Here we critically evaluate the role of elevated hematocrit as the principal determinant of thrombotic risk in polycythemia and erythrocytosis, defined by an expansion of red cell mass. Since red cell volume determination is no longer readily available, in clinical practice, polycythemia and erythrocytosis are defined by elevated hemoglobin and hematocrit. Thrombosis is common in Chuvash erythrocytosis and polycythemia vera. Although the increased thrombotic risk is assumed to be due to the elevated hematocrit and an associated increase in blood viscosity, thrombosis does not accompany most types of erythrocytosis. We review studies indicating that the occurrence of thrombosis in Chuvash erythrocytosis is independent of hematocrit, that the thrombotic risk is paradoxically increased by phlebotomy in Chuvash erythrocytosis, and that, when compared to chemotherapy, phlebotomy is associated with increased thrombotic risk in polycythemia vera. Inherited and environmental causes that lead to polycythemia and erythrocytosis are accompanied by diverse cellular changes that could directly affect thrombotic risk, irrespective of the elevated hematocrit. The pressing issue in these disorders is to define factors other than elevated hematocrit that determine thrombotic risk. Defining these predisposing factors in polycythemia and erythrocytosis should then lead to rational therapies and facilitate development of targeted interventions.

Introduction

Polycythemia and erythrocytosis

There are several different parameters for diagnosis of polycythemia and erythrocytosis based on a blood count: the number of red blood cells, the hematocrit, and the hemoglobin concentration. Elevations in these measures can occur on a primary or secondary basis (Table 1). Primary polycythemia results from functional abnormalities intrinsic to erythroid progenitors, causing them to be hypersensitive to or independent of erythropoietin. This category includes polycythemia vera (PV), which is associated with acquired somatic mutations in the Janus kinase 2 gene (JAK2), dominantly inherited primary familial and congenital polycythemia or erythrocytosis, caused by germline gain-of-function erythropoietin receptor (EPOR) mutations, and erythrocytosis due to \( \text{SH2B3} \) mutations. Primary familial and congenital polycythemia or erythrocytosis predisposes patients to cardiovascular disorders, perhaps due to chronic augmented erythropoietin signaling in all tissues bearing EPOR. In contrast, in secondary erythrocytosis, functionally normal erythroid progenitors are exposed to increased levels of circulating erythropoiesis-stimulating factors. In most instances, the erythropoiesis-stimulating factor is erythropoietin, but cobalt, insulin growth factor 1, increased angiotensin signaling and manganese may also stimulate erythropoiesis.

Acquired causes of secondary erythrocytosis include erythrocytosis of pulmonary disease, high altitude erythrocytosis, Eisenmenger syndrome, smoking, carboxyhemoglobinemia, erythropoietin-producing tumors, doping with erythropoietin, post-
reduced renal transplant erythrocytosis, exogenous testosterone use, and cobalt and manganese toxicities.12,13 Congenital secondary erythrocytosis can be caused by high oxygen affinity hemoglobin variants, inherited low 2,3-diphosphoglycerate leading to high hemoglobin oxygen affinity, congenital methemoglobinemia, and a recently described gain-of-function mutation of the gene encoding erythropoietin (EPO).1 Other congenital conditions include rare germline mutations in hypoxia sensing pathway genes, including loss of function mutations of VHL encoding von Hippel Lindau (VHL) protein and EGLN1 encoding prolyl hydroxylase 2 (PHD2), and gain-of-function mutations of EPAS1 encoding hypoxia inducible factor (HIF)-2α.1

Chuvash erythrocytosis (CE) is an autosomal recessive condition, endemic to Chuvashia in Russia and Ischia in Italy, which results from homozygosity for a C→T missense mutation of VHL (VHL c.598C>T or VHL E200K).8,10 The mutated protein impacts interactions of VHL with the HIF-α subunits, thereby reducing the rate of ubiquitin-mediated HIF-α degradation by the proteasome. As a result, the levels of HIF-1 and HIF-2 heterodimers increase, leading to increased expression of their target genes, including EPO, vascular endothelial growth factor (VEGF), and GLUT1, tissue factor (F3) and a plethora of other genes.9,11 In endothelial cells, more than 3% of genes are upregulated by HIF-1.11 CE erythrocytogenitors are hyper-sensitive to erythropoietin, a feature of primary polycythemia, but affected subjects also have increased erythropoietin levels mediated by increased HIF-2, a feature of secondary erythrocytosis.11,12 Similar combined features of both primary and secondary elevations in hematocrit are seen in certain other germline mutations of VHL (loss-of-function mutations) and EPAS1 (gain-of-function mutations).1

Viscosity, hematocrit and blood volume
Both PV and erythrocytosis secondary to hypoxia or upregulated hypoxia sensing are characterized by an increased red cell mass and total blood volume, but the two conditions may at times be divergent with regard to plasma volume. The plasma volume is increased in PV, potentially causing the hematocrit to underestimate the degree of erythrocytosis, whereas the plasma volume may not be increased in all types of erythrocytosis secondary to hypoxia or to upregulated hypoxia sensing.11,12 Some clinical manifestations of erythrocytosis, such as headaches and tinnitus, appear to be related to increased viscosity of blood resulting from the expanded red cell mass and elevated hematocrit. An increase in blood viscosity at higher hematocrits is associated with blood volume in the normal range impairs blood flow and reduces the transport of oxygen.17 In vitro, the viscosity of blood increases exponentially with an increase in hematocrit. However, mitigating factors in patients with erythrocytosis serve to improve oxygen transport, a process that is dependent on both cardiac output and hemoglobin concentration.14 Most importantly, the increase in blood volume accompanying erythrocytosis enlarges the vascular bed, decreases peripheral resistance and increases cardiac output. In addition, the blood flow is axial, with a central core of circulating red cells sliding over a peripheral layer of lubricating plasma. Therefore, optimum oxygen transport with increased blood volume occurs at a higher hematocrit value than with normal blood volume,15,16 and a moderate increase in hematocrit may be beneficial despite the increased viscosity. This may not hold true when there is a more pronounced increase in hematocrit, a circumstance in which high viscosity causes reduced blood flow19,20 that may be responsible for cerebral and cardiovascular impairment in some high-altitude dwellers21 or in patients with severely elevated hematocrit.22,23 In those instances, hematocrit has been reported to reach extreme values, sometimes exceeding 90%.24 In normovolemic individuals, cerebral blood flow decreases at a certain point of hematocrit elevation.25 However, blood flow is also influenced by the oxygen demand of tissues through incompletely understood mechanisms26 and cerebral blood flow remains high at high hematocrits when oxygen delivery is impaired. This was elegantly illustrated in six patients with high hemoglobin oxygen-affinity variants whose cerebral blood flow was 81% higher than that of 11 subjects of comparable age, matched for hematocrit and viscosity, but without the hemoglobin variant.27 Furthermore, cerebral blood flow decreases at much higher levels of hematocrit with any accompanying increased percentage of fetal hemoglobin,28 which also has high oxygen-affinity.29

Elevated hematocrit and thrombosis
Thrombotic events are well documented in patients with PV and CE, apparently less so in those with primary familial and congenital polycythemia or erythrocytosis and HIF-2α gain-of-function mutations, but not in patients with secondary erythrocytosis such as Eisenmenger syndrome,30,31 other cyanotic heart disorders,32,33 high altitude dwellers,34

Table 1. Classification of polycythemia and erythrocytosis.

| Primary - functional abnormalities expressed in erythroid progenitors | Familial | Secondary to increased erythropoietin | Acquired |
|---|---|---|---|
| Primary familial & congenital polycythemia or erythrocytosis | Acquired Polycythemia vera (JAK2 mutations) | Acquired Carboxyhemoglobinemia | Smoking |
| (EPOR mutations) | | Erythropoietin-doping Erythropoietin-secreting tumor | Lung or heart disease |
| Erythrocytosis due to SH2B3 mutations | Acquired to increased exposures other than erythropoietin | High altitude | Smoking |
| | | | |
| | | | Secondary to increased erythropoietin |
| | | | Acquired Smoking |
| | | | Carboxyhemoglobinemia |
| | | | Erythropoietin-doping |
| | | | Erythropoietin-secreting tumor |
| | | | Lung or heart disease |
| | | | Smoking |
| | | | High altitude |

| Table 1. Classification of polycythemia and erythrocytosis. | |
|---|---|---|---|
| | | | |
and subjects with high oxygen-affinity hemoglobins. Several lines of evidence suggest that an isolated elevation in hematocrit does not, per se, lead to thrombosis. For example, cerebral infarction in young children with cyanotic heart disease is attributed to iron deficiency and relative anemia rather than to erythrocytosis.34,51 In the Framingham study hematocrit was associated with risk of stroke but this association disappeared in multivariate analysis when smoking, a well-established risk factor for stroke,36 was removed.37 In a UK study of 7,346 men, an increased risk of stroke was not seen at higher hematocrit levels (≥51%) in normotensive men but was apparent in hypertensive individuals.28 Coronary blood flow is decreased in secondary erythrocytosis,22 but there is equivocal evidence as to whether the risk of coronary thrombosis is increased in patients with a high hematocrit.6,38 Secondary erythrocytosis reportedly does not pose a thrombotic risk in surgical patients.61

Studies of the influence of elevated hematocrit on the risk of thrombosis in animal models of PV and erythrocytosis secondary to elevated erthropoietin have failed to find a consistent positive relationship.62,63 A study of a murine model in which erythrocytosis was induced by transfusing packed red blood cells, with evaluation of thrombotic risk 24 hours later, found that an elevated hematocrit promoted arterial thrombus formation.64 However, acute erythrocytosis induced by transfusion may not reflect the physiology of the chronic elevation of hematocrit seen in PV and secondary erythrocytosis.7 Furthermore, it is not certain how well the ferric chloride-induced thrombosis model in mice reflects thrombosis formation in humans. Thus, in this review, we focus on thrombosis in human conditions of chronic elevation in hematocrit.

**Chuvash erythrocytosis and polycythemia vera share thrombosis as the principal cause of morbidity and mortality**

**Chuvash erythrocytosis**

The propensity to thrombosis is even higher in CE than in PV.65 Although endemic in Chuvashia and Ischia, CE is distributed worldwide.29,66 This form of erythrocytosis is characterized by a high risk of both arterial and venous thrombosis in subjects living near sea level. It protects from anemia in heterozygotes20 but causes augmented hypoxia sensing with elevated hematocrit in homozygotes.7,22 The VHLV617F variant is not associated with tumors characteristic of the VHL tumor predisposition syndrome. Thrombosis largely accounts for the morbidity and mortality of CE although affected individuals have lower body mass index, systolic blood pressure, glucose and HbA1c levels, and white blood cell and platelet counts compared to controls.62,23 The high rate of thrombosis in CE begins in childhood1 and increases with age.66 However, higher hematocrit is not an independent predictor of thrombotic risk in either children or adults.31 Furthermore, a history of therapeutic phlebotomy in CE is associated with an increased risk of thrombosis.48 Thus, the thrombotic risk in CE appears to be independent of viscosity, but rather to be related to changes in the upregulated hypoxic responses associated with the homozygous VHLV617F mutation. We found many HIF-regulated transcripts to be differentially upregulated in CE peripheral blood mononuclear cells, including IL1B, encoding interleukin 1β (2.1-fold), TSP1, encoding thrombospondin-1 (1.5-fold), NLRP3, encoding NLR family pyrin domain containing 3 (1.4-fold), SERpine1, encoding plasminogen activator inhibitor-1 (PAI-1) (1.2-fold), and F3 encoding tissue factor (1.1-fold).11 We also found differential gene expression in granulocytes and reticulocytes, and increased TSP-1 concentrations in plasma.68 Thus, increased HIF may cause a pro-thrombotic milieu in CE.44,54,68 The positive association of phlebotomy with thrombosis in CE parallels observations in the Polycythemia Vera Study Group (PVSG) 01 and 05 studies.69 We postulate that the heightened thrombotic risk is likely due to upregulation of HIF-controlled prothrombotic genes such as tissue factor64,65 and thrombospondin.46 It is likely that other HIF-regulated plasma or vascular factors also play contributory roles.70 In aggregate, these data demonstrate that the thrombotic risk in CE is independent of hematocrit.

**Polycythemia vera**

Thrombosis is the most common complication of PV.50-62 One-half to three-quarters of these events are arterial.63 Ischemic strokes and transient ischemic attacks account for the majority of thrombotic complications, followed in frequency by myocardial infarction, deep vein thrombosis, and pulmonary embolism. Cerebral venous thrombosis and splanchic thrombosis, including Budd-Chiari syndrome, occur with increased frequency in PV. While it is not unusual for Budd-Chiari syndrome to present as the first indicator of PV, we have been unable to find the exact prevalence of this complication in any large published study of PV.

Studies of the influence of elevated hematocrit on the risk of thrombosis in animal models of PV and erythrocytosis secondary to elevated erthropoietin have failed to find a consistent positive relationship.62,63 A study of a murine model in which erythrocytosis was induced by transfusing packed red blood cells, with evaluation of thrombotic risk 24 hours later, found that an elevated hematocrit promoted arterial thrombus formation.64 However, acute erythrocytosis induced by transfusion may not reflect the physiology of the chronic elevation of hematocrit seen in PV and secondary erythrocytosis.7 Furthermore, it is not certain how well the ferric chloride-induced thrombosis model in mice reflects thrombosis formation in humans. Thus, in this review, we focus on thrombosis in human conditions of chronic elevation in hematocrit.

**Hematocrit and thrombotic risk in erythrocytosis**
chlorambucil and $^{32}$P arms if the hematocrit was $>45\%$ despite the chemotherapy regimen. In 1987, with a maximal follow-up of 19 years, 37.8% of the patients had experienced thrombosis as a major study outcome and 14.8% had died from thrombosis. Overall, therapeutic phlebotomy was independently and significantly associated with an increased risk of thrombosis compared to chemotherapy, but hematocrit level was not independently associated with thrombotic risk. The increased risk of thrombosis in patients undergoing phlebotomy compared to that in patients treated with myelosuppressive therapy seemed to be limited to the first 3 years of therapy.\(^{57}\) The increased thrombotic risk did not seem to be related to poorer disease control as reflected by hematocrit and platelet count: in a retrospective analysis that paired patients with thrombosis to those without thrombosis within the same treatment group, neither hematocrit nor platelet count was associated with thrombosis.\(^{53}\) As of 1987, 10.2% of the patients in the PVSG 01 study had developed acute leukemia and 11.8% had died from a hematologic malignancy. Acute leukemia had developed in 11.8% of the PVSG 01 study patients with PVSG 01 study who had developed acute leukemia and 11.8% of the PVSG 01 study patients who had developed acute leukemia. Acute leukemia was more common in the $^{32}$P arm (9.6%) and the chlorambucil arm (13.5%) than in the phlebotomy alone arm (1.5%), and this contributed to the finding that the overall survival of patients treated with phlebotomy was comparable to that of patients treated with $^{32}$P and slightly better than that of patients treated with chlorambucil.\(^{57}\)

The increased risk of thrombosis with phlebotomy compared to chemotherapy observed in the PVSG 01 study was followed up in the PVSG 05 study. Patients were initially phlebotomized to achieve a hematocrit $\leq 40\%$ and then randomized to treatment with phlebotomy and the combination of aspirin (300 mg) and dipyridamole (75 mg) three times daily ($n = 88$) versus $^{32}$P ($n = 90$) to maintain the hematocrit $<45\%$.\(^{57}\) The study was stopped at a median follow-up of $<2$ years when seven (8.0%) patients in the phlebotomy, aspirin and dipyridamole group had experienced a major thrombosis versus two (2.2%) in the $^{32}$P group, providing further evidence of a higher rate of thrombosis with therapeutic phlebotomy versus chemotherapy for PV.

The European Collaboration on Low-Dose Aspirin in the Polycythemia Vera study (ECLAP), which included 1,638 patients, found that survival of PV patients correlated negatively with leukocytosis, older age, venous thrombosis, and atypical karyotype.\(^{56}\) It was also reported that PV may be associated with tissue factor expression in polymorphonuclear leukocytes in the absence of any in vitro challenge, and that expression is decreased after treatment with hydroxyurea.\(^{56}\) An additional risk for thrombotic events in PV may be environmental hypoxia. We found that PV patients residing in Salt Lake City at approximately 1,400 meters have a higher rate of arterial and venous thromboses than that of patients residing at sea level in Baltimore,\(^{51}\) even though they are only exposed to modest hypoxia.\(^{51}\) In a multivariate analysis, living in Salt Lake City was an independent thrombotic risk factor in PV.\(^{51}\) This may be explained by the recent observation that hypoxia decreases protein S levels in normal subjects by an HIF-1-mediated mechanism.\(^{51}\)

**Conclusion**

Certain disorders with elevated hematocrit, such as PV, CE, primary familial and congenital polycythemia or erythrocytosis (EPOR mutation), and EPAS1 gain-of-function mutations, are associated with thrombotic complications. These conditions are characterized by diverse cellular and metabolic changes that could be directly associated with thrombotic risk, irrespective of hematocrit level. The challenge in these conditions is to elucidate factors for the thrombotic risk other than the elevated hematocrit, and to define what, if any, role that viscosity plays in thrombotic risk. Defining these thrombosis-predisposing factors would provide the basis for iden-
tifying and developing novel targeted therapies for these disorders. The evidence we have presented here points to favoring the use of myelosuppressive therapy for intermediate- and high-risk PV, as this approach has been proven to decrease the risk of thrombosis in PV. Furthermore, we trust that the urge to correct any abnormal laboratory data by a therapeutic intervention should be tempered by consideration of the risk-benefit ratio of any such intervention. The routine practice of phlebotomy for elevated hematocrit, with its inevitable iron deficiency (which leads to inhibition of PHD2, increased HIF, and increased erythropoietin) and potential detrimental thrombotic effects, should be re-evaluated. We hope that this review will encourage more studies to pursue the challenge of defining the specific molecular basis of thrombosis in diverse types of polycythemia and erythrocytosis. Improved knowledge of the pathophysiolo-

gy of these entities should be extended to the development of targeted approaches for the prevention and therapy of thrombotic complications. A review of potential molecular mechanisms contributing to thrombosis in myeloproliferative neoplasms was published at the time of the submission of this manuscript.87

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