital. This finding suggests the existence of a not-yet-defined familial genetic predisposition to somatic mutations that lead to the development of these disorders.

H&O What are the symptoms of erythrocytosis?

JP The symptoms are hugely variable, depending on the cause. Erythrocytosis may cause no symptoms at all, or it may be highly symptomatic and detrimental to health. The symptoms of polycythemia vera may or may not be present. When they do occur they are quite specific, and include aquagenic pruritus, erythromelalgia, symptoms of arterial or venous thromboses, and gout. In addition, the risk for transformation to myelofibrosis in patients who have polycythemia vera is approximately 15%; in such cases, they present with fatigue, bone pain, sweating, and symptoms of splenomegaly, such as early satiety and/or splenic pain. The risk for transformation to acute leukemia is lower, at 3% to 5%, in which case the symptoms are the same as those in any acute leukemia.

A different set of symptoms is seen in patients who have an increased number of red cells because of an underlying pheochromocytoma, cerebellar or ophthalmic hemangioblastoma, or renal cancer; these patients have tumor-specific symptoms that are not the same as those of erythrocytosis. Resection of the tumor can resolve the condition.

Symptoms of other polycythemias/erythrocytoses are nonspecific, with most patients who have an increased number of red cells experiencing no symptoms. Rare patients may have symptoms such as fatigue and headaches resulting from hyperviscosity; these symptoms should resolve with phlebotomy.

Blood clots are the major complication in patients with polycythemia vera, and blood clots are even more common in those with Chuvash polycythemia. Common dogma dictates that a high hematocrit is the cause of blood thickening and blood clots, but I am skeptical that this is the major cause. No evidence exists that a high hematocrit is harmful and a direct cause of thrombosis; in addition, many conditions that lead to a very high hematocrit are not associated with thromboses. These include Eisenmenger complex, testosterone-induced erythrocytosis, and erythrocytosis resulting from a high hemoglobin affinity for oxygen. Growing evidence now indicates that other factors in polycythemia vera and Chuvash polycythemia contribute to blood clots. The best single blood cell parameter that correlates with thrombosis in polycythemia vera is the leukocyte count.

H&O How do hematologists go about determining the cause of erythrocytosis in a particular patient?

JP The first step is to determine how long the patient has had the condition. Does the red cell elevation go back to childhood? If so, the condition is likely congenital. Of course, it is often impossible to determine how long the elevation has been present because it may be that the patient's hemoglobin level is being tested for the first time in adulthood.

The next step is to address the family history. If erythrocytosis affects just one of the patient's parents and about half of the patient's siblings, the condition is likely dominantly inherited. If neither parent is affected but the patient has multiple siblings and approximately half of the siblings are affected, the condition is likely autosomal recessive. This information helps to narrow the diagnosis.

In cases of acquired erythrocytosis, I first look carefully at the pulmonary function. Is lung disease present? Is the patient a smoker? We measure arterial blood gases and how much oxygen is bound to hemoglobin. We also

measure carboxyhemoglobin because it is possible even for nonsmokers to have elevated carbon monoxide levels if they have a garage that is contaminated with car exhaust fumes or use equipment that is contaminated with carbon monoxide. Another form of hemoglobin is methemoglobin, in which the iron is in the ferric state rather than in the normal ferrous state; methemoglobin does not deliver oxygen to tissues. Methemoglobinemia can be either congenital or acquired. Both carboxyhemoglobin and methemoglobin bind oxygen so tightly that they are useless as oxygen carriers. All these conditions are either confirmed or ruled out by arterial blood gas testing. However, polycythemia vera may develop even in a smoker with pulmonary hypoxia and carboxyhemoglobinemia. When the clinician is in doubt, the presence of a *JAK2* mutation confirms the diagnosis of coexisting polycythemia vera.

If I am able to exclude arterial hypoxia, elevated carbon monoxide, and methemoglobinemia, I measure the erythropoietin level. This is generally low in polycythemia vera and even lower in primary familial and congenital polycythemia. If the erythropoietin level is high, or is inappropriately normal when the hemoglobin concentration is high, the patient may have a tumor that produces erythropoietin, or an abnormality in which the blood supply to the kidney tissue is inadequate and the tissue begins to produce erythropoietin.

If the subject has an inherited condition, it is possible that the patient was born with abnormal hemoglobin that binds oxygen too tightly, or even more rarely with low levels of 2,3-diphosphoglycerate (DPG) in red cells resulting from a congenital disorder of 2,3-DPG synthesis. Because less oxygen is released into the tissues from hemoglobin, the body compensates for the oxygen deficiency by increasing erythropoiesis until sufficient oxygen is delivered to the tissues. Phlebotomy is counterproductive in these patients, inevitably causes iron deficiency, and further elevates the erythropoietin level. The test to identify congenital forms of erythrocytosis is measurement of the hemoglobin-oxygen dissociation curve. If this curve is left-shifted (low P_{50}), the patient has a high-oxygen-affinity hemoglobin mutant; if it is right-shifted (high P_{50}), the patient has a low-oxygen-affinity hemoglobin mutant and the hemoglobin concentration may be decreased. If the instrument for measuring the hemoglobin-oxygen dissociation curve is not available, one can estimate the hemoglobin affinity for oxygen by measuring the partial pressure of oxygen, the oxygen saturation of hemoglobin, and the pH from venous blood gases, not arterial blood gases.

Other causes of congenital erythrocytosis with increased or inappropriately normal erythropoietin levels are inherited disorders of hypoxia sensing, in which a mutation in the hypoxia-inducible pathway that regulates erythropoietin production causes the body to increase red cell production. Hypoxia-inducible factors (HIFs; the 2 best-understood isoforms are HIF-1 and HIF-2) are master transcription factors that regulate multiple genes, including the erythropoietin gene. Patients with increased HIFs produce too much erythropoietin, which leads to the production of too many red cells. Increased HIFs can be caused by mutations resulting from loss of function of HIF negative regulators. The result is Chuvash polycythemia, caused by an alteration in the von Hippel-Lindau (*VHL*) gene or a prolyl hydroxylase mutation. Other mutations are from gain of function of HIF-2α, the principal regulator of erythropoietin production.

H&O Now that we have the ability to measure erythropoietin levels and test for *JAK2* mutations, do physicians still need to measure red cell and plasma volume?

JP I agree that the availability of *JAK2* mutation testing enables a specific diagnosis of polycythemia vera in most patients rapidly and accurately. However, as previously discussed, occasional cases of spurious polycythemia or hidden polycythemia cannot be diagnosed with a routine blood cell count. Measurement of the red cell and plasma volume is not available in most medical centers in the United States because of what I consider to be an exaggerated concern about radioactivity. The test does require the use of radioactive chromium, but the amount of radiation is very small—comparable to the natural radioactivity that a person is exposed to on a long airplane flight.

Fortunately, it is faster and easier to test for *JAK2* mutations when patients have elevated levels of red cells. Virtually all patients with polycythemia vera—approximately 99%—have this somatic mutation, which increases the production of red cells and sometimes platelets and neutrophils as well.

H&O Are any causes of secondary polycythemia known besides high altitude, smoking, heart disease, lung disease, and testosterone?

JP We used to think that sleep apnea was a cause, which makes sense because someone who stops breathing at night would produce more erythropoietin—at least in theory. The evidence does not support this association, however. Some patients develop polycythemia after kidney transplant, which is known as *post-transplant erythrocytosis.* Patients may develop increased levels of cobalt and manganese, or tumors that secrete erythropoietin. Some patients engage in surreptitious doping with erythropoietin.

H&O Do you treat symptoms of severe iron deficiency in phlebotomized patients with polycythemia?

JP Yes, I absolutely do. We have a lot of evidence indicating that ignoring iron deficiency is bad medicine. Hemoglobin requires iron, as do the muscles, the brain, and all other tissues. In addition, iron is essential for the degradation of HIF (iron is a cofactor of HIF's principal negative regulators; ie, prolyl hydroxylases), and so iron deficiency further increases erythropoietin production and erythropoiesis. If someone who has pulmonary hypertension and too many red cells in a high-altitude environment (chronic mountain sickness) is treated with phlebotomy, we have created iron deficiency and worsened the pulmonary hypertension. I believe that always using phlebotomy to treat all forms of polycythemia is misguided and can even be harmful. It can improve the laboratory test results, such as the hemoglobin level, which makes us physicians feel better but is bad for the patients. If symptomatic iron deficiency develops in a patient following phlebotomy, we can address that with a short course of oral iron supplementation, and the patient will usually experience an immediate decrease in fatigue and improvement in quality of life. I generally prefer to normalize the blood cell counts in polycythemia vera with hydroxyurea or another treatment. The 2 other options currently available are pegylated interferon and the JAK2 inhibitor ruxolitinib (Jakafi, Incyte). No proven therapy exists for congenital disorders of hypoxia sensing or high hemoglobin affinity for oxygen.

H&O Could you explain the mechanisms of Chuvash polycythemia and what it has taught us about erythropoiesis?

JP This syndrome was first described among people of Asian origin living in a European area of Russia, the Chuvash Autonomous Soviet Socialist Republic; however, it is present worldwide, with another area of endemicity in the Italian island of Ischia. Chuvash polycythemia is caused by a germline mutation in the *VHL* gene that is inherited in an autosomal-recessive manner from both parents. This disorder of hypoxia sensing, the first to be described, results from a loss-of-function mutation in a negative regulator of HIFs, the *VHL* gene. HIFs have 2 subunits; either HIF-1α or HIF-2α combines with HIF-β to form either an HIF-1 or HIF-2 dimer. Only intact HIF dimers have function. In the presence of oxygen, the subunits of HIF-α undergo prolyl hydroxylation by prolyl hydroxylase; to function, this enzyme requires the presence of iron and it is inhibited by cobalt and manganese. Hydroxylation of the proline in the subunits of HIF-α changes the configuration of these proteins, which then bind to the von Hippel-Lindau protein, resulting in their ubiquitination and rapid proteasomal degradation. Thus, people with Chuvash polycythemia have a congenital defect leading to high HIF levels and thus the production of excessive amounts of erythropoietin.

Intriguingly, other *VHL* mutations cause tumor predisposition syndrome, whereas the Chuvash *VHL* mutation causes congenital polycythemia, but not tumors. This mutation not only results in a very high level of HIF and increased erythropoietin (secondary erythrocytosis) but also causes erythroid progenitor hypersensitivity to erythropoietin, a feature of primary polycythemia. The morbidity and mortality of Chuvash polycythemia result principally from an increased occurrence of thrombosis that is not relieved and is even increased by phlebotomy; however, the cause is not the high hematocrit but too much HIF, which dysregulates genes in the thrombotic pathway (protein S, tissue factor, thrombospondin 1, and likely others).

A least 2 other disorders in addition to Chuvash polycythemia are congenital disorders of hypoxia sensing—caused by an HIF-2a (encoded by the *EPAS1* gene) mutation. The same molecular and pathophysiologic defects associated with the inherited *EGLN1* mutation (encoding prolyl hydroxylase 2) can also be acquired from cobalt and manganese poisoning, as well as iron deficiency. These conditions block the activity of prolyl hydroxylase and increase HIFs.

Disclosure

Dr Prchal has no conflicts to declare.

Suggested Readings

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