Diagnostic Evaluation of the Chronic Myeloproliferative Disorders

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Polycythemia vera, idiopathic myelofibrosis, essential thrombocytosis, and chronic myelogenous leukemia are traditionally classified under the rubric of the chronic myeloproliferative disorders. The rationale is that these disorders share several features: an origin in a multipotent hematopoietic progenitor cell, clonal dominance of the malignant progenitor cells over their normal counterparts, overproduction of one or more of the formed elements of the blood, extramedullary hematopoiesis, and transformation to acute leukemia – but at widely varying rates. Not surprisingly, there is substantial phenotypic mimicry among the chronic myeloproliferative disorders as well as between them and other clonal and non-clonal hematopoietic disorders. Therefore, distinguishing between the chronic myeloproliferative disorders clinically as well as between them and other benign or malignant conditions can be difficult [Table 1]. The problem is compounded because the clinical presentation of these disorders is variable, and of the four, only chronic myelogenous leukemia has a specific diagnostic marker, the 9-22 chromosomal translocation that gives rise to the chimeric tyrosine kinase, Bcr-Abl. This review discusses how to resolve the diagnostic dilemma with respect to the other three chronic myeloproliferative disorders – polycythemia vera, idiopathic myelofibrosis, and essential thrombocytosis.

Polycythemia vera

Polycythemia vera is unique among the chronic myeloproliferative disorders because it is the only one with increased red cells, white cells and platelets. However, in an era of widely available medical care and routinely measured blood counts, thanks to automated blood counters, patients with PV are frequently encountered before they have developed classical tri-lineage hematopoietic cell hyperplasia. In two large epidemiologic studies of PV, only 39% of patients presented with an elevation of red cells, white cells and platelets, 30% presented with an elevation of red cells and white cells, and 13% with elevation of red cells and platelets, while 18% presented with erythrocytosis alone [1,2]. Some PV patients may even present with myelofibrosis [3]. William Osler was the first to recognize the clinical issue of phenotypic mimicry with respect to polycythemia vera [Table 1] and the first to propose diagnostic criteria, which have stood the test of time. These diagnostic criteria were expanded by the Polycythemia Vera Study Group to encompass leukocytosis and thrombocytosis [4], features that Osler did not observe in his patients. The diagnostic criteria of the PVSG have continued to prove useful, but are perhaps as important for what they do *not* specify as for what they do.

PV = polycythemia vera

Bone marrow examination is not part of the PVSG diagnostic criteria. This is appropriate since if all the diagnostic criteria are met, bone marrow examination is unnecessary; and if they are not – for example, if erythrocytosis is absent – bone marrow examination is of little help since the histologic findings may be similar to those in the other chronic myeloproliferative disorders. Furthermore, PV is a clonal disorder and histology is not a sufficient surrogate for clonality. It should also be remembered that in the PVSG experience, bone marrow histology was normal in 13% of patients meeting the diagnostic criteria for polycythemia vera [5]. While a bone marrow specimen is the best source of cells for conventional cytogenetic studies, at the time of diagnosis no more than 25% of patients will have a non-random chromosomal abnormality [6]. Thus, for this approach to be successful, four to five patients would have to be evaluated to find one with positive cytogenetics, and since not all patients being evaluated for erythrocytosis will have PV, it is likely that twice as many would have to be studied to obtain a positive result. It should also be noted that neither a cytogenetic abnormality [6] nor even the presence of myelofibrosis, which was observed in 11% of patients at the time of diagnosis [5], has any prognostic significance.

An assay for serum erythropoietin was also not part of the PVSG diagnostic criteria, an omission that can be attributed to the fact that a sensitive and specific assay for this protein was not available at the time the diagnostic criteria were formulated. Although we now have such an assay, it cannot be relied upon to distinguish between PV and erythropoietin-driven erythrocytosis because of the physiology of erythropoietin. Although tissue hypoxia is the only physiologic signal for increasing erythropoietin production, there are several independent mechanisms for reducing erythropoietin production or down-regulating the circulating erythropoietin level. These include catabolism of the hormone by its target cells [7] and feedback inhibition of its synthesis by amelioration of tissue hypoxia, as well as the increase in whole-blood viscosity caused by the expanded red cell mass [8]. Thus, while tissue hypoxia increases erythropoietin synthesis, the effect of the expanded erythroid progenitor cell pool and red cell mass is to suppress this process. Consequently, it is not surprising that the serum erythropoietin level is not only normal or low in polycythemia vera, but may also be normal in patients with a hypoxic cause for erythrocytosis if the

PSVG = Polycythemia Vera Study Group

Table 1. Diagnostic considerations in the evaluation of the chronic myeloproliferative disorders

Causes of Myelofibrosis	Causes of Erythrocytosis	Causes of Thrombocytosis
Malignant	**Relative Erythrocytosis**	**Tissue inflammation**
Acute leukemia (lymphocytic,	Hemoconcentration secondary to	Collagen vascular disease
myelogenous, megakaryocytic)	dehydration, androgens or	Inflammatory bowel disease
Chronic myelogenous leukemia	tobacco abuse	**Malignancies**
Hairy cell leukemia		**Infection**
Hodgkin's disease	**Absolute erythrocytosis**	**Myeloproliferative Disorders**
Idiopathic Myelofibrosis	**Hypoxia**	Polycythemia vera
Lymphoma	Carbon monoxide	Idiopathic myelofibrosis
Multiple myeloma	High affinity hemoglobin	**Essential thrombocytosis**
Myelodysplasia	High altitude	Chronic myelogenous leukemia
Metastatic carcinoma	Pulmonary disease	Myelodysplastic disorders
Polycythemia vera	Right to left shunts	5q-syndrome
Systemic mastocytosis	Sleep apnea syndrome	Idiopathic refractory
	Neurologic disease	sideroblastic anemia
Non-malignant		**Postsplenectomy or**
HIV infection	**Renal disease**	**hyposplenism**
Hyperparathyroidism	Renal artery stenosis	**Hemorrhage**
Renal osteodystrophy	Focal sclerosing or membranous	**Renal disease**
Systemic lupus erythematosis	glomerulonephritis	**Iron deficiency anemia**
Tuberculosis	Renal transplantation	**Surgery**
Vitamin D deficiency		**Rebound**
Thorium dioxide exposure	**Tumors**	Correction of vitamin B12 or
	Hypernephroma	folate deficiency, post-ethanol
	Hepatoma	abuse
	Cerebellar hemangioblastoma	**Hemolysis**
	Uterine fibromyoma	
	Adrenal tumors	
	Meningioma	
	Pheochromocytoma	
	Drugs	
	Androgens	
	Recombinant erythropoietin	
	Familial (with normal	
	hemoglobin function)	
	VHL mutations	
	Polycythemia vera	

hypoxia is not severe [9]. This is also true when there is inappropriate erythropoietin production, since the normal range for serum erythropoietin is wide and physiologic erythropoietin production would be down-regulated. Thus, while an elevated serum erythropoietin level excludes PV as a cause of erythrocytosis, a normal erythropoietin level does not exclude a hypoxic, non-clonal or tumor-related cause.

Similarly, although *in vitro* erythropoietin-independent colony formation is a characteristic feature of PV erythroid progenitor cells and has been used as a diagnostic assay, it cannot be recommended clinically for this purpose. Erythropoietin-independent erythroid colony formation is not specific for polycythemia vera [10] and its absence does not exclude the diagnosis. The test is also not standardized, nor is it widely available.

Interestingly, although PV is a clonal disorder, the PVSG diagnostic criteria do not specify a demonstration of clonality as a requirement. Presumably, this omission acknowledged the low frequency of non-random cytogenetic abnormalities at the time of diagnosis and the lack of other suitable clonal assays. For example, clonality assays involving random inactivation of X-chromosome genes are useful only in women who are heterozygous with respect to specific polymorphisms of these genes, an occurrence that is not only low in frequency but also further limited diagnostically by age-linked X-chromosome gene inactivation. Recently, however, two potential clonal diagnostic assays have been developed for PV. The first exploits the observation that compared to patients with erythrocytosis from other causes, expression of the thrombopoietin receptor, Mpl, in polycythemia vera platelets and megakaryocytes is impaired due to a defect in post-translational processing [11]. Quantitative densitometry of Mpl in immunoblots of whole platelet lysates can distinguish PV from other forms of erythrocytosis with a specificity of 95% and a sensitivity of 96% [12]. Since the Mpl post-translational processing defect was present in polycythemia vera CD34+ cells [13], this defect appears to tract with the abnormal clone. Immunohistochemistry of bone marrow megakaryocyte Mpl is an alternative to platelet Mpl immunoblotting as a diagnostic test [14].

A second potential clonal assay for PV is real-time reverse transcriptase polymerase chain reaction to measure the expression of PRV-1, a member of the uPAR receptor family that is uniquely expressed in PV granulocytes [15]. PRV-1 over-expression was identified in polycythemia vera granulocytes by subtractive hybridization. Interestingly, the spectrum of its expression in the chronic myeloproliferative disorders was similar to that of impaired Mpl expression, supporting the contention that these assays have clonal relevance and should be studied as diagnostic tests for PV in a prospective fashion.

With respect to the specific diagnostic criteria stipulated by the PVSG, the most important is measurement of both the red cell mass and the plasma volume. While a hematocrit of 60% or higher in a man or 50% or higher in a woman indicates an elevated red cell mass, it cannot be emphasized too strongly that at lower hematocrits one cannot exclude an elevation of the red cell mass when polycythemia is a diagnostic consideration [16] or distinguish between absolute erythrocytosis and plasma volume contraction except by direct measurement of both. In the former instance, this is due to the unique pathophysiology of polycythemia vera. In this disorder, in contrast to hypoxic or erythropoietin-driven erythrocytosis, as the red cell mass expands there is concomitant expansion of the plasma volume. This is accentuated with splenomegaly. As a result, elevation of the red cell mass can be masked by the plasma

volume expansion and will be missed if the hematocrit alone is relied upon to assess the presence of erythrocytosis. With hypoxia or administration of recombinant erythropoietin, expansion of the red cell mass is associated with plasma volume contraction, an effect also seen with androgen administration. Plasma volume contraction is also a feature of other disorders and can be provoked by a number of drugs. Direct measurement of both the red cell mass and plasma volume is thus the only means to sort out the underlying cause for a high hematocrit. Measurement of the red cell mass alone provides no information about the plasma volume, while derivation of the red cell mass from the plasma volume is inaccurate because in disease these vary independently of each other. Furthermore, since erythrocytosis is the sole diagnostic feature that distinguishes polycythemia vera from its companion myeloproliferative disorders, its diagnosis can only be established by measuring the red cell mass. Even if there were a validated clonal assay for PV, a red cell mass determination would still be mandatory as this measurement also has therapeutic implications insofar as phlebotomy therapy is needed. Finally, if no discernible cause for an isolated acquired erythrocytosis can be found, there is no need to rush any decision. Survival with PV is not greatly different from normal [17], and the only appropriate treatment for erythrocytosis of unknown etiology is phlebotomy to a hematocrit of 42% or less in women and 44% or less in men.

Idiopathic myelofibrosis

Idiopathic myelofibrosis is the least common of the chronic myeloproliferative disorders and the most difficult to diagnose – not only because its hallmark characteristic, marrow fibrosis, is a reactive phenomenon complicating many other hematologic as well as non-hematologic disorders [Table 1], but because this very characteristic frustrates the diagnostic process by preventing marrow aspiration and, often, the collection of an adequate biopsy specimen. However, it is crucial to establish the basis for marrow fibrosis since the clinical course and prognosis of each of the various disorders causing myelofibrosis differs, as does their treatment. Since there is no clonal marker for idiopathic myelofibrosis, like its companion myeloproliferative disorders, polycythemia vera and essential thrombocytosis, the diagnosis of idiopathic myelofibrosis must be established clinically. Unlike the other two disorders, however, a bone marrow biopsy is mandatory for diagnosis as the presence of fibrosis cannot be established any other way. Attempts to diagnose idiopathic myelofibrosis histologically in the absence of marrow fibrosis are illogical and can only lead to the misdiagnosis of other myeloproliferative disorders. Even the presence of myelofibrosis is no guarantee that the illness is idiopathic myelofibrosis as polycythemia vera can also present in this manner. Indeed, if the hematocrit is normal in a patient with marrow fibrosis, particularly in the presence of splenomegaly, a red cell mass determination is essential.

Marrow fibrosis is not the only mandatory abnormality for establishing the diagnosis of idiopathic myelofibrosis; splenomegaly is as well. In the various published series describing patients with idiopathic myelofibrosis, palpable splenomegaly was present in 98% (range 89–100). Thus, while splenomegaly does not establish that idiopathic myelofibrosis as the basis for marrow fibrosis, its absence should raise the possibility of another cause for this abnormality [Table 1]. Autoimmune diseases and certain infections can complicate the diagnostic picture because patients with idiopathic myelofibrosis are prone to deep-seated tissue infections and autoimmune phenomena.

The incidence of cytogenetic abnormalities is approximately 30% in idiopathic myelofibrosis. Thus, this test has more diagnostic value than in polycythemia vera or essential thrombocytosis, although none of the chromosomal abnormalities is specific for the disease and there is still a large diagnostic gap. To resolve this dilemma, a set of diagnostic criteria has been proposed in which marrow fibrosis and the absence of Bcr-Abl expression are mandatory [18]. Unfortunately, splenomegaly is an optional criterion and is not required in the presence of other evidence of extramedullary hematopoiesis. However, like marrow fibrosis, extramedullary hematopoiesis can be a reactive or compensatory phenomenon [19] and thus should not be accorded the diagnostic specificity it receives with this approach. Recently, it was observed that the number of circulating CD34+ cells in patients with idiopathic myelofibrosis greatly exceeds that in other myeloproliferative disorders [20] and this may prove helpful in the diagnostic process. In the end however, until a clonal marker is identified, idiopathic myelofibrosis remains a diagnosis of exclusion since so many of the diseases that it mimics have specific and effective treatments that are still lacking for this disorder.

Essential thrombocytosis

The diagnosis of essential thrombocytosis encounters the same difficulties as for the other chronic myeloproliferative disorders – namely, lack of a clonal marker and phenotypic mimicry – and even more, since all these disorders can present with thrombocytosis but no more than 20% of patients with thrombocytosis will actually have a chronic myeloproliferative disorder as the cause. As might be expected, this is most likely to be the case when the platelet count is greater than 1,000,000/mm^3 [21]. As with the other chronic myeloproliferative disorders, diagnostic criteria have been proposed as surrogates for the lack of a clinically applicable clonal marker. However, even when clonality was established in informative women patients heterozygous for an X-chromosome gene polymorphism, no clinical differences were apparent between these patients and their polyclonal counterparts with thrombocytosis, with the exception of an increased incidence of thrombosis in one series [22]. Furthermore, none of the diagnostic criteria proposed are specific for essential thrombocytosis, and there has been uncertainty as to what degree the platelet count needs to be elevated before a diagnosis of essential thrombocytosis can be considered. The height of the platelet count is, however, irrelevant since thrombocytosis in the chronic myeloproliferative disorders is progressive and the degree of thrombocytosis merely reflects the stage at which the patient is first seen. At the same time, it should be recognized that the lower the platelet count the more likely that its cause is not a chronic myeloproliferative disease.

From a clinical perspective, essential thrombocytosis is a disorder compatible with a normal life span [17], and it is more

important to recognize diseases causing thrombocytosis that have serious prognostic implications or complications if not recognized. These include chronic myelogenous leukemia, carcinomas, myelodysplasia, and polycythemia vera. Therefore, although the frequency of cytogenetic abnormalities is no higher in essential thrombocytosis than in PV, it is likely that the elevated platelet count could be the result of either chronic myelogenous leukemia or myelodysplasia, and thus bone marrow examination is more appropriate diagnostically in this situation. While bone marrow morphology cannot establish a diagnosis of essential thrombocytosis, it can certainly exclude it, and cytogenetic analysis is also best performed using marrow cells. With respect to the controversy over Bcr-Abl expression in essential thrombocytosis, there is no question that some patients with chronic myelogenous leukemia present with clinical findings identical to essential thrombocytosis [23]. At the same time, RT-PCR may be too sensitive a method for establishing this, and Bcr-Abl fluorescent *in situ* hybridization should be the method of choice. There is, of course, no point in performing *in vitro* culture studies of erythroid or megakaryocytic progenitor cells as diagnostic tests, since these will not distinguish essential thrombocytosis from polycythemia vera [10]. Iron deficiency can cause thrombocytosis and iron deficiency can mask erythrocytosis. Furthermore, since essential thrombocytosis can evolve into PV, diagnostic complacency needs to be avoided. Finally, it should be remembered that like erythrocytosis, a cause for thrombocytosis may not always be forthcoming, but that does not establish the thrombocytosis as clonal. Inappropriate treatment of patients with isolated erythrocytosis has led to the development of acute leukemia [24], and this has also been true for patients with essential thrombocytosis [25]. Since there is no proof that treating asymptomatic thrombocytosis prolongs survival, when the platelet count is less than 1,000,000/mm^3 treatment should be dictated by patient symptoms, and when greater than that either by symptoms or evidence of a hemorrhagic diathesis. In either instance, mutagenic agents should be avoided whenever possible.

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RT-PCR = reverse transcriptase polymerase chain reaction