

Polycythemia Vera

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Polycythemia rubra vera (PV) is a clonal, myeloproliferative, stem cell disorder characterized by erythrocytosis frequently associated with leucocytosis and thrombocytosis of varying magnitude. The clonal nature of the disease and its origination at the multipotent hematopoietic stem cell level have been proven by G6PD isoenzyme analysis in female heterozygous patients¹.

The pathogenesis of this disease has attracted a lot of attention over the last thirty years. It was initially found that PV marrow erythroid cells could synthesize hemoglobin *in vitro* in the absence of exogenous erythropoietin (EPO)². Levels of serum and urine EPO were also found to be normal or depressed in PV,³⁻⁵ suggesting that in this disease there is an abnormal regulation of erythropoiesis due to an abnormality in the hematopoietic stem cell. Subsequent studies on erythroid progenitors, colony and burst forming units (CFU-E, BFU-E), have shown that in PV there are two types of erythroid progenitor cells, one type (normal clone) exhibiting normal response to erythropoietin and another type (PV clone) that shows independence from EPO^{6,7}. The latter is responsible for the ability of PV erythroid progenitors to generate even in serum-free media erythroid colonies *in vitro* in the absence of exogenous EPO (endogenous, intrinsic or spontaneous erythroid colony formation)⁸. The independence from or the hypersensitivity of PV erythroid progenitors to EPO is due neither to an increase in the number or affinity of EPO receptors⁹ nor to any mutation in the EPO receptor gene¹⁰. Actually, in PV only low affinity EPO receptors could be detected on erythroid progenitor cells (CFU-E)⁹. In addition, early erythroid precursors show increased sensitivity *in vitro* to interleukin 3, granulocyte-macrophage colony stimulating factor, insulin growth factor I and stem cell factor, whereas PV myeloid and megakaryocytic progenitors exhibit increased sensitivity *in vitro* to interleukin 3, granulocyte-macrophage colony stimulating factor and thrombopoietin¹¹⁻¹⁴. The recent claim that in PV hypersensitivity to EPO can be attributed to IGF-1¹² has not been confirmed and new studies using the same serum-free conditions of cultures have confirmed the hypersensitivity of PV erythroid progenitors to EPO even in purified CD34+ cells¹⁵. The hypersensitivity/independence of PV erythroid cells to/from EPO has been further confirmed by the recent demonstration that PV erythroid precursors overexpress Bcl-x_L¹⁶, an anti-apoptotic protein that is induced by EPO but not serum alone (source of IGF-1) in erythroid precursor cells¹⁷. It is not known at this time whether the overexpression of Bcl-x_L is the primary abnormality leading to growth factor hypersensitivity/independence or it is secondary to a yet unknown genetic defect in the PV clone.

The increased sensitivity of PV hematopoietic progenitors to a variety of growth factors have led to a search for an abnormality in the signal transduction pathway common to all these factors. The most reasonable candidate was thought to be the hematopoietic cell tyrosine phosphatase SHP-1, which is normally a negative regulator of many growth factor signaling¹⁸. This enzyme is genetically missing from the lymphoid and hematopoietic cells of motheaten mice that exhibit hematological abnormalities in their spleen resembling those commonly observed in PV marrow¹⁹. The structure and transcriptional integrity of the SHP-1 gene was examined in granulocytes of PV patients and found to be intact²⁰. Other investigators measured mRNA and protein levels of SHP-1 phosphatase in CD34+ and peripheral blood PV cells and found them to be normal²¹, whereas another group reported an increased phosphatase activity in erythroid progenitors from three patients with PV²². The practical difficulty with these studies is that cells tested for SHP-1 activity represent a mixture of cells derived from both the normal and the PV abnormal clone, and normal levels do not exclude the possibility of an existing but undetectable abnormality under such conditions. More recently, diminished levels of SHP-1 were reported in erythroid progenitors of 8 out of 13 patients with PV, which were grown *in vitro* under conditions favoring "endogenous" colony formation both at the mRNA and protein level¹⁵. If the levels of SHP-1 in erythroid cells are independent of the concentration of growth factors under the influence of which they grow, the latter study may be the first to indicate an involvement of the hematopoietic tyrosine phosphatase SHP-1 in the pathogenesis of PV.

The hypersensitivity of PV erythroid cells to IGF-1 has led to studies on the status of IGF-1 receptor, which was found to be constitutively activated in peripheral blood mononuclear cells of PV patients²³. This activation of the receptor was not associated with high levels of serum IGF-1 but with a fourfold increase in the level of serum IGF-1 binding protein-1. The latter has been found to be enhancing the effect of IGF-1 on peripheral blood BFU-E²⁴. The reason for the elevation of IGF-1 in PV and its relation to the pathogenesis of this disease remains unknown.

The diagnosis of PV has been traditionally relied on the criteria established by the Polycythemia Vera Study Group more than 30 years ago. These criteria were devised to guarantee a homogeneous population of patients in whom comparison of different types of treatment would be meaningful. The criteria were not set up for routine clinical use. The routine measurement of red cell mass does not seem necessary in all patients. The test is expensive, may not be available in all hospitals, and requires vigorous standard-

ization. It allows the differentiation of true from relative erythrocytosis. Measurement of red cell mass may not be necessary in patients with an elevated hematocrit equal to or higher than 59%; with splenomegaly; persistent leucocytosis or thrombocytosis; pruritus; or with a hypercellular marrow showing trilinear hyperplasia.

In recent years, measurement of serum EPO levels, detection of endogenous erythroid colony formation by peripheral blood mononuclear cells, detection of low levels of TPO receptors on platelets and detection of a high percentage of glycophorin-positive nucleated marrow cells expressing Bcl-x_L have been proposed as laboratory tests for the differential diagnosis of PV from secondary polycythemia.

Serum EPO levels are in general low or normal in PV and normal or elevated in secondary polycythemia³⁻⁵. A significantly elevated serum EPO level is indicative of secondary polycythemia and exclusive of PV, but a normal level does not exclude either. In almost 40-50% of cases, serum EPO levels overlap between the group with PV and the group with secondary polycythemia^{4,5}. Even with the use of immunoassays that can detect very low levels of serum EPO, a single determination cannot reliably assign almost half of patients to either the PV or the secondary polycythemia group⁵. In addition, it is necessary for each laboratory to establish its own values that provide a specificity of equal or greater than 95% as far as assigning patients to either group⁵. Phlebotomy has no significant effect on the level of EPO in PV, but in secondary polycythemia it tends to increase after phlebotomy and the levels fluctuate even during a steady state^{5,25}. For all these reasons the measurement of a single serum EPO level has limited clinical utility and should be interpreted with caution.

Endogenous erythroid colony formation (without addition of exogenous EPO) is a feature of myeloproliferative disorders and is primarily seen in PV, essential thrombocythemia and myelofibrosis²⁶. This assay is not available in all laboratories and its final interpretation requires a high level of expertise since, in more than half of PV cases, spontaneous erythroid colonies are so unusually small and poorly hemoglobinized that they may escape detection. In addition the specificity and sensitivity of the test does not seem to be high and cannot distinguish with certainty between PV and secondary polycythemia. In the larger study published endogenous colony formation was detected in 70% of cases of PV but also in 25% of cases classified as secondary polycythemia²⁷. A modification of the assay using a two-stage liquid culture system and detection of erythroid cells by flow cytometry has been proposed but its sensitivity and specificity has not yet been tested²⁸. Detection of endogenous colonies for the differential diagnosis of polycythemia should be restricted to cases where all other tests have failed to provide the answer and there is access to a laboratory with experience in this technique.

Two relatively new observations may provide reliable tests to differentiate primary from secondary polycythemia. Receptors for thrombopoietin are very decreased or absent

from PV platelets whereas they can be detected in platelets from normal controls and patients with secondary polycythemia²⁹. Although the significance of this finding and its relation to the pathogenesis of the disease remain unclear, it may provide an easy and clinically useful assay for the differential diagnosis of polycythemia. Also, the finding that marrow erythroblasts in PV express Bcl-x_L protein in a much higher percentage (22±4, n=14) than normal marrow erythroblasts or erythroblasts from patients with secondary polycythemia (7±2, n=12) may prove to be clinically useful for the diagnosis of PV¹⁶. The clinical utility of both these assays needs to be tested in a large number of patients before their implementation in clinical practice.

The treatment of PV continues to follow to a great extent the recommendations made by the Polycythemia Vera Study Group, which were based on the results of the studies run by this group^{30,31}. Phlebotomy, hydroxyurea or ³²P and antiplatelet agents are progressively added to control the disease and prevent new episodes of thrombosis. Today, 35 years after these studies were designed, it is important to recognize their limitations.

Phlebotomy, although the least toxic of all treatments, may not be ideal for a young (<50) patient with a strong family history of vascular disease, hyperlipidemia, hypertension or diabetes. Considering the fact that during the first three years of phlebotomy the occurrence of thrombotic events is high and that the PV studies did not stratify patients into groups with high- and low-risk factors for vascular disease, it may be wise in high-risk patients to use hydroxyurea in combination with phlebotomy. However, such an approach has not yet been tested. In addition, the lack of leukemogenicity of hydroxyurea when used in younger patients for many years is not yet proven beyond any doubt and conflicting reports exist in the literature³¹.

The role of thrombocytosis in the pathogenesis of thrombosis and the role of anti-platelet agents in the treatment of PV remain unclear. Despite the fact that no study so far has incriminated thrombocytosis as a risk factor for thrombosis in PV, it is worth noticing that the thrombosis-free survival has been superior for patients receiving alkylating agents or radioactive phosphorus compared to those treated by phlebotomy alone. This may indicate that control of the red cell mass alone may not provide the optimal prophylaxis from thrombosis. Thus, reduction of platelet numbers to almost normal by hydroxyurea, anagrelide or interferon- α and decrease of platelet function by low-dose aspirin or other anti-platelet agents may be necessary. However, the platelet number above which such a treatment is necessary, the target platelet count for such a therapy, and its effectiveness are not well established. Extrapolating data from studies on essential thrombocythemia may be equally misleading since these studies did not account for all risk factors and did not assess the pretreatment risk for thrombosis³². Thus, further studies are needed in which patients should be stratified according to the risk of thrombosis based on risk factors and non-invasive tests of the cerebral and coronary circulation, which will address the efficacy of phle-

botomy alone versus phlebotomy in combination with hydroxyurea, interferon, or anagrelide plus/minus anti-platelet drugs. Until the results of such studies become available, the practicing hematologist should individualize the treatment of PV according to the risk factors of the patient.

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