The Differential Diagnosis of the Polycythemic States

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ABSTRACT

The clinical and laboratory characteristics that are essential for the differential diagnosis of the polycythemic states are reviewed. The various forms of polycythemia that present as increases in hemoglobin concentration or packed red cell volumes can often be distinguished on the basis of pathophysiology, predisposition to neoplasia, hematological and viral parameters, chromosomal patterns, and response to therapy. Polycythemia vera differs from secondary and relative polycythemic states in its basic pathophysiology and etiology. It appears to be a clonal disorder that is characterized by a generalized bone marrow panmyelosis. Although the primary event leading to this stem cell disorder is not known, recent studies suggest a possible etiology. Therapeutic regimens based on the parameters inherent in making a differential diagnosis of the polycythemic states are discussed. Prognosis for polycythemia vera also appears to be predicated by the choice of drugs used to treat the disease. The consequences of making the correct diagnosis and of choosing appropriate therapy in light of the differences in underlying pathophysiology and etiology of the polycythemic states are stressed.

Polycythemia Vera as a Preneoplastic State

In this review of the polycythemic states, polycythemia is characterized clinically by an increase in hemoglobin (Hb) or increase in packed cell volume (PCV) and includes polycythemia vera (PV), secondary polycythemia, and relative or stress polycythemia. The pathophysiology of polycythemia vera (PV) will be considered first. In contradistinction to secondary polycythemia whether of the appropriate or inappropriate type and relative (stress) polycythemia, PV is a preneoplastic state that is characterized by bone marrow panmyelosis with increased erythropoiesis, granulopoiesis and megakaryocytopenia. Recent studies of the isozymes of glucose-6-phosphate dehydrogenase (G-6-PD) indicate that PV is a clonal disorder. PV will frequently evolve into myelofibrosis and myeloid metaplasia, and approximately 10 to 15
percent of the patients die of acute leukemia. Patients with PV who are treated with either radioactive phosphorus ($^{32}$P) or chlorambucil (leukeran) have an increased incidence of both leukemia and solid tumors.\textsuperscript{5}

An important morphologic characteristic of the bone marrow in PV is an intense megakaryocytic hyperplasia. In this regard, PV bears a superficial resemblance to the murine disease induced by the polycythemia strain of Friend virus, at least with regard to splenomegaly and bone marrow morphology. A major difference is that high titers of both the Friend and Rauscher viruses are initially associated with thrombocytopenia, whereas PV is generally associated with thrombocytosis.\textsuperscript{3}

The megakaryocyte and platelet appear to be major reservoirs for retroviral activity in the viral-induced animal leukemias as well as PV. Electron micrographs have revealed that retroviruses (C-type) are characteristically present in vacuoles of megakaryocytes and platelets.\textsuperscript{5,5} Reitz et al.\textsuperscript{15} have demonstrated budding of retroviruses from platelets of a gibbon ape with lymphoma. Electron microscopic studies in our laboratory combined with reverse transcriptase assays have demonstrated the presence of retroviral-like activity in the platelets of patients with PV and the closely allied disorders essential thrombocytopenia, myelofibrosis and myeloid metaplasia (MF).\textsuperscript{4} A possible specific chromosomal abnormality, i.e., a 21q-, has recently been demonstrated.\textsuperscript{8} It has also been demonstrated by us that therapy with busulfan results in a disappearance of viral particles and reverse transcriptase activity in the platelets. Chromosomal patterns in the bone marrow return to normal, and a subjective and objective remission in the disease follows.\textsuperscript{4}

The possibility that retroviral activity would be found in the platelets of patients with PV was first raised in an editorial\textsuperscript{3} in the Journal of the National Cancer Insti-
tute in 1973. In that editorial we postulated that PV might be an early, viral-induced, preneoplastic state with the potential to evolve into acute leukemia. This hypothesis has now begun to receive experimental support. Tambourin et al.,\textsuperscript{17} in a review of the physiopathology of Friend leukemia, concluded that both the Friend and Rauscher murine leukemias appear to be multiple step (at least two steps), virus-induced, malignant diseases. The first step is a preneoplastic transformation, with the second step resulting in the ultimate neoplastic process. Tambourin\textsuperscript{17} and his co-workers conclude that the evolution of these experimental leukemias appears similar to the two phased evolution of certain human differentiated leukemias, such as PV or chronic myeloid leukemia in which an indolent chronic phase evolves into an acute aggressive disease, namely acute myelocytic or, in some cases lymphocytic leukemia.

The symptoms, signs and laboratory findings of PV are due to the pancytopenia which leads to an expansion of the erythrocytic, myelocytic, and megakaryocytic compartments of the bone marrow and peripheral blood. Many of the major symptoms of the disease are due to increased red blood cell volume, with an attendant increase in blood viscosity, as well as increased numbers and turnover of platelets and white blood cells. Patients may complain of fatigue, malaise, headache, and pain in the peripheral extremities and intolerance to heat. Thromboembolic and hemorrhagic episodes may occur. Thrombosis and secondary hemorrhage have been noted in the coronary arteries and in the cerebral and peripheral vascular circulations. In fact, thromboembolic episodes are the major cause of early death in untreated patients. A hypercoagulable state exists in PV which is caused by increased platelet turnover and an associated increased fibrinogen turnover.\textsuperscript{2} For this reason, PV cannot be treated by phlebotomy alone. Phlebotomy will reduce blood volume
and the red blood cell mass, and may alleviate some symptoms. However, phlebotomy will not prevent and may accelerate the incidence of thrombotic complications. Successful treatment of PV requires a combination of phlebotomy and marrow suppression.

The leukocytosis and increase in the white blood cell compartment is generally responsible for such symptoms as gastrointestinal distress, heartburn, abdominal pain, and the rather characteristic pruritus following bathing. Increased levels of histamine in the white blood cells may be responsible in part for some of the symptoms, but other factors such as the vasoactive kinins are probably also involved.9

Physical examination of patients with PV demonstrates a characteristic plethora and, most importantly, an enlarged spleen. Splenomegaly will be noted in approximately 75 percent of patients with PV. In order to be certain that the spleen is not enlarged even though it is not palpable, splenic scans should be obtained.

**Polycythemia Vera vs. Secondary and Relative Polycythemia**

With the previous considerations in mind, the differential diagnosis of the polycythemic states is presented in tables I through IV. The laboratory studies are grouped under four major categories: (1) erythropoiesis, (2) myelopoiesis, (3) thrombopoiesis, and (4) bone marrow, splenic size, and uric acid turnover.

All polycythemic states are characterized by a peripheral erythrocytosis, evidenced by an elevated hemoglobin concentration (Hb), packed cell volume (PCV), and red blood cell count. The diagnosis of erythrocytosis, i.e., polycythemia, cannot be made without first measuring the red blood cell (RBC) mass. The simplest direct measure, of the red blood cell mass is done with radioactive chromium (51Cr), but an indirect measure of RBC mass can also be obtained by performing a radioactive iron (59Fe) clearance study. In PV and in secondary polycythemia, the red blood cell mass is significantly increased. In relative (stress) polycythemia, the red cell mass is normal or only top normal. It is essential to rule

**TABLE I**

<table>
<thead>
<tr>
<th>Studies</th>
<th>Polycythemia Vera</th>
<th>Secondary Polycythemia*</th>
<th>Relative Polycythemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red blood cell mass (Cr51 and Fe59)</td>
<td>Increased</td>
<td>Increased</td>
<td>Normal</td>
</tr>
<tr>
<td>Fe59 T1</td>
<td>Decreased</td>
<td>Normal or decrease</td>
<td>Normal</td>
</tr>
<tr>
<td>Serum Iron</td>
<td>Decreased</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>PIT (Plasma iron turnover)</td>
<td>Increased</td>
<td>Normal to increased</td>
<td>Normal</td>
</tr>
<tr>
<td>Marrow iron stores</td>
<td>Decreased</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Hemoglobinopathy</td>
<td>Absent</td>
<td>Absent or present</td>
<td>Absent</td>
</tr>
<tr>
<td>Arterial oxygen saturation and tension</td>
<td>Normal</td>
<td>Decreased or normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Erythropoietin</td>
<td>Normal to decreased</td>
<td>Normal to increased</td>
<td>Normal</td>
</tr>
</tbody>
</table>

*Appropriate (physiologic); decreased oxygen saturation and tension, i.e. pulmonary and cardiovascular disease with right to left shunt; tissue hypoxia, i.e. hemoglobinopathy or CO toxicity with left shift in oxygen dissociation curve. Inappropriate (nonphysiologic); increased erythropoietin levels.

**TABLE II**

<table>
<thead>
<tr>
<th>Studies</th>
<th>Polycythemia Vera</th>
<th>Secondary Polycythemia</th>
<th>Relative Polycythemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocytosis</td>
<td>Present</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Serum vitamin B12</td>
<td>Increased</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Serum U12RC*</td>
<td>Increased</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Leukocyte alkaline phosphatase</td>
<td>Increased</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>blood histamine</td>
<td>Increased</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Basophil count</td>
<td>Increased</td>
<td>Normal</td>
<td>Normal</td>
</tr>
</tbody>
</table>

*Serum unsaturated B12-binding capacity.
out the diagnosis of relative polycythemia. Individuals who have relative or stress polycythemia may present with an increased Hb and PCV, which suggest the diagnosis of PV. These people are usually short, mesomorphic, and have a history of essential hypertension. The increased Hb and PCV are spurious and are due to a contraction in the total plasma volume. These patients have no evidence for increased marrow, erythropoiesis, myelopoiesis or thrombopoiesis. However, some clinicians consider an Hb concentration in excess of 17 grams per dl and a PCV of greater than 50 percent as risk factors for the development of coronary artery disease and cerebral vascular disease.\textsuperscript{1,20} Thus, phlebotomy has been suggested as therapy, even though the RBC mass is normal.

Secondary polycythemia is associated with erythrocytosis and an increase in the red blood cell mass. Secondary polycythemia can be divided into two groups, (1) appropriate (physiologic), or (2) inappropriate (nonphysiologic).\textsuperscript{7}

**Physiologic Secondary Polycythemia**

Appropriate or physiologic secondary polycythemia is due either to a decrease in the arterial oxygen tension or an inability to deliver oxygen normally to the tissues and results in a compensatory (physiologic) increase in erythropoietin levels. Decreased arterial oxygen tension is most commonly caused by residing at high altitudes, pulmonary disease or cardiovascular disease with a right to a left shunt. Ineffective release of oxygen to the tissues is caused by a shift of the oxygen dissociation curve to the left. Cigarette smoking has been associated with secondary polycythemia, presumably on the basis of low grade carbon monoxide toxicity leading to a left shift in the oxygen dissociation curve.\textsuperscript{16} Certain abnormal hemoglobins will cause a similar phenomenon. The low oxygen tension is sensed by the juxtaglomerular apparatus in the kidney, and this leads to a compensatory increase in erythropoietin output resulting in increased marrow erythropoiesis and an elevated Hb concentration, PCV and red blood cell count.\textsuperscript{7}

**Nonphysiologic Secondary Polycythemia**

Inappropriate or nonphysiologic secondary polycythemia is associated with increased erythropoietin levels with no evidence of arterial oxygen desaturation.
or defective oxygen release to the tissues. The most common disease associated with this phenomenon is hypernephroma. It has also been reported with other tumors such as hepatoma, leiomyoma, hemangioblastoma, pheochromocytoma, and some benign disorders of the kidney, such as hydronephrosis, cystic disease, renal artery stenosis, transplantation renal artery stenosis, transplantation rejection, and Bartter’s syndrome (table V).

Criteria for Diagnosis of Polycythemia Vera

In 1968, the Polycythemia Vera Study Group (PVSG) established criteria for the diagnosis of PV, and the distinction between this condition and secondary polycythemia. These criteria are presented in table VI. A key parameter that is required for diagnosis is an elevated red blood cell mass. The diagnosis of PV would only be acceptable if (1) all three parameters in category A are present, or (2) the combination of an elevated red blood cell mass and normal oxygen saturation is present with any two parameters from category B. In essence, the latter consideration would apply only in those patients who do not have demonstrable splenomegaly.

To establish the diagnosis of PV in a patient with an elevated Hb or PCV, the following laboratory studies are useful. The red cell mass can usually be determined by the \( ^{51}\text{Cr} \) labeling of red blood cells. However, it can also be determined indirectly by means of the \( ^{59}\text{Fe} \) T½. With this methodology, the plasma volume can be calculated from the following equation: total plasma volume is equal to the total activity \( ^{59}\text{Fe} \) administered divided by the activity in one ml of plasma at zero time. The total red blood cell mass can then be calculated from an estimate of plasma volume correcting the venous packed cell volume by multiplying by 0.91. The \( ^{59}\text{Fe} \) T½ can also be used to assess indirectly marrow erythropoiesis in PV. In patients with a normal or elevated Hb and PCV, a \( ^{59}\text{Fe} \) T½ of less than 40 minutes is highly suggestive of PV.

### TABLE V
**Nonphysiologic Secondary Polycythemia**

<table>
<thead>
<tr>
<th>Location</th>
<th>Pathologic Condition</th>
<th>Number of Case Reports Until 1972</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>Hypernephroma</td>
<td>118</td>
</tr>
<tr>
<td>Other tumors</td>
<td></td>
<td>13</td>
</tr>
<tr>
<td>Hydronephrosis</td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>Cystic disease</td>
<td></td>
<td>35</td>
</tr>
<tr>
<td>Renal artery stenosis</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Transplantation rejection</td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>Bartter's syndrome</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Liver</td>
<td>Hepatoma</td>
<td>64</td>
</tr>
<tr>
<td>Uterus</td>
<td>Leiomyoma</td>
<td>24</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>Hemangioblastoma</td>
<td>50</td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>Pheochromocytoma</td>
<td>5</td>
</tr>
</tbody>
</table>


### TABLE VI
**Criteria Adopted in 1968 by the National Polycythemia Vera Study Group**

Category A

A1. Increased red cell mass (measured with \( ^{51}\text{Cr} \)-labeled red cells): Male \( \geq 36 \text{ ml per Kg} \); Female \( \geq 32 \text{ ml per Kg} \).

A2. Normal arterial oxygen saturation \( \geq 92 \) percent.


Category B

B1. Thrombocytosis: platelets \( \geq 400,000 \text{ per mm}^3 \).

B2. Leukocytosis: white count \( \geq 12,000 \text{ per mm}^3 \) (in absence of fever or infection).

B3. Elevated leukocyte alkaline phosphatase score: \( > 100 \) in absence of fever or infection.

B4. Elevated serum \( B_{12} \) or unbound \( B_{12} \)-binding capacity: \( B_{12} > 900 \text{ pg per ml} \); \( VB_{12} > 2,200 \text{ pg per ml} \).

Diagnosis of polycythemia vera is acceptable if [1] all three parameters from category A are present, or [2] the combination of an elevated red cell mass and normal oxygen saturation is present with any two parameters from category B.

The rapid iron clearance in PV is due to a combination of the marked increase in erythropoiesis in association with relative iron deficiency. Marrow iron stores in PV are usually depleted, and this depletion in association with the increased erythropoiesis is the major reason for reported increased iron absorption from the gastrointestinal tract. Even though the serum iron is usually low in PV, the plasma iron turnover is generally increased. The plasma iron turnover can be calculated from the following formula:

\[
\text{Plasma iron turnover (mg per 24 hours per 100 ml whole blood)} = \frac{\text{Plasma iron (\(\mu g\) per 100 ml) \times 100 - PCV}}{T_{1/2} \text{(minutes)} \times 100}
\]

Normal values for the \(^{59}\text{Fe} T_{1/2}\) and plasma iron turnover (PITR) are 60 to 120 minutes and 0.45 to 0.75 per 24 hours per 100 ml of whole blood, respectively. In a series of patients studied by Brodsky et al., ferrokinetic studies and estimation of marrow iron stores were useful in differentiating polycythemia vera from both relative and secondary polycythemia. Ferrokinetic studies estimated the degree of marrow erythropoiesis and, indirectly, the total red blood cell mass. Ninety percent of the patients with PV had an iron \(^{59}\text{Fe} T_{1/2}\) of less than 40 minutes, and 85 percent had one less than 30 minutes. The PITR was increased in 85 percent of the patients, while only one patient with PV had stainable iron in the marrow. In patients with relative polycythemia, the total red blood cell mass was either normal or slightly elevated. \(^{59}\text{Fe} T_{1/2}\) was greater than 50 minutes in all patients. The PITR was normal, and all had stainable marrow iron.

Hemoglobinopathies resulting in a shift to the oxygen dissociation curve to the left lead to tissue hypoxia and secondary erythrocytosis. Some of these hemoglobin abnormalities can be diagnosed by abnormal electrophoretic mobility, such as hemoglobin Chesapeake. Others can only be diagnosed by determination of the oxygen dissociation curve and the \(P_{50}\). Both alpha chain variants (such as hemoglobin Chesapeake and hemoglobin Capetown) and beta chain variants (such as hemoglobin Yakima and hemoglobin Rainier) have been described. Prior to the discovery of these abnormal hemoglobins, patients in either category were frequently referred to as familial secondary polycythemia. In these abnormal hemoglobins, the amino acid substitution occurs in the contact area between the alpha and beta chains. Substitutions of this type may prevent normal conformational or allosteric changes which take place during oxygenation. Other substitutions in these interface areas may result in unstable molecules which lead not to polycythemia but to hemolytic anemia.

In secondary polycythemia of the physiologic or appropriate variety, the major abnormality will be a decreased arterial oxygen tension and saturation. In chronic pulmonary disease associated with arterial oxygen unsaturation the increase in red cell mass is not as great as anticipated. This is clearly reflected by the fact that the \(^{59}\text{Fe} T_{1/2}\) is frequently within normal limits, and that the serum iron is elevated. The reason for this phenomenon is poorly understood, but may reflect the presence of chronic inflammation.

Erythropoietin levels are elevated in cases of secondary polycythemia of the nonphysiologic or inappropriate type (table V). One would expect erythropoietin levels to be normal or low in the other states.

Increased myeloid activity and leukocytosis are common in PV but do not occur in either secondary polycythemia or relative polycythemia (table II). In PV, the unsaturated vitamin B\(_{12}\) binding capacity is markedly elevated and may be associated with increased serum vitamin B\(_{12}\) levels. Vitamin B\(_{12}\) is carried in the
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serum by transcobalamins of which there are three types: TCI, TCII, and TCIII. Preliminary studies suggest that TCIII is elevated in PV and TCI in chronic myelogenous leukemia. TCII, a β globulin, is the normal carrier of vitamin B₁₂, whereas TCI and III are α-globulins. TCI and III are produced by elements of the myeloid marrow (hence their elevations in PV and CML). TCII, on the other hand, is made by the reticuloendothelial system.

Leukocyte alkaline phosphatase levels (LAP) in the circulating granulocytes are increased in PV. Histamine levels and basophil counts are also elevated (table II). The LAP is normal in the other polycytemic states unless an associated inflammatory response or pregnancy elevates it.

Of major significance in the pathophysiology of PV is the level of the platelet count, platelet function, and platelet turnover. Thrombocytethemia (thrombocytosis) is present in a large percentage of patients with PV. In order to evaluate the hypercoagulable state and platelet function in PV adequately, one must determine not only the total platelet count but also platelet survival, platelet turnover, platelet function as well as fibrinogen survival and fibrinogen turnover. In PV, platelet survival is generally decreased and the platelet turnover increased. The platelet turnover is calculated by dividing the total platelet count by the platelet survival in days. With the selenomethionine (75Se) method, the normal platelet survival is 10.5 days, and the platelet turnover is 38,000 per day. In PV, the mean platelet survival is six to seven days with the turnover averaging 100,000 platelets per day. It is important to recognize that even though the platelet count may be within normal limits, for example, 400,000 per μl, a shortened platelet survival will lead to a substantial increase in platelet turnover. Fibrinogen levels tend to be within the normal range in PV, but the fibrinogen survival is decreased resulting in an increased fibrinogen turnover. These results strongly suggest a hypercoagulable state.

Although hypercoagulability, presumably initiated by increased platelet turnover and consumption, is common in PV, in vitro tests of platelet function demonstrate defective platelet function. In vitro platelet aggregation with epinephrine and, frequently, collagen is markedly decreased. Receptors for epinephrine on the platelet membrane in the myeloproliferative states and, in particular, PV are absent or decreased. One might anticipate that such an abnormality would lead to a hemorrhagic diathesis rather than a hypercoagulable state. There is no clear explanation for this paradox.

Recent studies in our laboratories have also demonstrated the association of reverse transcriptase activity and viral-like particles in platelets obtained from patients with PV in whom chromosomal abnormalities are present in the bone marrow, and the platelet count is usually elevated. The bone marrow in PV demonstrates a great increase in the number and the size of the megakaryocytes. The marrow in PV shows a panmyelosis with virtually no fat.

Chromosomal analysis of the bone marrow is important in the initial evaluation of all patients with PV. Recent data indicate that those patients who have chromosomal abnormalities at diagnosis are more likely to develop solid tumors and leukemia when treated with alkylating agents such as chlorambucil.

PV is one of the most common causes of secondary gout. Uric acid levels are frequently elevated owing to increased turnover of marrow elements, and gout may be one of the presenting manifestations of the disease.

The majority of patients presenting with PV can be distinguished from secondary and relative polycythemia by adherence to the criteria established by
the PSVG (table VI). However, there are borderline cases in which the packed red cell mass is only slightly elevated or within normal range. In some of these patients the major initial presentation may be a slight elevation of the platelet count and increased platelet turnover. Frequently, determination of reverse transcriptase activity, marrow chromosomes as well as examination of bone marrow biopsies, will be helpful in differential diagnosis. The most difficult problem is a patient who presents with an elevated red cell mass with no abnormalities in either myelopoiesis or thrombopoiesis. In such cases, it is essential to establish that the arterial oxygen saturation and tension are within normal limits. It is also important to rule out a hemoglobinopathy or an abnormal shift of the oxygen dissociation curve to the left, i.e., increased affinity of hemoglobin for oxygen. The PSVG should be calculated. Erythropoietin levels should be increased in patients with inappropriate (nonphysiologic) polycythemia.

Some hematologists are convinced that there is a subset of patients with PV whose only abnormality is indeed an elevated red cell mass, and in whom the only therapy required is occasional phlebotomy to maintain the red cell mass within normal limits. With the development of more sophisticated tools and perhaps probes for viral-like activity, clinical subsets of PV will be diagnosed. Obviously, such conditions may exist. However, in our department a diagnosis of PV is not made unless abnormalities can be demonstrated in megakaryocytopoiesis, thrombopoiesis or platelet function.

Families have been described in which erythrocytosis was found as an inherited trait unrelated to any abnormalities in oxygen transport. In some of these families, erythropoietin production was increased. The suggestion was made that such a disorder is caused by a genetic defect in erythropoietin-producing cells or in response to erythropoietin.

Finally, one must be alert to the possibility of an iatrogenic stimulation in erythropoiesis owing to drugs such as androgens and cobaltous chloride. Androgens can stimulate erythropoiesis and increase the red blood cell mass by two mechanisms: (1) direct stimulation of bone marrow, and (2) secondary increase in erythropoietin level. Cobalt chloride causes secondary polycythemia probably by shifting the oxygen dissociation curve to the left and increasing the erythropoietin production.

References

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