Chapter 1
Polycythemia Vera and Other Polycythemia Syndromes

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1.1 Introduction

Polycythemia vera (PV) is an acquired clonal hematopoietic stem cell disorder characterized by the overproduction of red and white blood cells and platelets in the absence of any appropriate stimulus for these events. For this reason, it is considered one of the four myeloproliferative diseases, which include essential thrombocythemia, primary myelofibrosis (PM), and chronic myeloid leukemia, all diseases which reflect varying degrees of erythroid, myeloid, and megakaryocytic marrow hyperplasia. Polycythemia, in general, can be defined as an increase in the volume of circulating red cells per kilogram of body weight or, equivalently, an increase in the red blood cell mass. Clinically, this is expressed as an absolute increase in the number of red cells, usually but not always accompanied by corresponding increases in the hemoglobin and hematocrit. Polycythemia might occur as a result of a primary disease of unknown cause, PV, or as a secondary manifestation of other illnesses. In the past, the diagnosis of PV was often an exclusionary diagnosis; now it is made more easily because of the *JAK2*^{V617F} molecular abnormality (*vide infra*) found in this disease. Untreated PV leads to thrombohemorrhagic complications and eventually to progressive myelofibrosis, anemia, and splenomegaly.

1.2 Definitions

The terms "erythremia" and "erythrocytosis" are often used to refer to primary and secondary polycythemia, respectively. Others use erythremia as a classification for patients whose only abnormality is an increased red cell volume. The term "relative" polycythemia is a misnomer. A better term is "false" polycythemia, because it is not polycythemia since the red cell volume per kilogram of body weight is normal. In this instance, the increased hematocrit is related to a decrease in plasma volume. In PV, the plasma volume may be increased, normal, or decreased. Other terms referring to false or relative polycythemia include stress polycythemia, stress erythrocytosis, pseudopolycythemia, and benign polycythemia (1).

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The determination of red cell volume per kilogram of body weight separates both primary and secondary polycythemia from false polycythemia. PV is differentiated from secondary polycythemia by other means (to be described) but *not* by a red blood cell volume determination.

1.3 Presenting Signs and Symptoms

Polycythemia vera is twice as common in men as in women. It is usually a disease of middle age, but its range extends broadly from 20 to 60 or 70 years. Forty percent of our patients are under age 50 (1); rare cases of PV have been described in children (2). PV has been reported rarely in African Americans and is said to occur with increased frequency in Jews (3). Whether the latter observation is related to selection by virtue of specific hospital populations is not clear. The development of symptoms, which is often insidious, is related to increased red cell mass and whole-blood viscosity and an increased number of platelets. These symptoms include easy fatigue, weakness, shortness of breath, dizziness, tinnitus, bone pain, visual disturbances, headaches, erythromelalgia, pounding in the ears, and pruritus. The pruritus increases in intensity after a tub bath and less often after showering. It is worse when the temperature of the water is warm rather than cool. Burning or throbbing pain in the legs, feet, or hands might occur, which might be accompanied by a mottled redness.Abnormalities of coagulation are responsible for the hemorrhagic tendency and paradoxically contribute to thrombotic episodes. Either might be the presenting manifestation of PV, but thrombotic events predominate. Most often, these episodes result from qualitative and quantitative platelet abnormalities or to acquired abnormalities of Von Willebrand's factor (4), which resembles type II v WF disease.

The most common source of major hemorrhage is the upper gastrointestinal tract, where peptic ulcer has been reported in about 10% of patients (5). Thus, iron deficiency might distort the overall picture because of anemia, hypochromia, and microcytosis. Sometimes iron deficiency occurs in a previously polycythemic woman owing to menometrorrhagia producing the same distorted picture. In these instances, the nature of the underlying polycythemia is revealed when hemorrhage is controlled and the anemia has been treated with iron.

Patients referred because of small vessel thrombosis of the hands or feet may suffer from polycythemia. Erythromelalgia might be observed in PV, especially in those patients with significant thrombocythemia (6). Thrombosis of larger vessels may involve the arterial and venous circulations of the heart, brain, liver, spleen, gastrointestinal tract, and lung. Thrombophlebitis is common. Acute gouty attacks can accompany excess production of uric acid (7).

Particularly serious thrombotic events typically associated with PV include abdominal vein thrombosis (e.g., Budd-Chiari syndrome) and obstruction of the portal, mesenteric, and/or splenic system (8). In this case, red cell mass studies in addition to a *JAK2*^{V617F} determination can be extremely helpful because erythropoietin

levels may be increased (9) and the presence of splenomegaly might not be diagnostically helpful. If the *JAK2*^{V617F} abnormality is found and the red cell mass is increased, phlebotomy is usually required (10).

1.4 Physical Findings

In the early stage of PV, the conjunctivae are injected. The liver is palpable in about 50% of patients, and the spleen is palpable in about 60% of patients (1). The size of the spleen depends on the stage of the illness and ranges from barely palpable below the left costal margin to massive enlargement filling the left half of the abdomen. Extremely large spleens might herald the future development of myelofibrosis and myeloid metaplasia.

1.5 Hematologic Manifestations

Red cell counts of 7–10 million cells/μL, hematocrit values of 55–70%, and hemoglobin concentrations of 18–24 mg/dL have been observed (1). Often the hemoglobin is not increased as much as the red cell count; thus, the hematocrit might be slightly reduced in proportion to the erythrocyte count. If there has been hemorrhage, the hemoglobin content of the red blood cells is reduced more than their size, and the red cells appear hypochromic and microcytic. In general, however, the individual erythrocytes appear normocytic and normochromic. A rare normoblast may be found. The number of reticulocytes is usually normal except after hemorrhage.

In about half of the patients, there is a moderate increase in the peripheral leukocyte count, ranging from 10,000 to 30,000 cells/μL. Values greater than 50,000 cells/μL have been reported (11). Metamyelocytes may be seen in the peripheral blood smear. An increase in the absolute basophil count may be seen and might be related to certain features of PV, such as peptic ulcer and pruritus.

The number of blood platelets is increased above 400,000/μL in more than 80% of patients at the time of diagnosis and assumes diagnostic importance (12). In most of these patients, the platelet count ranges between 500,000 and 1 million/μL, but in some, the initial platelet count may be more than 1 million/μL. On occasion, the platelet count may increase after spontaneous bleeding or phlebotomy, although there has been no specific correlation between platelet count before phlebotomy and thrombosis.

The bone marrow in patients with PV is hypercellular, and marrow fat is reduced, findings particularly well appreciated in biopsy sections (13–15). Each of the three developmental series, erythroid cells, granulocytes and megakaryocytes, typically participate in the hyperplasia, although erythroid hyperplasia is paramount. (In contrast, the hyperplasia in *secondary* polycythemia is typically confined to the erythroid

series.) There is no abnormality in erythroid maturation; orthochromic normoblasts predominate. There may be a slight shift to the left in the neutrophilic series. Eosinophils and basophils are prominent.

Bone marrow examination by itself cannot unequivocally distinguish primary and secondary polycythemia. However, a hyperplastic granulocyte series with evidence of cellular immaturity and an increase in megakaryocytes of variable size favor PV (16). Marrow iron stores are depleted more often in primary rather than in secondary polycythemia, a finding of diagnostic importance. However, because secondary iron deficiency is so common in women, the absence of marrow iron might not always be of significant differential diagnostic value in the female patient.

Normally, reticulin fibers, also known as type 3 collagen, extend throughout the marrow and provide a supporting stroma for hematopoietic cells. In disease states, two different patterns of the reticulin network can be recognized. The first consists of an increased prominence of the normal network, which probably occurs in response to an increase in functioning hematopoietic tissue. It is a nonspecific change, because it is found in many hematologic disorders (13–16). This second, or "fibroblastic," pattern, consisting of type 1 collagen, results in coarsening of the network, resulting from the formation of fibers that are thicker than normal. These thick fibers tend to form bundles or fascicles that follow a waving or swirling course. The fibroblastic pattern is seen in patients with PV and other myeloproliferative diseases (14–16).

1.6 Hyperuricemia

Hyperuricemia, a characteristic of all myeloproliferative diseases, occurs in many patients with PV. A good correlation between serum urate concentration and red and white cell counts has not been demonstrated. The incidence of gout in patients with PV has ranged from 5% to 10%. Although gout may precede the development of PV, it usually occurs 5–10 years after the onset of the disease (1).

Increased urate production and excretion may result in the precipitation of uric acid in the kidneys, causing stones or uric acid nephropathy. Renal colic is an unusual presenting manifestation of patients with PV, but it has been observed (1). Although serum uric acid may be increased in secondary polycythemia, it is rare to find the same high concentrations that occur in the primary disease.

1.7 Natural History

An appreciation of the natural history of PV is required because a patient might be observed during a stage when the disease is in transition. PV is an illness of reasonably long duration, usually ranging from 10 to 20 years. Although phlebotomy and other treatment modalities influence the course of the disease and its clinical

manifestations, unsuccessful treatment and/or the natural history of the disease after a number of years is associated with a fall in hemoglobin concentration and the gradual development of anemia. The spleen (and sometimes the liver) progressively enlarges and may fill the entire abdomen. Splenic infarcts, causing mild to severe left upper quadrant pain, may mimic an acute abdominal crisis or renal colic. Rarely, the enormous spleen causes large bowel obstruction. Erythrocytes of variable size and shape, teardrop forms, nucleated red cells, and immature granulocytes appear in the peripheral blood. The white blood cell count, often normal or moderately increased throughout the early and middle phases of the illness, continues to rise. Blood platelets may increase or decrease in number. Bone marrow examination at this time reveals a prominent "fibroblastic" and reticulin network, myelofibrosis, and osteosclerosis. Although the degree of extramedullary hematopoiesis (myeloid metaplasia) generally is proportional to the duration of the disease, some patients display features of it early in the course of the illness and even relatively early during the polycythemic phase. Without an antecedent history of PV, it might appear that the patient is suffering from primary myelofibrosis (PM) with myeloid metaplasia. Sometimes the clinical and hematologic picture resembles chronic myeloid leukemia, which is readily excluded by examining the peripheral blood or marrow for the *BCR-ABL* gene rearrangement. Moreover, the Philadelphia chromosome, the hallmark of chronic myeloid leukemia, is never present in PV. Acute myeloid leukemia occurs as a terminal event in PV (17), indistinguishable from that which occurs in myelofibrosis with myeloid metaplasia or myeloid blast-phase chronic myeloid leukemia.

1.8 Diagnostic Tests

1.8.1 JAK2 ^{V617F}

Within the past few years, the finding of a specific mutation in the JAK-STAT signaling pathway in PV, essential thrombocythemia, and primary myelofibrosis with myeloid metaplasm has revolutionized our diagnostic abilities and our understanding of the pathophysiology of these disorders.

There are four members of the Janus kinase (JAK) family, Janus kinases 1, 2, and 3 and tyrosine kinase 2 (JAK1, JAK2, JAK3, and TYK2, respectively), with slightly different functions. Each has a kinase domain and a catalytically inactive pseudokinase domain, which has an important regulatory function. To some, the presence of these two similar domains in the protein, one active and the other inactive, suggested the Roman god of gates and passageways, Janus, who had the ability to look simultaneously in two directions (18).

The JAK proteins function as intermediates between membrane receptors and signaling molecules. When particular cytokines or growth factors bind to their receptors on JAK proteins, the kinases associated with the cytoplasmic regions of these

receptors become phosphorylated and thereby activated. This activation causes docking sites for downstream molecules, notably those of the STAT system (signal transducers and activators of transcription), which become activated and enter the nucleus, where they function as transcription factors. JAK2, the third Janus kinase discovered, is activated particularly when receptors bind to hematopoietic growth factors, also known as type 1 cytokine receptors, such as erythropoietin, granulocyte-colony stimulating factor (G-CSF), granulocyte macrophage-colony stimulating factor (GM-CSF), and thrombopoietin (18, 19).

The *JAK2*^{V617F} mutation lies within the negative regulatory domain of JAK2 known as the pseudokinase or Janus homology 2 (JH2) domain. In this mutation, phenylalanine is substituted for valine in codon 617 (V617F) of the JAK2 nucleotide 1849, which leads to independent activation of cellular pathways in erythropoietic receptor signaling and without need for erythropoietin. The mutation might be either heterozygous or homozygous. Homozygosity is thought to be related to more advanced disease (20) and to predict for major vascular events (21).

Our studies (22) and those of others (21–25) have shown that JAK2 is present in more than 95% of patients with the clinical phenotype of PV (19–23). It is also found in approximately 50% of patients with essential thrombocythemia (ET) or primary myelofibrosis, about 3% to 5% of patients with myelodysplasia, acute myeloid leukemia, systemic mastocytosis, and chronic neutrophilic leukemia, and 10–30% of normal individuals (20–25). Loss of the V617F gene has been observed in previously positive PV patients who develop acute myeloid leukemia (26).

The search for new mutations in members of the JAK family, particularly in those patients with V617F negative polycythemia has been the subject of intense scrutiny. New mutations, indeed, have been found in members of the JAK and STAT families in patients with V617F negative PV or idiopathic erythrocytosis. Four somatic gain of function mutations affecting JAK2 exon 12 have been described in 10 patients negative for *JAK2*^{V617F} (27). These were found in marrow samples from patients who presented with an isolated erythrocytosis and distinctive bone marrow morphology. The platelet counts were less than 450,000/μL and the neutrophil counts were normal. Some also had reduced serum erythropoietin levels. Erythroid colonies could be grown from blood samples in the absence of exogenous erythropoietin. These erythroid colonies were heterozygous for the mutation, whereas colonies homozygous for the mutation occurred mostly in patients with V617F-positive PV. Three of the exon 12 mutations included a substitution of leucine for lysine at position 539 of JAK2.

The discovery of the JAK2 abnormality has broad implications for the present and the future both for diagnosis and treatment. There is now an intense search for new therapeutic agents that may affect JAK2 expression and might be effective in the treatment of PV and related disorders. The sensitivity of the techniques for detecting and measuring *JAK2*^{V617F} or its variants will improve in the immediate future. Other mutations, similar to those described earlier, may soon be discovered. In the meantime, it is still important to review a recommended method for the diagnosis of PV in those patients with the phenotypic clinical and hematologic

Table 1.1 Clinical classification of polycythemia

Primary polycythemia (polycythemia vera)
JAK2^{V617F} – Positive *95+ % (and variants)*
JAK^{V617F} – Negative *< 5 %*
Secondary polycythemia – JAK2^{V617F} negative
Related inadequate oxygen delivery to tissues with respect to need
Due to increased arterial oxygen tension
With physiologic or anatomic cardiopulmonary abnormalities
Abnormalities of lungs, chest, bellows, or ventilator control mechanisms
Right-to-left vascular shunts
Without physiologic or anatomic cardiopulmonary abnormalities
Impaired oxygen-carrying capacity of hemoglobin
Normal oxygen-carrying capacity of hemoglobin
Truncated erythropoietin receptor
Congenital polycythemias (e.g., "Chubash")
Due to increased blood flow-congestive heart failure
Unrelated to inadequate oxygen delivery of need usually accompanied by increased serum levels of erythropoietin and associated with benign or malignant lesions:
Kidney—cysts, hydronephrosis, hypernephroma, sarcoma
Cerebellum—hemangioblastoma
Uterus— myoma
Liver—hepatoma, hamartoma
Other—adrenal (pheochromocytoma), lung

characteristics of PV in whom a *JAK2^{V617F}* abnormality cannot be demonstrated. Until recently, the diagnosis of PV was essentially an exclusionary one, usually based on the criteria of the Polycythemia Vera Study Group (PVSG) or its many suggested modifications (28, 29). An alternative schema, which we have used and validated, is shown in Table 1.1.

1.8.2 Endogenous Erythroid Colonies

For many years, the measurement of endogenous erythroid colonies had been an important marker for diagnosing PV (30). However, this is a laborious test usually restricted to research laboratories, and from a diagnostic standpoint, this has fallen out of diagnostic favor because of the use of the JAK2 determination. However, it is described here because of its biologic importance.

In normal individuals, erythroid colonies grow only in culture media that are supplemented with erythropoietin (EPO). Erythroid progenitor cells obtained from blood or marrow from PV patients grow in semisolid serum-containing cultures in the absence of EPO (27, 31). This spontaneous growth of erythroid colonies, known as endogenous erythroid colony formation, is observed in some cases of essential thrombocythemia and idiopathic myelofibrosis but not in normal subjects. The formation of these cultures was first thought to be to the result of hypersensitivity

of the cells to tiny amounts of EPO in the culture media and to sensitivity to other growth factors such as interleukin (IL)-3, stem cell factor, GM-CSF, and thrombopoietin, and GM-CSF insulin-like growth factor-1 (31,32). The augmented response of erythroid progenitors to growth factors suggested there was an abnormality in EPO signaling that controlled the process of proliferation, maturation, and cell death. However, subsequently, it became clear that growth of these colonies was truly independent of EPO because it was not blocked by anti-EPO neutralizing antibodies (31).

1.8.3 PRV-1 mRNA Overexpression

A molecular marker of interest in PV is the measurement of granulocyte levels of PRV-1 mRNA. Several studies suggested that overproduction of PRV-1 mRNA could be a marker of clonal hematopoiesis in PV (33–36). The frequency of finding this marker could be attributed to differences in sampling technique (37). Furthermore, elevations in granulocyte levels of PRV-1 mRNA are not specific for the chronic myeloproliferative diseases as initially believed. PRV-1 values, like the leukocyte alkaline phosphatase score, are higher in pregnancy, infection, and after G-CSF administration (36, 37). Thus, it appears to be a secondary phenomenon to myeloproliferation rather than a primary event, similar to high serum B_{12} levels and increased leukocyte alkaline phosphatase values (36–38).

1.8.4 Thrombopoietin Receptor (c-MPL)

Abnormalities of thrombopoietin receptor, c-MPL, have been involved in our understanding of the myeloproliferative diseases. Intuitively, one might think the expression of c-MPL would be increased in PV and ET; in fact its expression is reduced in ET and PV (39). This is related to impaired c-MPL glycoslation rather than c-MPL gene disruption or repression of transcription factors (40). Moreover, because c-MPL expression is also reduced in Idiopathic Myelofibrosis (IMF), it is not valuable as a diagnostic marker, notwithstanding its biologic interest.

1.8.5 Hematocrit

Polycythemia is first suspected most commonly because of abnormalities of hematocrit values. However, the extent of reliance on the hematocrit for the diagnosis of polycythemia is the source of much confusion both at the bedside and in published texts. Despite a reasonably good correlation between the hematocrit and the circulating total red cell volume over a wide range of hematocrit

determinations, the value of a single hematocrit (HCT) measurement for predicting total red cell volume is poor (28) unless the hematocrit is more than 60% (41–44). At this level, there is excellent correlation between the hematocrit determination and actual red cell mass. For values of HCT ≤ 60%, the red cell mass must be measured with radioactive chromium (Cr^{51}) to ascertain whether it is increased. This is especially true for modest increases in HCT. To compare total red cell volume from one individual to another, it is best to express it in terms of corrected cubic centimeters per kilogram of body weight. Because fat tissue is relatively avascular, obese individuals might have a falsely low red cell mass. A satisfactory and widely used method for determining red cell volume is the direct tagging of red cells with Cr^{51}. The method is easy to perform and is reproducible with a coefficient of variation of 1.5% (28, 42–44). The red cell mass for a normal man is less than 36 cc/kg and for a woman is less than 32 cc/kg. Adjustments are made for obesity (42–44). A normal red blood cell mass might be found on occasion in patients with PV (e.g., after recent hemorrhage). In JAK2-negative patients with the phenotypic characteristics of PV, the diagnosis still relies upon the clinical criteria that has been discussed (*vide infra*).

The World Health Organization (WHO) recently revised its criteria for the diagnosis of PV (45) and recommended Cr^{51} red cell mass values as just described among other criteria for the diagnosis of polycythemia (45). However, it also allowed other surrogate markers for this test, including a hemoglobin value in men of 18.5 g/dL and a value of 16.5 g/dL or more in women, as published earlier (46). It is not clear how some of the hemoglobin values were selected (44). The use of a single hemoglobin value is no more reliable than the use of single hematocrit value, particularly in the lower ranges of presumed red blood cell mass increase (41). Moreover, hemoglobin values are often comparatively lower than hematocrit and red blood cell values because of iron deficiency. It is unfortunate that the use of these hemoglobin values has replaced the red cell mass in many reports and studies of PV (47) because it permits the addition of patients to studies who might not have had the disease. Moreover, the detection of endogenous erythroid colonies is a laborious process restricted to certain research laboratories. Thus, it is difficult to understand the reason why it was recently listed as a minor diagnostic criterion for PV by WHO, as its use will be so restricted.

1.8.6 Erythropoietin

Even in the JAK2 era, the easy availability of a relatively routine test for measuring serum erythropoietin remains important in the diagnosis of PV. Decreased arterial oxygen tension typically leads to increased red cell production, presumably mediated by erythropoietin. A major exception is in patients with emphysema who have diminished arterial oxygen tension but normal red cell mass (48). An increase in erythropoietin has, nevertheless, been demonstrated in some of these patients,

implying that these patients are not polycythemic because of an inability to produce erythropoietin.

Polycythemia unrelated to tissue hypoxia might be due to autonomous erythropoietin production by benign or malignant tumors (49, 50). Biologically active erythropoietic substances, which have been demonstrated in plasma, urine, and tissue extracts from such patients, are not subject to physiologic control mechanisms and are independent of the red cell mass. The causal relationship between polycythemia and tumor is indicated by the fact that, in many patients, remission of polycythemia has occurred following removal of the tumor (51, 52). This type of polycythemia has been most frequently associated with renal abnormalities, especially neoplasms and benign cysts (51). From a diagnostic standpoint, it is important to stress that with polycythemia accompanying tumors, there might be increases in the platelet and white cell counts; even splenomegaly has been reported. To confound the situation even more, the coexistence of primary and secondary polycythemia (e.g., PV and chronic pulmonary disease) can occur. Therefore, a high level of diagnostic vigilance must be maintained.

In contrast to the observations in polycythemia secondary to anoxia and tumors, the polycythemia of PV is not oxygen dependent and is not usually associated with increased erythropoietin in plasma or urine. In fact, erythropoietin output is less than normal, because red cell production by the marrow is autonomous and erythropoietin output is suppressed by the increased red cell mass.

Despite the aforementioned, our data suggest that only low erythropoietin levels are of value in diagnosis. In analyzing our own data, we were surprised to find the number of normal or elevated EPO values in *JAK2*^{V617F}-positive patients at diagnosis; the cause for this is not clear (Table 1.2). The relation between erythropoietin and polycythemia can be summarized as follows:

1. Increased, but regulated, erythropoietin production is proportional to the stimulus in anemic but normal individuals and secondary hypoxemic polycythemia.
2. Autonomous erythropoietin production irrespective of red cell mass is observed in secondary polycythemia accompanying erythropoietic related tumors.
3. Autonomous erythropoiesis by the marrow, unrelated to erythropoietin production, and usually decreased levels of serum erythropoietin in PV.

Table 1.2 EPO levels determined in 77 PV patients at diagnosis at New York Presbyterian Hospital

EPO Level (mU/mL)	Number (%)
<5	39 (51%)
5–10	24 (31%)
10–15	7 (9%)
>15	7 (9%)

1.8.7 Arterial Oxygen Saturation

Causes of secondary polycythemia can be classified for clinical use as (1) those related to inadequate oxygen delivery that does not meet tissue needs and (2) those that are not related to oxygen delivery. Customarily, an arterial oxygen saturation of 92% or more has been considered characteristic of PV, whereas a lower saturation has been regarded as evidence of polycythemia related to hypoxemia. Recent studies have cast doubt on the usefulness of arterial oxygen saturation measurements in subtle cases in differentiating primary and secondary polycythemia, even when the latter is related to impaired arterial oxygen supply (48, 53). For example, some patients with pulmonary disease have respiratory alkalosis due to hyperventilation. This relates to the influence of pH on the oxyhemoglobin dissociation curve. Oxygen saturation in these patients might be normal even though arterial oxygen tension is significantly reduced. Of course, in primary PV, the JAK2 molecular abnormality is found, whereas in those cases of PV due to inadequate oxygenation the JAK2 test will be "negative."

1.8.8 Vitamin B and Its Binding Proteins

In PV and other myeloproliferative diseases, concentrations of serum vitamin B and vitamin B_{12} capacity are increased more than normal, paralleling the degree of leukocyte proliferation. Serum vitamin B_{12} levels tend to be modestly increased in PV and greatly increased in chronic myeloid leukemia (54). A similar, but more striking, increase occurs in the unbound B_{12} binding capacity (54). This test also had diagnostic value in days prior to JAK2.

1.8.9 Leukocyte Alkaline Phosphatase

Because of the ready availability of other tests to exclude chronic myeloid leukemia, including cytogenetics, fluorescent *in situ* hybridization tests, and analysis of the peripheral blood or marrow for the abnormal *BCR-ABL* gene by polymerase chain reaction (PCR), the LAP test is rarely used now. It is described briefly because of references to it in the older literature. The test was used as a diagnostic criterion of PV by the PVSG and to distinguish patients with PV from those with chronic myeloid leukemia (55). The principle of the LAP test is based on the amount and staining intensity of a dye precipitated at presumed sites of enzyme activity within mature and band neutrophils, which are the only granulocytes with significant LAP activity in the peripheral blood or bone marrow. Usually, peripheral blood is used and scored semiquantitatively; 100 neutrophils are graded 0 to 4+. The total score thus ranges from 0 to 400.

Abnormally low or absent LAP activity is observed in chronic myeloid leukemia and in some patients with myelofibrosis with myeloid metaplasia. A marked increase both in LAP activity and in the number of neutrophils showing such activity may be seen in PV, essential thrombocythemia, leukemoid reactions, and some patients with myelofibrosis with myeloid metaplasia (primary myelofibrosis).

The normal LAP score ranges from 25 to 50. In leukemoid reactions and in PV, it could rise to more than 200, whereas in chronic myeloid leukemia, it is usually less than 25 (55).

1.9 Differential Diagnosis of Polycythemia Vera

The JAK2 abnormality has made the diagnosis of PV much easier. As previously mentioned, it is found in 95% of patients with PV. However, because it is also found in approximately 50% of patients with ET and 50% of patients with PM, if the *JAK2*^{V617F} abnormality is found, and if the HCT value is slightly elevated above normal median values, it is mandatory to perform a Cr^{51} red blood cell study to determine whether there is an increase in red cell mass. (In our institution we also routinely perform a radioactive iodinated albumin plasma volume study, which thus allows us to measure total blood volume.) This is especially important in patients with ET who have the *JAK2*^{V617F} mutation for these patients have been reported to have a higher hemoglobin level than those without the mutation (56); it has been suggested these patients might evolve into PV. Of course, unless a red cell mass study is initially performed, it is impossible to unequivocally exclude PV at the onset of the illness, as the true red cell volume will not be known.

What is the approach for patients who are polycythemic but do not have the molecular JAK2 abnormality? As previously mentioned, the *JAK2*^{V617F} mutation is found in approximately 95% of PV patients, a figure consistent with the findings in our own institution (Table 1.1). Among the 5% JAK2-negative PV patients, some carry the JAK2 exon 12 mutation. These patients have a different phenotype, because they do not have thrombocytosis or splenomegaly and the bone marrow shows only slight erythroid hyperplasia. However only one of our four *JAK2*^{V617F}-negative PV patients carried this mutation; therefore, other causes of polycythemia have to be considered.

In most patients with secondary polycythemia due to hypoxemia, the structural abnormalities of the heart and lung are sufficiently apparent to indicate the underlying cause of the polycythemia. In patients with familial high-affinity hemoglobin, the diagnosis is relatively easy because most, but not all, of these patients have a positive family history. The initial laboratory tests should, of course, include a hemoglobin electrophoresis and an oxygen tension at which hemoglobin is 50% saturated (P50). A low P50 suggests the presence of a high-affinity oxygen (57, 58) or bisphosphoglycerate (2,3-BPG) deficiency (59). High-affinity hemoglobins which include hemoglobin J Capetown, Yakima, Kemsey, Rainier, Ypsi, and Chesapeake may be found on electrophoresis (57). If the P50 is normal, mutations

might involve the von Hippel-Lindau (VHL), tumor suppressive gene (60) or truncated EPO receptor (EPOR) (61). Another well-recognized cause of polycythemia is the so-called endemic Chubash polycythemia (62). Variations of these interesting polycythemias have been described and are detailed elsewhere (59–63).

1.10 Treatment of Polycythemia Vera

Considerable controversy surrounds the treatment of polycythemia vera ever since it was described 100 years ago. Current recommendations for its management are based upon a small number of randomized clinical trials, and a series of prospective and retrospective studies evaluating different treatment programs pertaining to different aspects of the disease. These include survival, reduction in phlebotomy requirements, thrombotic events, and evolution of the disease into myelofibrosis and acute leukemia.

1.10.1 Phlebotomy

Reduction of the red cell mass and maintaining it at a safe level by phlebotomy is the first principle of therapy in PV. Improperly treated, PV becomes a disease with serious consequences related to morbidity and mortality; this was recently emphasized by Marchioli et al, (64), who reported a death rate of 3.7 per 100 patients per year, far exceeding the normal. Important physiologic and pathologic components of the disease, which must be dealt with therapeutically, relate to the erythroid cell and the megakaryocyte, both of which play essential roles in causing complications of the disease. Hematologists agree that the mainstay in treatment is phlebotomy, with target levels of the hematocrit at <45% for men and <42% for women. Although a recent publication based on a retrospective review suggested higher red cell levels might be permissible (65). During pregnancy, the HCT should be <36%. Reduction of the red cell mass and maintaining it at a level close to normal will help to remove a major source of complications. A progressive increase in the incidence of vascular occlusive episodes at HCT levels >45% (66) has been shown as well as abnormal changes in blood viscosity above 45% (67). Iron replacement should not be used. Phlebotomy does not relieve pruritus. Repeated phlebotomy, overtime, causes severe iron deficiency. Venous access sites become exhausted. However, in a recent multivariate analysis of a large retrospective cohort of patients, a hematocrit in the evaluable range of 40–55% was not associated with the occurrence of an increased rate of thrombotic events or mortality (68). Although an appropriate controlled study to establish the real target range in PV is needed, it seems prudent to continue to recommend to the target of <45% (68) in men and <42% in women (69).

There is very little data in the literature pertaining to the frequency or the rate of phlebotomy which can be considered a manifestation of the aggressiveness of disease. In general, I believe that patients who have high phlebotomy requirements are those who might have a more aggressive form of the disease and should receive some form of myelosuppression prior to the development of splenomegaly and myelofibrosis. This is because a higher rate of phlebotomy may be associated not only with increased erythroid activity but also with more active megakaryocytic proliferation, leading to the earlier development of myelofibrosis (69).

Little quantifiable data exists, however, with respect to the phlebotomy requirements of patients with PV. It has been suggested that measurement of a Cr^{51} red cell mass provides an indication for the exact measurement of the blood to be removed (66). This statement, however, does not take into account that no study has correlated initial red blood cell mass with subsequent phlebotomy requirements. Additionally, as blood is removed by phlebotomy, it is replenished by the hyperactive bone marrow. Thus, each patient must provide sole source criteria on a measure of activity of the disease. In our series of 55 patients (71), the annual number of phlebotomies per year per patient ranged from 1 to 25, with a mean of 8 and a median of 9 phlebotomies per year. However, more than half the patients required 8–25 phlebotomies per year and thus became candidates for myelosuppressive therapy.

If the phlebotomy requirement is minimal (i.e., requiring 2 to 3 or even 4 units a year), a patient can be managed with phlebotomy only (PHO-O). All patients should receive low-dose aspirin (81– 100 mg daily). The use of acetylsalicylic acid (ASA) was originally discouraged because of the findings by the PVSG evaluating ASA 900 mg daily combined with dipyridamole (72). The trial was stopped because of an excess of major bleeding without preventing thrombotic events. The European Collaboration on Low-dose Aspirin in Polycythemia Study (ECLAP) provided compelling evidence for the efficacy and safety of low-dose ASA (for instance, 100 mg daily) in a double-blind, placebo-controlled, randomized clinical study (73). Five hundred eighteen patients without a clear indication or contraindications to ASA were enrolled. The median age was 61 years. Previous cardiovascular events were reported in only 10% of cases in this subgroup, so that the trial reflected an asymptomatic, low-risk population. Median follow-up was only 2.8 years. ASA significantly lowered the risk of a primary combined endpoint, including cardiovascular death, nonfatal myocardial infarction, nonfatal stroke, and major venous thromboembolism. Total and cardiovascular mortality were also reduced by 46% and 59%, respectively. Major bleeding was only slightly increased. The results of this trial eliminated the concerns raised by the PVSG about the risk:reward ratio with ASA. Different doses of ASA, mostly on the low side, have confirmed the value of this drug in treating microvascular symptoms such as erythromelalgia, transient ischemic attacks and other neurologic and ocular disturbances, dysarthria, scintillating scotoma, and so forth (74).

Should a patient have relatively frequent phlebotomies (more than six per year) and/or develop signs and symptoms of severe iron-deficiency anemia, progressive splenomegaly, or evidence of increasing marrow fibrosis or complain of the

morbidity and discomfort of venesection in conjunction with frequent phlebotomy, myelosuppressive treatment should be considered (*vide infra*). Selection of the type of treatment for those patients who require some form of myelosuppression because of the frequency of phlebotomy and or its complications provides the basis for major discussion, confrontation, and disagreement since the days of the PVSG (72). For the most part, alkylating agents (including busulfan) are avoided because of the established risk of secondary leukemia, but these drugs and radioactive phosphorous (P^{32}) still play a role in treating the very elderly patient or those who have significant comorbid conditions. P^{32}, in particular, is associated with long periods of remission-free survival after 1 or 2 doses of 5 mCi intravenously (75).

The first systematic approach to myelosuppressive therapy of PV began with the PVSG in 1970 (72). Since then, a number of medications have been used for cytoreduction, including newer treatments such as recombinant interferon alpha (rIFN-α) and imatinib, which are therapeutically effective, have a biologic basis for their use and effect, and can modify JAK2 expression in some patients.

The first study of the PVSG (72), the 01 trial, included 431 patients who were randomized to receive either phlebotomy-only, phlebotomy plus radioactive phosphorous (P^{32}), or phlebotomy plus chlorambucil. The median survival of patients receiving phlebotomy-only (PHL-O), about 13.9 years, was superior to those receiving phlebotomy plus P^{32} (11.8 years) or phlebotomy plus chlorambucil (8.9 years). In the first 3 years of treatment, however, PHL-O was associated with an increased risk of thrombosis. Those in the second two groups experienced a higher rate of acute leukemia and other malignancies, which developed during the follow-up period. The frequency of myelofibrosis with myeloid metaplasia was essentially the same in all three groups.

Because treatment with chlorambucil predisposed patients to the development of acute myelogenous leukemia (AML), it is safe to presume other alkylating agents would do likewise. In general, such drugs should not be used in patients with PV. After the experience with chlorambucil, the PVSG tried to find a presumably nonmutagenic myelosuppressive drug. Hydroxyurea (HU) was selected (76); this agent is an antimetabolite affecting DNA synthesis by inhibiting the enzyme ribonucleotide reductase. In the first report by the PVSG, 51 patients were treated for a median of 8.6 years. The HU group showed a tendency for the development of acute leukemia in 5.8% of patients. This study was compared to the 134 patients treated with PHL-O in the PVSG 01 protocol. This noncontemporaneous, nonrandomized study is obviously statistically faulty, yet this information has been used to cite that HU is not leukemogenic (76). The limitations of the HU study of the PVSG include the fact that the patients were followed for a median of 8.6 years (maximum: 15.3 years) so that the true incidence of acute leukemia, which would increase by continuous exposure to HU, could not been determined (77, 78).

Few hematologists have followed serially and reported the treatment results of HU over the course of years; thus, the issues regarding potential leukemogenicity (69, 79, 80), although very suggestive, are not completely resolved. Likewise, it has also been presumed that HU is not leukemogenic because a recent study (64) indicated no increased risk of leukemia after HU; however, the median follow-up

was only 2.8 years. Presumably, the development of leukemia in patients with PV occurs after more than 10 years of treatment (78). Others have observed acute leukemia developing in approximately 11.5% of PV patients after 10 years when HU was used as a myelosuppressive agent (79, 80). In another report, HU was not considered leukemogenic (69) because no increased leukemia risk was cited when compared with pipobroman, a drug not used in the United States. Although there was no difference in leukemia risk between the two drugs, there was a 10% frequency at the 13th year of follow-up in both, suggesting that both pipobroman and HU were associated with an increased risk of leukemia (78). Moreover, long-term maintenance therapy with HU was frequently ineffective, with complications appearing generally 5 years or more after initiation of treatment. These late occurrences explain why the frequency of complications is often underestimated. Indeed, the frequency of HU-induced leukemia was similar in that study to P^{32} in the PVSG studies!

Moreover, chromosomal abnormalities have been observed in cell cultures incubated with HU, in patients treated with HU for lung cancer (81), and in 36% of patients treated for PV with HU (82). Additional limitations of using HU in PV include an increased risk of squamous cell cancer, skin rash, and buccal aphthous ulcers, failure to control blood counts (high platelet count and macrocytic anemia), or leukopenia and thrombocytopenia in 15–30% of 109 patients reported by the PVSG; moreover, constitutional symptoms (night sweats and pruritus) persist and other toxicities of the drug (gastrointestinal, renal) were reported. Moreover, although PV customarily is thought of as a disease of older patients, the finding of this illness in patients under 50 years is noteworthy. In our series, the median age was 50 years and 23% were younger than 40 years (71). Obviously, selection of appropriate therapy for this younger group of patients is particularly important.

Further, HU, other than by general myelosuppressive does not affect abnormal megakaryocyte dysfunction, an abnormality leading to fibrosis as the disease evolves (70, 83). Unfortunately, clinical tradition has hardened into habit, and the therapeutic effects and the additional risk factors of therapy with HU have either been ignored or misinterpreted. The limitations of HU for treating PV have not received adequate attention. Even though the PVSG reported that only 61% of 51 patients treated with HU achieved long-term control (72, 76), the criteria for HU failure used by the PVSG allowed for the HCT to increase to more than 55% and permitted more than six phlebotomies in any consecutive 52-week period. This broad window for treatment failure would not be considered satisfactory in this era. Nevertheless, HU is considered by some to be very effective and should be considered the “drug of choice” in high-risk patients (69). An initial starting dose ranges between 500 and 1500 mg daily depending on the counts, with adjustment depending on response. In addition to its antiplatelet effect, HU might have additional mechanisms of activity, including myelosuppression, qualitative and quantitative changes in leukocytes, decreased expression of endothelial molecules, and increased nitric acid generation; all of which play a role in thrombosis in PV (83).

Hydroxyurea can be used with less reluctance for patients who require myelosuppression, particularly with a high platelet count, and in older patients, especially for whom interferon is contraindicated.

1.10.2 Interferon

Since the initial studies of the use of recombinant interferon-α (rIFN-α) in treating patients with PV (85–88), many reports of its beneficial results have been published (89–91). Using rIFN-α avoids many of the problems and complications associated with PHL-O and/or chemotherapy and relieves pruritus and other constitutional symptoms. An update concerning long-term effects has recently been published (71).

The use of rIFN-α in PV is based on its broad effects on the hematopoietic system. Although PV, as noted, is a clonal stem cell disorder, there exist case reports of the resumption of polyclonal hematopoiesis after rIFN-α therapy (92, 93). rIFN-α inhibits erythroid progenitors and erythroid burst-forming units in vitro (94) and produces morphologic and biochemical changes in megakaryocytes (95), including a decrease in megakaryocyte density, size, and proliferation. rIFN-α inhibits megakaryocyte progenitor proliferation and reduces thrombopoietin-induced Mpl receptor signaling (96). The biochemical abnormalities of platelets in PV characterized by impaired conversion of exogenous arachidonic acid by platelet thromboxane B_2 and its correction by rIFN-α have been reported (97). rIFN-α also antagonizes platelet-derived growth factor (PDGF) (98). This is important because PDGF and other fibrogenic cytokines such as transforming growth factor β (TGF-β) and basic fibroblast growth factor play a major role in the pathogenesis of myelofibrosis (70, 84, 98). rIFN-α also inhibits the growth of marrow-derived fibroblasts (70, 84). rIFN-α, therefore, might retard the development of myelofibrosis because of its effect on megakaryocytes, thrombopoiesis, and various cytokines.

Recombinant IFN-α thus offers a physiologic basis for its use in PV. Compared with nonspecific myelosuppressive drugs or PHL-O, rIFN-α represents a treatment modality that could fundamentally alter the course of the disease and addresses the unmet therapeutic needs (64) of patients with PV.

Systematic studies in 55 patients with PV treated with rIFN-α have been reported recently (71); however, more than 70 patients have been treated in our clinic. In our studies, the criteria for the diagnosis and response to treatment were those of the PVSG (72). Response criteria were phlebotomy-free, HCT ≤ 45%, and platelets ≤ 600,000/μL. Our treatment program began with phlebotomy and rIFN-α 2b. Initially, we used doses of 3.0 MU/m^2 three times a week (tiw), but this was accompanied by frequent side effects over the long term. Beginning with low doses (1 MU tiw regardless of body surface area) and a gradual increase over the course of months, patient tolerance was enhanced. Then the dose was increased by 0.5 MU every 2–4 weeks until the target dose of 3 MU tiw was achieved, if necessary. Afterward, the dose was adjusted up or down depending on response until the

patient remained phlebotomy-free at the target HCT. Supplemental antipyretics and analgesics were used liberally to treat side effects of rIFN-α. Aspirin, 81 mg daily, was given to all patients. Patients achieved partial response of their disease by 6 months and complete response by 1–2 years (phlebotomy free, HCT ≤ 45%, platelets 600,000/μL). Spleen size was reduced in 27 of 30 patients with prior splenomegaly. In our series (71), with a median disease duration of 7 years (range: 20 months to 20 years), the median disease-free survival was 11.9 years (range: 1.7–30 years). Strikingly, no thrombohemorrhagic events were observed.

The ubiquitous side effects of rIFN-α are described in detail elsewhere (99), and those that occurred in our patients are shown in Table 1.3. Although toxicity is acceptable for the majority of patients, 15–20% will not tolerate its long-term side effects, especially patients over the age of 65 years. Those patients who suffered influenzalike symptoms to a greater or lesser degree were in our early group of patients who had received higher initial doses of rIFN-α (3.0 MU/m^2 tiw). At lower doses (0.5–1.0 MU tiw), virtually no significant side effects were noted in many patients, but some degree of headache, fatigue, chills, and low-grade fever should be anticipated. Secondary hypothyroidism was common and easily treated. Hair loss and nail changes might occur. Neurological disorders such as demyelinating diseases or a history of seizures or other neuropsychiatric problems, including depression, are contraindications for rIFN-α (100).

A common error in treating PV patients with rIFN-α is to assume that it will replace the use of initial phlebotomy; it usually does not. rIFN-α suppresses the need for phlebotomy, once the target HCT values have been achieved by phlebotomy. The second major issue relates to the initial dose. In the majority of studies, the dose has been too high, modeling dosing used in the treatment of chronic myeloid leukemia. By way of contrast, the doses I advise suppresses the need for phlebotomy. I prefer nonpegylated forms of interferon, for this allows greater flexibility in dosing to avoid or reduce the frequency of side effects and to improve issues of quality of life.

Table 1.3 Toxicity of interferon seen in our patients with PV and an index of treatment continuation

	Treatment Continued?	
Type of toxicity	Yes	No
Initial influenzalike symptoms	√	
Liver function abnormalities	√	
Hypothyroidism	√	
Severe asthenia		√
Skin rash	√	
Complex seizures		√
Depression		√
Peripheral neuritis		√
Parkinson's syndrome		√
Bone pain	√	
Blurred vision		√

1.10.3 Imatinib Mesylate

Because of the side effects of interferon, alternative treatments are required and have been evaluated. Of interest, therefore, is the effect of imatinib mesylate (Gleevec®; Novartis Pharmaceuticals, Basel, Switzerland) in treating patients with PV. Reasons for using imatinib include inhibition of c-kit, which is involved in both normal and abnormal hematopoiesis (101, 102). Inhibition of c-kit reduces both erythroid and granulocyte/macrophage progenitor cells in patients with PV. Imatinib may also inhibit PDGF (101, 102), previously mentioned as involved in the production of marrow fibrosis. We initially reported that the erythrocytosis of PV was controlled with imatinib mesylate (101). We then reported treating an expanded cohort of 37 PV patients with imatinib in a multi-institutional phase II trial (103). All patients had an increased Cr^{51} red blood cell mass determined at diagnosis and fulfilled the other stipulated criteria for the diagnosis of PV. Men were initially phlebotomized to an HCT ≤45% and women to ≤42%. Imatinib was started at 400 mg/day, escalating by 100 mg every 2 weeks to a maximum of 800 mg/day for those with persistent phlebotomy requirements or platelet counts greater than 600,000/μL. All patients received 81 mg/day of aspirin and prophylactic allopurinol. The study included 21 men and 16 women with a median age of 51 years (range: 29–83). Prior to imatinib, mean disease duration was 60.2 months; the patients had received a mean of nine phlebotomies per year (range: 0–23). After 1 year of imatinib, the mean phlebotomy requirement was only two per year, range: 0–9. There were 9 complete remissions (phlebotomy-free, platelet count <600,000/μL, no splenomegaly) and 10 partial remissions (phlebotomy and/or splenomegaly decreased by 50% of initial values, platelets less than 600,000/μL, or platelets more than 600,000/μL but no phlebotomy requirement or splenomegaly). There was one protocol violation. Thus, of 36 evaluable patients, 19 (53%) had a complete recovery (CR) or partial recovery (PR). Of the remaining 17, 10 showed disease progression: myelofibrosis (n = 2); platelets >1,000,000/μL, and/or persistent phlebotomy requirements or splenomegaly (n = 2) or increasing phlebotomy requirements (n = 2); progressive cytogenetic abnormalities (n = 1); increasing thrombocytosis (n = 1); and transient ischemic attacks (n = 2). Of these same 17, 7 developed toxicity: grade 3 dermatologic effects (n = 2); grade 3 diarrhea (n = 2); grade 3 bone pain (n = 1); liver toxicity (n = 1); and heart failure (n = 1). The median duration of response to date is 17.2 months. We concluded that imatinib is useful for treating erythrocytosis and controlling splenomegaly in a significant proportion of patients with PV, but it is less effective for controlling thrombocytosis or splenomegaly in others (101–103). These data suggest heterogeneity of hematopoietic stem cell proliferation in PV or patient variability with respect to imatinib metabolism. Dosage increases above 400 mg/day were usual and doses as high as 800 mg/day were associated with fluid retention, periorbital edema, and diarrhea. Clinical experience with imatinib facilitates its more effective use. The use of supplemental anagrelide could be considered, especially early in the course of treatment, particularly in those patients in whom HU had been used. Others have also reported the

effectiveness of imatinib in the treatment of PV (104, 105). Continued investigation of this or related drugs would be of interest to explore the reason for heterogeneity of response, to determine the optimum dose and side effects, and the duration of response.

1.11 The Nature of the Response to rIFN-α and Imatinib

Despite these encouraging results with rIFN-α and imatinib, it is evident that for the most part, the results are quantitative and not qualitative in nature. Despite maintenance of a normal HCT and platelet count, marrows have remained hypercellular even in those patients who developed clinical and hematologic remission. Expression of c-kit by interval marrow immunohistochemistry tests was not reduced. Three patients with long-standing disease did develop progressive myelofibrosis after rIFN-α. (Whether earlier institution of rIFN-α treatment would have prevented this is moot.) rIFN-α has been reported to induce cytogenetic remission in a few patients with PV, with conversion of monoclonal to polyclonal hematopoiesis, as already mentioned. Such events have not yet been reported with imatinib treatment. Because only 20–30% of patients with PV present with a chromosomal abnormality, in general it has not been possible to gauge the depth of response to treatment (106). The recent finding of the *JAK2*^{V617F} mutation in more than 95% of patients with PV suggests that this mutation might be exploited in order to measure the response to therapy in patients with PV (106). We examined change in gene expression in seven patients who received rIFN-α at an initial dose of 1 MU tiw increasing to 3 MU/day, with a median follow-up of 16 months (range: 13–132), and in 14 patients who received imatinib at initial doses of 400–800 mg/day, with a median follow-up of 17 months (range: 5–31). These patients were compared with 90 individuals who served as a control group; they had received phlebotomy only or HU, anagrelide, or both, or were untreated. We found that patients remained strongly positive for the *JAK2*^{V617F} mutation after treatment, but there was significant reduction in the median percentage of mutant alleles, which correlated with hematologic response ($p = 0.001$). Furthermore, individuals who had achieved complete hematologic response had lower levels of *JAK2*^{V617F} than those who did not. From a molecular standpoint, these results were modest, indeed. On the other hand, others have reported a more impressive molecular response to pegylated rIFN-α (107). These authors treated 40 patients, of whom 83% had a complete remission and 17% a partial remission, but judged after just 3 months of study. Three of the 40 patients were negative for JAK2 mutations at diagnosis (8%). Of 27 PV patients treated with pegylated IFN-α-2a, 24 (89%) had a mean decrease of 44% in the expression of mutated JAK2 allele by reverse transcription (RT)–PCR. There was no evidence of a plateau. In one patient, mutant JAK2 was not detected after 12 months. In three patients homozygous for the mutation, reappearance of 50% of wild-type allele was observed during treatment. Interferon has also been shown to preferentially target the malignant clone. These results suggest that

rIFN-α-2a significantly decreased the proportion of circulating clonal cells in the majority of PV patients. Whether pegylated interferon results in a qualitative difference compared with nonpegylated interferon still remains to be determined.

In comparison with the myelosuppressive drugs that have been used in treating PV, why might rIFN-α be unique? Inhibition of erythroid progenitors has been discussed. Several observations suggest that the megakaryocytic and granulocytic myeloproliferations play a unique role in the production of cytokines, leading to secondary myelofibrosis (70, 84). The fact that the great majority of our patients, even those treated previously with HU, had increased platelet counts prior to the use of rIFN-α is significant. Hyperplasia and clustering of small to giant (pleomorphic) megakaryocytes in the bone marrow is a characteristic feature of PV (13–15). Although PDGF is involved in fibroblastic proliferation, it does not fully account for the complex production of myelofibrotic stroma.

Additional growth factors (cytokine storm) produced by megakaryocytic and granulocytic/monocyte precursor cells must be involved in the etiology of secondary myelofibrosis in Myeloproliferative Disorders (MPD), the most important of which is probably TGF-β (70, 98). How TGF-β, PDGF, other cell lineages, and cytokines interact to cause marrow fibrosis is unknown; after rIFN-α therapy, TGF-β values return to normal levels. Moreover, TGF-β has powerful angiogenic properties (70, 98). That rIFN-α has activities against erythropoiesis, megakaryocytopoiesis, PDGF, and TGF-β and is also antiangiogenic suggests that it might have a unique role in the treatment of the MPDs, particularly PV. The differentiation of erythroid-megakaryocyte precursor cells into one or another lineage might involve a balance between two related transcription factors, NF-E2 and BACH-1, which could conceivably be affected by rIFN-α (108). Finally, the effect of rIFN-α on JAK2 in patients with PV clearly requires further elucidation. rIFN-α might be the first drug that can alter the natural history of the disease.

1.12 Treatment of Other Aspects of PV

It is also my practice to treat all patients prophylactically with allopurinol or probenecid, particularly in patients with increased serum uric acid levels. Many patients take multivitamins containing relatively large amounts of vitamin C. By acidifying the urine, this agent might cause precipitation of uric acid crystals and result in renal colic. Of interest is the observation that acute gouty arthritis involving the right hallux can occur in patients who drive an automobile long distances. This is related to the right foot pressure, as the foot is applied repeatedly to the accelerator pedal. Except for fatigue associated with general metabolic symptoms and weight loss, leukocytosis does not require treatment *per se*. Blood cells of patients with polycythemia have normal phagocytic function; patients do not have an increased frequency of bacterial infection.

It is extremely important, as previously mentioned, to provide "total care" for the patient undergoing phlebotomy to reduce the risk of thrombosis. Thus, the use of birth control pills or estrogen supplementation is contraindicated. All cigarette smoking is forbidden. Obesity, hypertension, and diabetes should be treated in the usual fashion.

1.13 Summary

1. Evidence is presented indicating that rIFN-α effectively reduces phlebotomy requirements for thrombocythemia, splenomegaly, and thrombohemorrhagic events. It is an effective drug for treating PV with acceptable toxicity.
2. Because PV is a chronic disease and the use of rIFN-α is long term, the initial dose must be small. An initial dose of rIFN-α-2b 1 MU tiw subcutaneously is suggested. A dose that is too high should be avoided.
3. Treatment with rIFN-α cannot be expected to lower the HCT. Phlebotomies must be continued as necessary to maintain the HCT at target levels (men: HCT ≤ 45%; women: HCT ≤ 42%).
4. Attention to the side effects of rIFN-α must be addressed from the onset. Thus, it is best to give the injection of rIFN-α at night, with adequate coverage with nonsteroidal anti-inflammatory drugs.
5. Dose adjustments must be made; most patients will require an increased dose of total weekly rIFN-α during the first year of treatment. This is done initially by gradually increasing the frequency of the dose. In general, 9–10 MU/week is the usual target dose the first year. After the first year of treatment, the dose can be gradually decreased, so that the minimum dose is achieved to suppress erythropoiesis with minimum toxicity.
6. The platelet count should lower by the end of the first year of therapy to ≤600,000/μL.
7. All patients should receive aspirin, 80–100 mg/day.
8. Probenecid or allopurinol should be given to a patient with an elevated serum uric acid level. Liberal fluid intake is encouraged. (It is not necessary to routinely alkalinize the urine.)
9. For patients who are in the older age group or intolerant of rIFN-α, HU is the drug of choice for marrow suppression. Imatinib mesylate can be of value in selected patients.
10. For those in whom the leukemogenic potential is not an issue, such as significant comorbid illness, P32, busulfan, or other alkylating agents might be considered because they induce a smooth remission, which is associated with a very good quality of life. Of course, full informed consent must be obtained from such individuals prior to use. Anagrelide is of value in younger patients with platelet counts unresponsive to other agents which are considered to be clinically threatening.

11. Attention must be paid to other details of medical care such as the treatment of hypertension, diabetes, and obesity. Estrogen-containing compounds should be avoided.

1.14 Conclusion

These are exciting times for the study of PV and allied diseases. The discovery of *JAK2*^{V617F} has lead to an enormous increase in activity in both understanding the pathobiology of this disease and attempting to find inhibitors which would affect the molecular abnormality. Hopefully, these advances will come soon.

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