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The Crux

BIMONTHLY FORUM FOR THE LABORATORIANS

CONTENTS

Editorial

Polycythemia or Polycythaemia vera (PV, PCV) (also known as erythremia, primary polycythemia and polycythemia rubra vera) is a neoplasm in which the bone marrow makes too many red blood cells. It may also result in the overproduction of white blood cells and platelets.

Most of the health concerns associated with polycythemia vera are caused by the blood being thicker as a result of the increased red blood cells. It is more common in the elderly and may be symptomatic or asymptomatic. Common signs and symptoms include itching (pruritus), and severe burning pain in the hands or feet that is usually accompanied by a reddish or bluish coloration of the skin. Patients with polycythemia vera are more likely to have gouty arthritis. Treatment consists primarily of phlebotomy.

Polycythemia veras occurs in all age groups, although the incidence increases with age. One study found the median age at diagnosis to be 60 years. Polycythemia vera (PCV), being a primary polycythemia, is caused by neoplastic proliferation and maturation of erythroid, megakaryocytic and granulocytic elements to produce what is referred to as panmyelosis. In contrast to secondary polycythemias, PCV is associated with a low serum level of the hormone erythropoietin (EPO). Instead, PCV cells have a mutation in the tyrosine kinase (JAK2), which acts in signaling pathways of the EPO-receptor, rendering those cells hypersensitive to EPO.

For complete clinico-diagnostic approach, please turn over. The **DISEASE DIAGNOSIS** section reveals all. **INTERPRETATION** clearly outlines the morphologies of all blood cells with colour pictures, sizes and features etc. Identification hereafter would be much easier. The **TROUBLESHOOTING** component highlights the erroneous results with their reasons that we often get while using automated cell counters.

Amidst all this **BOUQUET** proudly occupies its place. Happy READING!!

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DISEASE DIAGNOSIS

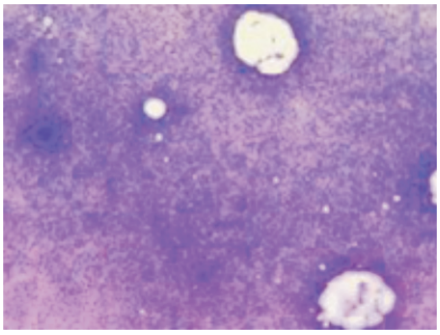
POLYCYTHEMIA VERA

Background

Polycythemia vera (PV) is a stem cell disorder characterized as a panhyperplastic, malignant, and neoplastic marrow disorder. Its most prominent feature is an elevated absolute red blood cell mass because of uncontrolled red blood cell production. This is accompanied by increased white blood cell (myeloid) and platelet (megakaryocytic) production, which is due to an abnormal clone of the hematopoietic stem cells with increased sensitivity to the different growth factors for maturation.

Pathophysiology

Normal stem cells are present in the bone marrow of patients with polycythemia vera (PV), but also present are abnormal clonal stem cells that interfere with or suppress normal stem cell growth and maturation. Evidence indicates that the etiology of panmyelosis is unregulated neoplastic proliferation. The origin of the stem cell transformation remains unknown. See the image below.



Bone marrow film at 100X magnification demonstrating hypercellularity and increased number of megakaryocytes.

Progenitors of the blood cells in these patients display abnormal responses to growth factors, suggesting the presence of a defect in a signaling pathway common to different growth factors. The observation that in vitro erythroid colonies grow when no endogenous erythropoietin (Epo) is added to the culture and the presence of a truncated Epo receptor in familial erythrocytosis indicate that the defect is in the transmission of the signal. The sensitivity of polycythemia vera progenitors to multiple cytokines suggests that the defect may lie in a common pathway downstream from multiple receptors. Increased expression of *BCLX* suggests an additional decrease in cellular apoptosis. Several reasons suggest that a mutation on the Janus kinase-2 gene (*JAK2*) is the most likely candidate gene involved in polycythemia vera pathogenesis, as *JAK2* is directly involved in the intracellular signaling following exposure to cytokines to which polycythemia vera progenitor cells display hypersensitivity. A recurrent unique acquired clonal mutation in *JAK2* has been found in most patients with polycythemia vera and other myeloproliferative diseases (MPDs), including essential thrombocythemia and idiopathic myelofibrosis. A unique valine-to-phenylalanine substitution at position 617 (V617F) in the pseudokinase *JAK2* domain has been identified. The substitution, called *JAK2V617F,* leads to a permanently turned-on signaling at the affected cytokine receptors. How these mutations interact with the wild-type kinase genes and how they manifest into different forms of MPDs need to be elucidated. Thrombosis and bleeding are frequent in persons with polycythemia vera, as a result of the disruption of hemostatic mechanisms because of (1) an increased level of red blood cells and (2) an elevation of the platelet count. There are findings that indicate the additional roles of tissue factor and polymorphonuclear leukocytes (PMLs) in clotting, the platelet surface as a contributor to phospholipid-dependent coagulation reactions, and the entity of platelet microparticles. Tissue factor is also synthesized by blood leukocytes, the level of which is increased in persons with MPD, which can contribute to thrombosis. Rusak et al evaluated the hemostatic balance in patients using thromboelastography and also studied the effect of isovolemic erythrocytapheresis on patients with polycythemia vera. They concluded that thromboelastography may help to assess the thrombotic risk in patients with polycythemia vera. Hyperhomocystinemia is a risk factor for thrombosis and is also widely prevalent in patients with MPD (35% in controls, 56% in persons with polycythemia vera). Acquired von Willebrand syndrome is an established cause of bleeding in persons with MPD, accounting for approximately 12-15% of all patients with this syndrome. von Willebrand syndrome is largely related to the absorption of von Willebrand factor onto the platelets; reducing the platelet count should alleviate the bleeding from the syndrome.

Epidemiology

Frequency: Polycythemia vera (PV) is relatively rare, occurring in 0.6-1.6 persons per million population.

Race: Many studies have shown that this condition occurs in all ethnic groups.

Sex: Polycythemia vera has no sex predilection, although the Polycythemia Vera Study Group (PVSG) found that slightly more males than females are affected.

Age: The peak incidence of polycythemia vera is age 50-70 years. However, this condition occurs in persons of all age groups, including early adulthood and childhood, albeit rarely.

History: Symptoms of polycythemia vera (PV) are often insidious in onset, and they are often related to blood hyperviscosity secondary to a marked increase in the cellular elements of blood. Subsequent sludging of blood flow and thrombosis lead to poor oxygen delivery, with symptoms that include the following: Headache, Dizziness, Vertigo, Tinnitus, Visual disturbances, Angina pectoris, Intermittent claudication. Bleeding complications, seen in approximately 1% of patients with PV, include epistaxis, gum bleeding, ecchymoses, and gastrointestinal (GI) bleeding. Thrombotic complications (1%) include venous thrombosis or thromboembolism and an increased prevalence of stroke and other arterial thromboses. Abdominal pain due to peptic ulcer disease may be present because PV is associated with increased histamine levels and gastric acidity or possible Budd-Chiari syndrome (hepatic portal vein thrombosis) or mesenteric vein thrombosis. Early satiety can occur in patients with splenomegaly, because of gastric filling being impaired by the enlarged spleen or, rarely, as a symptom of splenic infarction. Weight loss may result from early satiety or from the increased myeloproliferative activity of the abnormal clone. Pruritus results from increased histamine levels released from increased basophils and mast cells and can be exacerbated by a warm bath or shower. This occurs in up to 40% of patients with PV.

Physical

Physical findings in patients with polycythemia vera (PV) are due to the myeloproliferative process and excess concentrations of the cellular

elements of blood with extramedullary hematopoiesis. Splenomegaly is present in 75% of patients at the time of diagnosis. Hepatomegaly is present in approximately 30% of patients. Plethora or a ruddy complexion is characteristic of PV and results from the marked increase in total red blood cell mass. This manifests in the face, palms, nailbeds, mucosa, and conjunctiva. Hypertension is common in patients with PV. Measurement of the red blood cell mass should differentiate this condition from Gaisbock syndrome, which is hypertension and pseudopolycythemia (i.e. high hemoglobin levels due to low plasma volume).

Causes

The causes of polycythemia vera (PV) are unknown, but a number of approaches are now being studied to define the molecular lesion or lesions. The *JAK2* V617F mutation can give rise to a turned-on cytokine receptor, leading to pancytosis similar to the PV phenotype. This is similar to the biologic properties of the *BCR/ABL* abnormality in that they both mimic cytokine signaling. Clonality studies using a rare polymorphism in the *G6PD* gene demonstrate predominant expression of a single allele in all blood cell lines. X-chromosome inactivation studies have played a pivotal role in establishing current concepts of many hematologic malignancies. Approximately 90% of patients with PV show a skewed pattern of X inactivation in all their blood cell lines, indicating support for the concept of a transformed multipotential stem cell. Cytogenetic studies show the presence of an abnormal karyotype in the hematopoietic progenitor cells in approximately 34% of patients with PV, depending on which stage of the disease the study was performed at. Approximately 20% of patients have cytogenetic abnormalities at diagnosis, increasing to more than 80% for those with more than 10 years of follow-up care. The following genetic abnormalities, which are similar to the abnormal karyotypes observed in patients with myelodysplastic syndromes and other MPDs, have been observed in patients with PV: Deletion of 20q (8.4%), Deletion of 13q (3%), Trisomy 8 (7%), Trisomy 9 (7%), Trisomy of 1q (4%), Deletion of 5q or monosomy 5 (3%), Deletion of 7q or monosomy 7 (1%). Spivak and colleagues analyzed gene expression in CD34+ peripheral-blood cells from 19 patients with PV and found twice as many up-regulated or down-regulated genes in men as in women. In addition, these researchers found 102 genes with differential regulation that was concordant in men and women and that could be used to divide patients into two phenotypical groups. The groups differed significantly with respect to disease duration, clinical manifestations and prognosis.

Diagnostic Considerations

Diagnostic laboratory tests have been developed to increase the ability to diagnose primary myeloproliferative diseases (MPDs) and to differentiate them from reactive conditions associated with increased blood cell levels, which can mimic MPDs. Polycythemia is characterized by increased cell counts in all cell lines in the myeloid series (i.e. red blood cells, white blood cells [preferentially granulocytes] and platelets). Thus, if red blood cell levels are increased, several conditions must be excluded, including the following: Conditions that increase red blood cells secondary to systemic hypoxia or an artificial condition stimulating erythropoietin secretion in the kidneys. Granulocytosis from infections or mobilization by secondary causes, as in leukemoid reactions. Thrombocytosis from bleeding and iron deficiency. Once an MPD (Philadelphia chromosome negative [Ph–]) is documented, it must be differentiated from the following conditions, which have manifestations that overlap with polycythemia vera (PV): Essential thrombocytosis (ET), Chronic myelogenous leukemia (CML), Agnogenic myeloid metaplasia (AMM). Abdulkarim et al studied the long-term rate (15 years) of leukemic transformation (acute myelogenous leukemia [AML]) in 795 unselected patients with Ph– MPD in the regions of Gothenburg, Sweden, and the Côte d'Or, Burgundy, France. Fifty-six patients (7%) with Ph– MPD demonstrated transformation to AML, of whom six had never received cytoreductive agents and two had been exposed to interferon. The annual rate of AML transformation was 0.38% in patients with PV, 0.37% in those with ET, and 1.09% in individuals with idiopathic myelofibrosis (IMF). The average time from diagnosis to AML transformation was 88 +/- 56 months in the PV patients compared with 76 +/- 57 months in the ET patients and 42 +/- 33 months in those with IMF (significantly shorter than the PV and ET patients), and the investigators noted that it appeared to be a continuous event in all 3 MPDs. Another finding was that 17 of 18 patients with PV whose condition transformed to AML were females despite the fact that the male-to-female ratio of the entire PV group was 146:171. The other conditions (ET and IMF) showed a slight male preponderance (ET, 1.33; IMF: 1.13). The average survival for the 56 patients with MPD who developed AML did not differ among the 3 diseases (4.6 +/- 5.5 months).

Differential Diagnoses

Agnogenic Myeloid Metaplasia With Myelofibrosis, Chronic Myelogenous Leukemia, Essential Thrombocytosis, Polycythemia, Secondary.

Approach Considerations

The Polycythemia Vera Study Group (PVSG) was the first to set rigorous criteria for the diagnosis of polycythemia vera (PV) in the 1970s. With the establishment of polymerase chain reaction (PCR)–based methods for detecting the *JAK2*V617F mutation, this may become the first molecular diagnostic marker for PV, similar to *BCR/ABL* for chronic myelogenous leukemia (CML). However, because of a paucity of centers doing red blood cell mass measurements, demonstrating an elevated red blood cell mass continues to become more difficult. The diagnostic criteria set by the PVSG are organized into two categories, A and B. The diagnosis of PV is established if all three category A criteria are present, or if criteria A1 plus A2 plus any two criteria from category B are present.

Category A criteria are as follows:

1. Total red blood cell mass $\geq$ 36 mL/kg in males or $\geq$ 32 mL/kg in females
2. Arterial oxygen saturation $\geq$92%
3. Splenomegaly

Category B criteria are as follows:

Thrombocytosis, with platelet count > 400,000/µL

Leukocytosis, with a white blood cell count > 12,000/µL

Increased leukocyte alkaline phosphatase (ALP) > 100 U/L

Serum vitamin B-12 concentration > 900 pg/mL or binding capacity > 2200 pg/mL

Total red blood cell mass is measured by labeling the cells with chromium 51 (^{51}Cr). Documentation of an elevated total red blood cell mass with^{51} Cr-labeled red blood cells and, ideally, an iodine-131 (^{131} I) plasma volume dual technique differentiates true erythrocytosis from pseudoerythrocytosis (decreased plasma volume). However, the red blood cell mass is becoming difficult to obtain because the^{51} Cr isotope needed to perform the test is no longer readily available, and institutions willing to perform the test are few as a result of small demand and lack of

profit in performing the test. Diagnostic criteria for PV as per the 2008 revised World Health Organization (WHO) guidelines include both major and minor criteria. Diagnosis requires the presence of both major criteria and one minor criterion or the presence of the first major criterion together with two minor criteria.

Major WHO criteria are as follows:

1. Hemoglobin > 18.5 g/dL in men and > 16.5 g/dL in women, or other evidence of increased red blood cell volume
2. Presence of JAK2617V F or other functionally similar mutation, such as *JAK2* exon 12 mutation

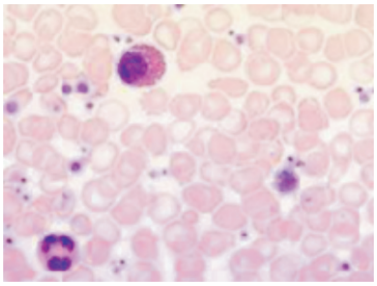
Minor WHO criteria are as follows:

Bone marrow biopsy showing hypercellularity for age with trilineage growth (panmyelosis) with prominent erythroid, granulocytic, and megakaryocytic proliferation. Serum erythropoietin level below the reference range for normal. Endogenous erythroid colony formation in vitro. The major diagnostic issue related to PV is distinguishing it from other forms of erythrocytosis, which are more common than PV. *JAK2* V617F mutation and erythropoietin (Epo) level are key in the diagnosis of erythrocytosis. If the *JAK2*V617F mutation is positive and Epo level is low, then it confirms the diagnosis of PV (*JAK2* V617F mutation is positive in 97% of PV patients). If the *JAK2* V617F mutation is absent but the Epo level is low, then testing for *JAK2* exon 12 and 13 mutations would be helpful is making a diagnosis of PV in the 2-3% of PV patients who are negative for *JAK2* V617F mutation. Patients who are negative for *JAK2* mutations and have a normal or high Epo level have secondary erythrocytosis.

Laboratory Studies

Automated red blood cell counts and hematocrit values (including hemoglobin levels) may be deceptive with regard to the total red blood cell mass in patients with polycythemia vera (PV). Direct measurement of the red blood cell mass should show an increase with a normal or slightly decreased plasma volume. This is a nuclear medicine test that uses radiochromium-labeled red blood cells to measure actual red blood cell and plasma volume. However, patients with hemoglobin concentrations of at least 20 g/dL or hematocrit values of at least 60% in males and 56% in females always have an elevated red blood cell mass.

The red blood cells in patients with PV are usually normochromic and normocytic, unless the patient has been bleeding from underlying peptic ulcer disease or phlebotomy treatment (wherein the cells may be hypochromic and microcytic, reflecting low iron stores). See the image below.



This blood film at 10,000X magnification shows a giant platelet and an eosinophil. Erythrocytes show signs of hypochromia as a result of repeated phlebotomies.

An elevated white blood cell count (>12,000/µL) occurs in approximately 60% of patients. It is mainly composed of neutrophils with a left shift and a few immature cells. Mild basophilia occurs in 60% of patients. The leukocyte alkaline phosphatase (LAP) score is elevated (>100 U/L) in 70% of patients. This technique is only semiquantitative and is susceptible to interobserver and laboratory errors unless it can be performed by flow cytometry, which is not routinely available. The platelet count is elevated to 400,000-800,000/µL in approximately 50% of patients. The release of potassium into the serum caused by the increased number of platelets during in vitro coagulation may cause a pseudohyperkalemia in the serum, whereas the true plasma potassium level in vivo is actually within the reference range, as shown by measuring plasma levels and by the lack of electrocardiography (ECG) changes. Morphologic abnormalities in platelets include macrothrombocytes and granule-deficient platelets. Abnormal platelet function (as measured by platelet aggregation tests with epinephrine, adenosine diphosphate [ADP], or collagen) may be demonstrated, but bleeding time may be normal. Some patients' platelet-rich plasma spontaneously aggregates without the addition of any of the above substances. This indicates a propensity for thromboses. Routine coagulation test results are normal, with a high turnover rate for fibrinogen. The prothrombin time (PT) and activated partial thromboplastin (aPTT) time may be artifactually prolonged, however, because the erythrocytosis results in the collection of a low amount of plasma in relation to the anticoagulant in the test tube. Thus, the volume of the ratio of anticoagulant to blood must be modified when drawing blood for coagulation tests in patients who are polycythemic. Vitamin B-12 levels are elevated to more than 900 pg/mL in approximately 30% of patients, and 75% of patients show an elevation in the unbound vitamin B-12 binding capacity greater than 2200 pg/mL. This is because of increased transcobalamin-III, a binding protein found in white blood cells, and it reflects the total white blood cell counts in the peripheral blood and bone marrow. Hyperuricemia occurs in 40% of patients and reflects the high turnover rate of bone marrow cells releasing DNA metabolites. The most important diagnostic tests are *JAK2* mutation analysis and the serum erythropoietin (Epo) level. A positive *JAK2* V617F mutation and a low Epo level confirms the diagnosis of PV. A low serum Epo level, which is decreased in nearly all patients with PV who have experienced no recent hemorrhage, distinguishes polycythemia from secondary causes of polycythemia in which the serum Epo level is generally within the reference range or is elevated. Each laboratory has its own reference range for serum Epo levels.

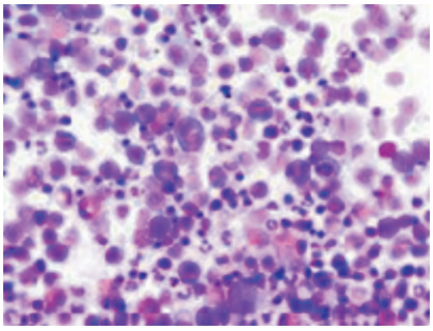
Imaging Studies

An enlarged spleen is often palpable and in such cases, imaging studies are not required. In some patients with posteriorly enlarged spleens or in those who are obese, ultrasonography or computed tomography scans may be able to detect splenic enlargement that was not evident on physical examination.

Other Tests

Measuring arterial oxygen saturation (SaO_2) and carboxyhemoglobin (COHb) levels is important to rule out hypoxia as a secondary cause for erythrocytosis. Pulse oximetry is the most convenient method for measuring SaO_2 however, in people who smoke cigarettes, the COHb must be determined directly and subtracted to give an accurate SaO_2 value. A value below 92% indicates a causal relationship with erythrocytosis. If the fall is due to increased COHb, this is less likely to cause erythrocytosis. Nocturnal oxygen desaturation due to sleep apnea is observed in 20% of patients. Bone marrow studies are not

necessary to establish the diagnosis of polycythemia vera. If such studies are performed, however, the finding of hypercellularity and hyperplasia of the erythroid, granulocytic, and megakaryocytic cell lines or myelofibrosis supports the diagnosis of a myeloproliferative process. See the image below.



Bone marrow film at 400X magnification demonstrating dominance of erythropoiesis.

Iron stores are decreased or absent because of the increased red blood cell mass, and macrophages may be masked in the myeloid hyperplasia that is present. Fibrosis is increased and detected early by silver stains for reticulin. Cytogenetics of the bone marrow cells show a clonal abnormality in 30% of patients who are not treated and in 50% of patients who are treated with alkylating or myelosuppressive agents. These chromosomal abnormalities include deletions of the long arm of chromosome 5 or 20 (5q-, 20q-) and trisomy 8 (+8) or 9 (+9). Leukemic transformation is usually associated with multiple or complex abnormalities. Measuring spontaneous growth of erythroid progenitors in cultures (burst-forming unit, erythroid [BFU-E]) in the absence of Epo is a very sensitive test for polycythemia vera (PV) or familial erythrocytosis. However, it is not routinely available for clinical use. The hemoglobin-oxygen dissociation curve may be useful in rare cases to detect a congenital hemoglobinopathy with increased oxygen affinity. This condition can occur in families.

Medical Care

The long-term risks of polycythemia vera (PV) include leukemic and fibrotic transformation, which occurs in fewer than 5% and 10% of patients, respectively, at 10 years. Current treatment modalities do not change these outcomes. Instead, treatment for PV is intended to decrease the risk of arterial and venous thrombotic events, which could be approximately 20%. Patients can be risk-stratified for their risk of thrombosis according to their age and history of thrombosis. Patients older than 60 years or with a previous history of thrombosis are considered to be high risk. Patients younger than 60 years and with no prior history of thrombosis are considered low risk. All patients with PV should undergo phlebotomy to keep their hematocrit below 45% and should take aspirin, 81 mg daily. In addition, if a patient is at high risk for thrombosis, cytoreductive therapy is added to the management plan. Hydroxyurea at a starting dose of 500 mg twice daily is the most commonly used cytoreductive agent. It can be titrated on the basis of blood counts. In patients who are refractory to or intolerant of hydroxyurea, interferon-alpha can be used as an alternative. Busulfan is also an option for patients older than 65 years.

Phlebotomy

Phlebotomy (bloodletting) has long been the mainstay of therapy for polycythemia vera (PV). The object is to remove excess cellular elements, mainly red blood cells, to improve the circulation of blood by lowering the blood viscosity. Because phlebotomy is the most efficient method of lowering the hemoglobin and hematocrit levels to the reference range, all newly diagnosed patients are initially phlebotomized to decrease the risk of complications. Patients can be phlebotomized once or twice a week to reduce the hematocrit to the range of less than 45%. A recent randomized trial demonstrated a significant difference in the rate of thrombotic events and cardiovascular deaths (2.7 % vs 9.8%) when the hematocrit goal was 45% versus 50%. Patients with severe plethora who have altered mentation or associated vascular compromise can be bled more vigorously, with daily removal of 500 mL of whole blood. Elderly patients with some cardiovascular compromise or cerebral vascular complications should have the volume replaced with saline solution after each procedure to avoid postural hypotension. The presence of elevated platelet counts, which may be exacerbated by phlebotomy, is an indication to use myelosuppressive agents to avoid thrombotic or hemorrhagic complications.

Maintenance therapy

Once the patient's hemoglobin and hematocrit values are reduced to within the reference range, implement a maintenance program either by inducing iron deficiency by continuous phlebotomies (the frequency of the procedure depends on the rate of reaccumulation of the red blood cells) or by using a myelosuppressive agent. The choice depends on the risks of secondary leukemias and the rate of thrombosis or bleeding. Patients must be cautioned to not take iron supplements. The risks for secondary leukemia depend on the type of therapy (eg, phlebotomy, radioactive phosphorus-32 [^{32} P], chlorambucil) or the type of myelosuppressive agents (eg, hydroxyurea [HU], anagrelide, interferon alfa) and duration of therapy. The Polycythemia Vera Study Group (PVSG) demonstrated a decreased survival rate and increased mortality rate from acute leukemia in the first 5 years, and a total of 17% of patients had leukemia after 15 years with chlorambucil and with ^{32}P. An increased incidence of thrombotic complications occurred in the phlebotomy arm. This indicates that phlebotomy is not ideal for patients with elevated platelet counts and previous thrombosis, as are observed in patients who are older. In this situation, using HU has decreased these complications. Hydroxyurea has been the mainstay therapy for PV since the PVSG results indicated it is an effective agent for myelosuppression; however, concerns have been raised regarding long-term risks for leukemic transformation.[16] In the PVSG trial, HU therapy reduced the risk of thrombosis compared with phlebotomy alone; the PVSG recommended that HU should be the drug of choice for patients older than 40 years. The role of HU in leukemic transformation is not clear. Several nonrandomized studies have supported or refuted a significant rise in leukemic conversion with the long-term use of HU in patients with essential thrombocythemia (from 0% to 5.5%) and in patients with PV (from 2.1% to 10%). The PVSG closed the chlorambucil arm because of increased rates of acute leukemia after 7 years. However, in the 15-year follow-up of the HU arm compared with the phlebotomy-alone arm, the trend for leukemic transformation was greater in the HU arm but the differences did not meet statistical significance. Followup for a median of 8.6 years and a maximum of 795 weeks showed that 5.4% of patients developed leukemia in the HU arm compared with 1.5% of patients treated with phlebotomy alone. Other case series have reported secondary leukemia in 3-4% of patients, which is relatively low compared with the benefits of preventing thrombotic complications. In an

open-label study by Huang and colleagues that included 136 patients with JAK2V617F mutation–positive PV, treatment with interferon alfa 2b (IFN α-2b) did not produce a superior overall hematologic response, compared with HU. However, IFN α-2b provided better 5-year progression-free survival (66.3% versus 46.7%, P< 0.01) and clinical improvement (in vasomotor symptoms, distal paresthesias, and erythromelalgia). No severe hematological adverse events were observed in patients receiving IFN α-2b. Do not administer alkylating agents to younger patients (< 40 y) who need long-term treatment. Alternative nonleukemogenic agents are needed for these patients. Low-dose aspirin suppresses thromboxane biosynthesis by platelets, which is increased in PV and essential thrombocythemia. The European Collaboration on Low-dose Aspirin in Polycythemia Vera (ECLAP) found that low doses of aspirin (40 mg/d) were effective for preventing thrombosis and controlling microvascular painful symptoms (erythromelalgia), which result from spontaneous platelet aggregation, in patients with PV and essential thrombocythemia, without creating a bleeding diathesis. **Ruxolitinib:** Ruxolitinib (Jakafi), a JAK1/JAK2 inhibitor, was approved by the FDA in December 2014 for the treatment of patients with polycythemia vera who have had an inadequate response to or are intolerant of hydroxyurea. Approval was based on data from the Phase III RESPONSE trial. In this trial, patients treated with ruxolitinib demonstrated superior hematocrit control and reductions in spleen volume compared to best available therapy. A greater proportion of patients on the ruxolitinib treatment arm achieved complete hematologic remission (ie, hematocrit control, lowering platelet and WBCs). Hematologic adverse reactions are prevalent with ruxolitinib (incidence >20%) and include thrombocytopenia and anemia. Ruxolitinib was initially approved in the United States in 2011 for patients with intermediate- or high-risk myelofibrosis including primary myelofibrosis, post-polycythemia vera myelofibrosis, and post–essential thrombocythemia myelofibrosis.

Surgical Care

Consider splenectomy in patients with painful splenomegaly or repeated episodes of thrombosis causing splenic infarction. Budd-Chiari syndrome occurs in patients with myeloproliferative disease (MPD) and most frequently in young women. Surgical approaches to the management of Budd-Chiari syndrome are, therefore, relevant to patients with polycythemia vera. Budd-Chiari syndrome is a liver-related condition associated with large-vessel thromboses and outflow obstruction with inferior vena cava or portal vein thrombosis. This is associated with the development of ascites, hepatosplenomegaly, abdominal pain, and gastrointestinal bleeding, but 20% of patients are asymptomatic. The diagnosis is made by using ultrasonography to identify portal vein patency. In addition to the standard computed tomography (CT) scan and magnetic resonance imaging (MRI), patients with Budd-Chiari syndrome may need invasive angiographic imaging to determine the hemodynamics of the liver and the intrahepatic and vena caval gradients to determine the best surgical procedure. The histology of the liver helps determine the acuteness of the problem, the presence of chronic changes, and the degree of cirrhosis. This determines whether a patient requires a shunt or a liver transplant. The following procedures have been used in patients with Budd-Chiari syndrome: Transjugular intrahepatic portosystemic shunt (TIPS). Side-to-side portocaval shunt or mesocaval shunt, portocaval/cavoatrial shunt, or mesoatrial shunt. These procedures have been reported to be successful in 38-100% of patients, with follow-up ranging from 9-98 months.

Consultations

Consultation with a hematologist is recommended in cases of polycythemia vera (PV). Long-term follow-up care of these patients and managing complications of the disease and its treatment can be difficult.

Medication Summary

One objective of therapy for polycythemia vera (PV) is to control the myeloproliferative activity of this disease. Evidence of an increase in levels of white blood cells and/or platelets and organomegaly indicate uncontrolled myeloproliferative activity that requires a myelosuppressive agent. Studies by the Polycythemia Vera Study Group (PVSG) have led to the abandonment of long-term therapy with Phosphorus-32 (^{32}P) and most alkylating agents (eg, busulfan, chlorambucil), and to the use of hydroxyurea (HU) instead. However, long-term data seem to indicate a possible slight late increase in cases of acute leukemia in patients with PV who are treated with HU for more than 15 years. Ruxolitinib is now approved in the United States for intermediate- or high-risk myelofibrosis, including primary myelofibrosis, post-PV myelofibrosis, and post–essential thrombocythemia myelofibrosis.

Antimetabolites: Class Summary: HU is a nonalkylating agent that inhibits DNA synthesis and cell replication by blocking the enzyme ribonucleoside diphosphate reductase. **Hydroxyurea (Droxia, Hydrea):** Inhibitor of deoxynucleotide synthesis and DOC for inducing hematologic remission in CML. Less leukemogenic than alkylating agents such as busulfan, melphalan, or chlorambucil. Myelosuppressive effects last a few days to a week and are easier to control than those of alkylating agents; busulfan has prolonged marrow suppression and can cause pulmonary fibrosis. Can be administered at higher doses in patients with extremely high WBC counts (>300,000/µL) and adjusted accordingly as counts fall and platelet counts drop. Dose can be administered as a single daily dose or divided into 2-3 doses at higher dose ranges. Droxia, available in smaller tabs of 200, 300, and 400 mg, is for patients with sickle cell disease. **Ruxolitinib (Jakafi):** JAK1/JAK2 kinase inhibitor indicated for polycythemia vera in patients who have had an inadequate response to or are intolerant of hydroxyurea. Janus-associated kinases (JAKs) JAK1 and JAK2 mediate the signaling of a number of cytokines and growth factors that are important for hematopoiesis and immune function. **Imidazole Quinazolines: Class Summary:** Imidazole quinazolines have been demonstrated to have powerful anti-aggregating effects on platelets and to cause thrombocytopenia. **Anagrelide hydrochloride (Agrylin):** Primary activity is to lower platelet levels but shows slight decrease in mean hemoglobin and hematocrit while WBC counts maintained. Effective in polycythemia vera with elevated platelet counts. Adjust dosage to lowest effective dose to reduce and maintain platelet counts, WBC count, and hemoglobin levels within reference range. **Interferons: Class Summary:** Recombinant interferon alfa is a biologic response modifier with myelosuppressive activity. **Recombinant alfa-2a (Roferon) or alfa-2b (Intron) interferon:** Protein product manufactured by recombinant DNA technology. Can lower counts and shrink enlarged spleens.

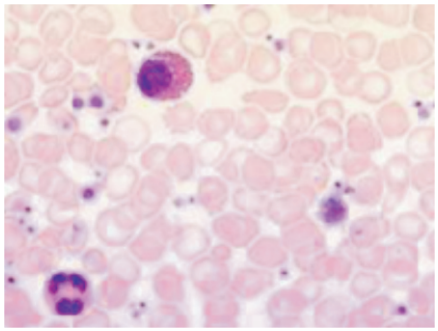
Further Inpatient Care

The optimum management of polycythemia vera (PV) remains elusive despite the findings of the Polycythemia Vera Study Group (PVSG).

However, certain diseases, such as familial erythrocythemia, secondary polycythemia, and relative polycythemia (a benign condition), should be differentiated, and the exact diagnosis of PV should be established. General principles in the management of PV include the following: Tailor therapy to suit the clinical needs of the patient; consider the status of the formed elements of the blood, bone marrow, and organomegaly. Normalize red blood cell mass with phlebotomy as rapidly as clinically possible (250-500 mL every other day); patients who are elderly or have cardiovascular compromise should be phlebotomized cautiously, and smaller amounts should be removed. Suppress myeloproliferative activity with chemotherapy (hydroxyurea) in all patients older than 50 years. In general, phosphorus-32 (^{32}P) should be reserved for patients older than 80 years or patients with comorbid conditions in whom life expectancy is less than 5-10 years and the convenience of ^{32}P dosing outweighs the substantial risks of developing acute leukemia 5-15 years after ^{32}P administration. Patients with thrombotic tendencies or those who develop thrombocytosis following phlebotomy should be treated with marrow suppression; consider anagrelide in younger patients (aged 50-70 y). Maintain blood values at reference range levels by regular examination and treatment. Avoid overtreatment and toxicity by careful and judicious use of chemotherapy and radiation; supplemental phlebotomy is preferred over excess marrow suppression. Postpone elective surgery until long-term control of the disease is established. Women of childbearing age should be treated with phlebotomy only. In young males, myelosuppressive therapy can lead to aspermia; thus, evaluate treatment carefully before using any chemotherapy or radiotherapy. The PVSG no longer recommends the use of alkylating agents because of the associated increased incidence of leukemia and certain types of cancer. Treat hyperuricemia with allopurinol (100-300 mg/d) until remission has been attained; for acute gouty attacks, colchicine or other anti-inflammatory agents are indicated.

Further Outpatient Care

Thrombosis in polycythemia vera (PV) is substantially more frequent in patients treated with phlebotomy alone without myelosuppression. This risk is believed to be related to thrombocytosis, which was not observed in the study. Platelet numbers alone are not likely to be the primary factor responsible for the increased risk of thrombosis; the presence of abnormal platelets is more likely. The initial PVSG study using antiplatelet drugs also used aspirin at 300 mg 3 times a day plus dipyridamole at 75 mg 3 times a day. This showed an increase in the incidence of hemorrhage. Lower doses of aspirin have been suggested to be more effective without increasing bleeding complications, although this has not yet been demonstrated in a prospective randomized trial. A syndrome specific to polycythemia vera (PV) and other MPDs is termed erythromelalgia, and it is associated with an increased risk of thrombosis. The symptoms are burning pain in the feet, hands, and digits, sometimes associated with pallor, erythema, or cyanosis of the distal portions of the extremities. Occasionally, it may progress to frank gangrene. In some instances, this is treated with aspirin (50-300 mg/d) and dipyridamole (75 mg orally 3 times a day). Myelosuppressive therapy plus phlebotomies, with the intent of normalizing the erythrocyte and platelet counts, also decreases or eliminates these symptoms. Proven thrombotic complications warrant the use of long-term anticoagulation with warfarin (see below).



This blood film at 1000X magnification shows a giant platelet and an eosinophil. Erythrocytes show signs of hypochromia as a result of repeated phlebotomies.

Complications

Bleeding complications (1%) in patients with polycythemia vera (PV) include epistaxis, gum bleeding, ecchymoses, and GI bleeding. Thrombotic complications (1%) include venous thrombosis or thromboembolism and an increased prevalence of stroke and other arterial thromboses. In young patients and during pregnancy, interferon alfa may be useful when HU is unsuitable. Anagrelide may be useful when interferon alfa is not tolerated. In the very elderly patients for whom regular clinic attendance is impractical,^{32}P or intermittent busulfan may still be used.

Prognosis

Polycythemia vera (PV) is a chronic disease and its natural history of 1.5-3 years of median survival in the absence of therapy has been extended to at least 10-20 years because of new therapeutic tools. The major causes of morbidity and mortality are as follows: Thrombosis has been reported in 15-60% of patients, depending on the control of their disease. It is the major cause of death in 10-40% of patients. Venous and arterial thromboses have resulted in pulmonary emboli, renal failure from renal vein or artery thrombosis, intestinal ischemia from mesenteric vein thromboses, or peripheral arterial emboli. Hemorrhagic complications occur in 15-35% of patients and lead to death in 6-30% of these patients. Bleeding is usually the consequence of vascular compromise resulting from ischemic changes from thrombosis or hyperviscosity. Peptic ulcer disease is reported to be associated with polycythemia vera (PV) at a 3- to 5-fold higher rate than that of the general population. This has been attributed to increased histamine serum levels. Myelofibrosis and pancytopenia occur in 3-10% of patients, usually late in the disease, which is considered the spent phase of polycythemia vera (PV). In these patients, infections and bleeding complications may be the most serious health threats, and red blood cell transfusions may be required to maintain adequate red blood cell counts and to improve fatigue and other anemia-related symptoms. Acute leukemia or a myelodysplastic syndrome develops in 1.5% of patients treated with phlebotomy alone. The transformation risks increase to 13.5% within 5 years with treatment using chlorambucil and to 10.2% within 6-10 years in patients treated with ^{32}P. At 15 years, the transformation risk for HU is 5.9%, which, although not statistically significant, is a worrisome trend.

INTERPRETATION

DEVELOPMENT OF BLOOD CELLS SITES OF BLOOD FORMATION

Normal Sites

Foetus: Less than 2 months—yolk sac.

From 2-7 months: Liver, with minimal haemopoiesisin spleen.

After 3 months: Haemopoiesis starts in bone marrow.

Full-term infant: Bone marrow is the only site for production of granulocytes and monocytes. Occurs mainly in the spleen, lymph nodes and other lymphoid tissues, though liver and bone marrow produce these in much less numbers.

After birth: Same as above except that the monocytes are provided by the bone marrow, spleen and lymphoid tissues contribute minimally.

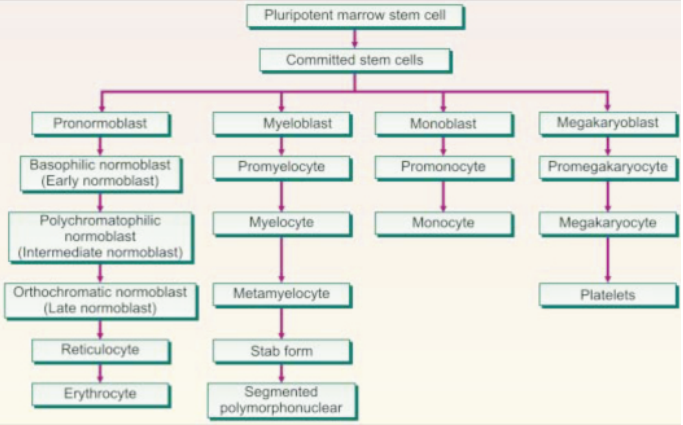
Abnormal Sites

Extramedullary haemopoiesis (myeloid metaplasia): In certain disorders the foetal organs revert to their old function supported by the reticulum cells which retain their potential haemopoietic activity. This occurs when bone marrow cannot any further fulfil the requirements or demand imposed upon it, e.g. in Growing children with haemolysis, Myelosclerosis, Secondary carcinoma of the bone.

Development of Blood Cells (Chart 1.1)

Blood formation has to undergo three stages: (1) Multiplication of precursor cells (1% of all marrow cells are in dividing phase). (2) Gradual maturation (both structural and functional).

Chart 1.1: Development of blood cells



(3) Release into the peripheral circulation. The exact release mechanism is ill understood, granulocytes achieve this by their motility and RBCs by diapedesis.

Erythropoiesis (Fig. 1.2)

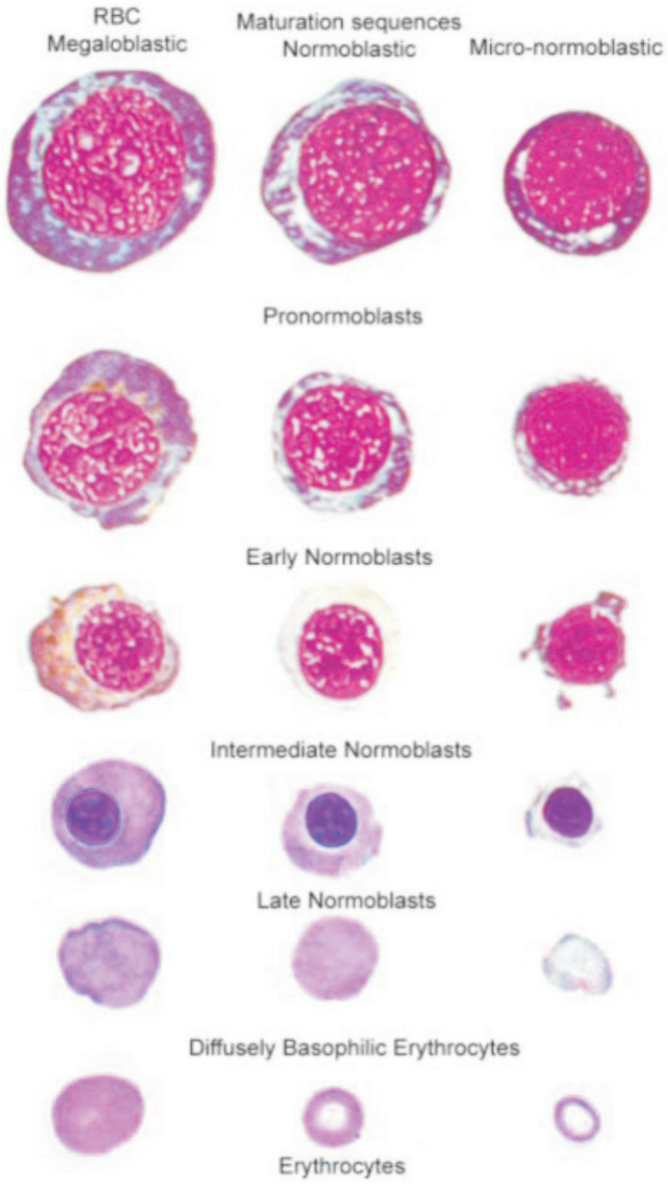
Erythroblast is a nucleated red cell. Normoblast implies normal (reaction) erythropoiesis. Normoblastic maturation involves: Reduction in cell size. Ripening of cytoplasm, i.e. haemoglobinisation. Maturation time from pronormoblast to RBC is 7 days. Mitotic division occurs till the intermediate normoblast stage.

Pronormoblast: 12-20 µm, large nucleus surrounded by a rim of deep basophilic cytoplasm and has a perinuclear halo. Nucleus is round and has several nucleoli.

Early normoblast: 10-16 µm, nucleus still large, chromatin coarser and deeply staining nucleoli disappear.

Intermediate normoblast: 8-14 µm, nucleus smaller, haemoglobinisation commences, cytoplasm takes an acidophilic tint, chromatin becomes coarser and very deeply staining.

Fig. 1.2: Erythropoiesis



Late normoblast: 8-10 µm, cytoplasm is acidophilic,nucleus becomes much smaller, later it becomes pyknotic and is eccentrically placed, ultimately it is lost by extrusion.

Reticulocyte: Flat, non-nucleated, disc shaped, slightly larger than mature RBC. It shows diffuse pale basophilia which appears in the form of a reticulum with supravital stains (brilliant cresyl blue or new methylene blue). In 1-2 days, it loses its basophilia and becomes a mature erythrocyte.

Control of erythropoiesis: Erythropoietin (formed in kidneys) is released in response to lowered tissue oxygen tension. Erythropoietin is a glycoprotein and stimulates primitive cell differentiation to pronormoblasts. It affects the rate of multiplication and maturation. It acts up to early normoblast stage and also affects the rate of haemoglobinisation.

Erythropoietin levels are reduced in:

- Acute starvation.
- Hypophysectomy.
- Transfusion-induced polycythaemia.

Erythropoietin levels are increased in:
- All anaemias except those of renal origin.
- Aplastic anaemia.
- Polycythaemia.

Leucopoiesis (Fig. 1.3)

The Myeloid Series: Specific granules are developed at the myelocyte stage which determine the nature of the mature cell.

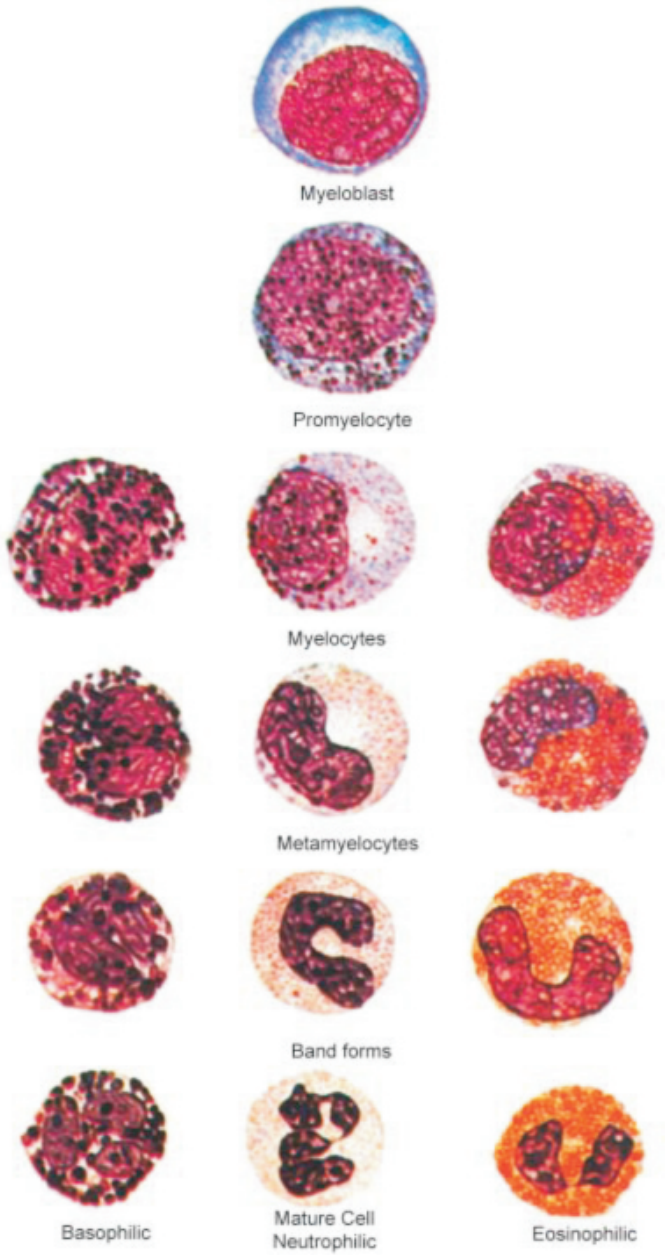


Fig. 1.3: Leucopoicsis

Development of a Mature Neutrophil: Maturation involves: (1) Development of specific granules. (2) Loss of basophilia of the cytoplasm. (3) Nuclear ripening till the segmented stage. (4) Ability to be motile and to phagocytose. (Mitotic division occurs till the myelocyte stage only).

Myeloblast: 15-20 µm has a large round or oval nucleus, evenly stained chromatin in strands or granules with reticular appearance, 1-6 nucleoli. The cell is peroxidase negative.

Promyelocyte: It is like myeloblast except that it contains azurophilic granules which are peroxidase positive. Nuclear chromatin becomes condenser and nucleoli are less well defined.

Myelocyte: Specific neutrophilic granules appear, nucleus shows no nucleoli. N:C ratio reduces, cytoplasm is pale pink, chromatin thicker and deeply stained.

Metamyelocyte: Nucleus is smaller and indented, cytoplasm is pink with neutrophilic granules (purplish).

Band or stab form: Cell becomes still smaller, nucleus has a deep indentation, chromatin is coarsely clumped. Cytoplasm is pink with purplish granules.

Segmented neutrophil: 12-14 µm in size, nucleus shows 2-5 lobes, chromatin in dark purple clumps, cytoplasm has numerous, fine, evenly distributed purplish granules. In female at least 6 neutrophils/500 should show drumsticks.

The mature eosinophil: 16 mm in size, granules are acidophilic and larger. Nucleus is bilobed and is not masked by granules.

The mature basophil: It usually has a bilobed nucleus, but the nucleus is masked by about 10 large basophilic granules.

Lymphocytic Series (Fig. 1.4)

Lymphoblast: 15-20 µm in size, resembles myeloblast, cytoplasm is agranular and moderately basophilic. Nuclear chromatin gives fine reticular appearance with up to 2 nucleoli. It is peroxidase negative.

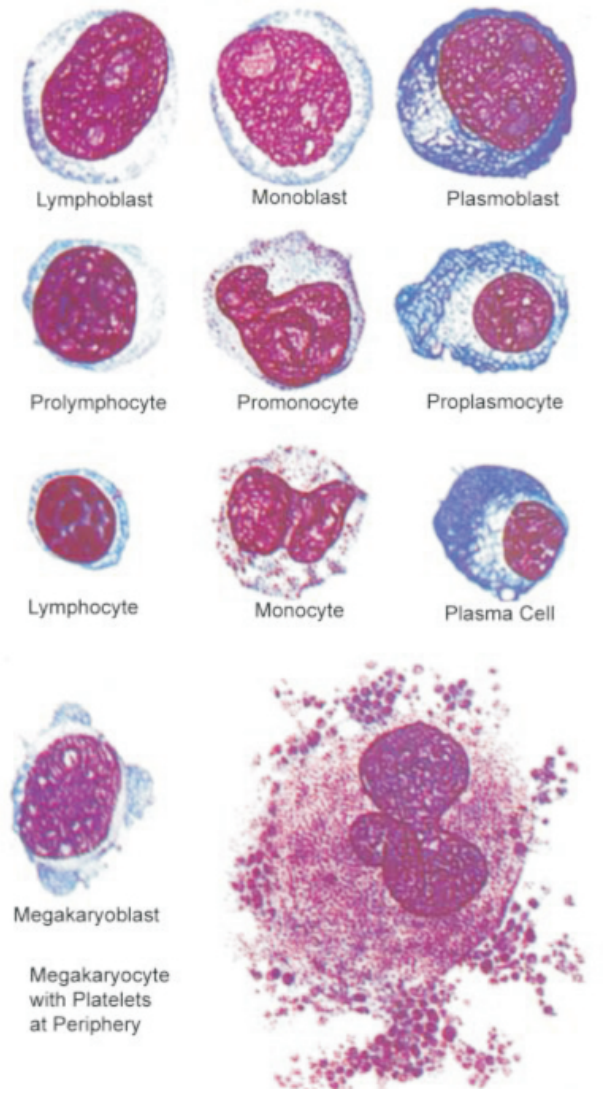


Fig. 1.4: Lymphocytic series

Large lymphocyte: 12-16 µm in size, has abundant pale sky blue cytoplasm with a few purplish red granules seen in about 33% of the cells.
Small lymphocyte: 9-12 µm in size, has scanty cytoplasm. Nucleus is usually round and shows heavily clumped chromatin.

Monocytic Series (Fig. 1.3)

Monoblast: It resembles myeloblast.
Promonocyte: Up to 20 µm in size, has a large convoluted nucleus, chromatin is seen in skein like strands. Cytoplasm is dull grey-blue and may contain a few azurophilic granules.
Monocyte: 15-20 µm in size, has abundant dull grey-blue cytoplasm with a ground glass appearance and may show vacuolation and fine azurophilic granules. It has a kidney-shaped nucleus.

Thrombopoiesis (Fig. 1.3)

Megakaryoblast: 20-30 µm in size, has a large, oval or kidney-shaped nucleus with several nucleoli. It possesses relatively small amount of agranular cytoplasm.
Promegakaryocyte: 30 µm in diameter, cytoplasm is intensely basophilic with fine azurophilic granules. Nucleus may show mild lobulation and chromatin appears denser.
Megakaryocyte: 30-90 µm in diameter, it contains a single multilobulated or indented nucleus. Nuclear lobes may vary from 4-16 in number. Cytoplasm is bulky, light blue with fine azurophilic granulation. The margin is irregular and may show fragmentation or budding, precursor of circulating platelets.
Mature platelet: 1-4 µm. It is formed by fragmentation of megakaryocytic pseudopods. In circulation they acquire a discoid shape. Cytoplasm stains light blue and contains purple reddish granules which may be clumped centrally.
Control of platelet production: Perhaps by a humoral factor called thrombopoietin, acts by a feedback mechanism.

BOUQUET

In Lighter Vein

There's this guy who had been lost and walking in the desert for about 2 weeks.

One hot day, he sees the home of a missionary. Tired and weak, he crawls up to the house and collapses on the doorstep.

The missionary finds him and nurses him back to health.

Feeling better, the man asks the missionary for directions to the nearest town.

On his way out the backdoor, he sees this horse. He goes back into the house and asks the missionary, "Could I borrow your horse and give it back when I reach the town?"

The missionary says, "Sure but there is a special thing about this horse. You have to say 'Thank God' to make it go and 'Amen' to make it stop."

Not paying much attention, the man says, "Sure, ok."

So, he gets on the horse and says, "Thank God" and the horse starts walking. Then he says, "Thank God, Thank God, " and the horse starts trotting.

Feeling really brave, the man say, "Thank God, Thank God, Thank God, Thank God, Thank God" and the horse just literally takes off.

Pretty soon he sees this cliff coming up and he's doing everything he can to make the horse stop. "Whoa, stop, hold on!!!!"

Finally he remembers, "AMEN!!"

The horse stops 4 inches from the cliff.

The man leans back in the saddle and says, "Thank God"

Wisdom Whispers

Thoughts to Introspect

A Lot Of Trouble Would Disappear If Only People Would Learn To Talk To One Another Instead Of Talking About One Another .....

When People Walk Away From You, Let Them Go. Your Destiny Is Never Tied To Anyone Who Leaves You. It Doesn't Mean They Are Bad People. It Just Means That Their Part In Your Story Is Over.. !

People nowadays are like Bluetooth, If you stay close they stay connected, If you go away they find new devices...

Human Life Would Be Perfect If... Anger Had A STOP Button
Mistakes Had A REWIND Button Hard Times Had A FORWARD Button And Good Times A PAUSE Button !!

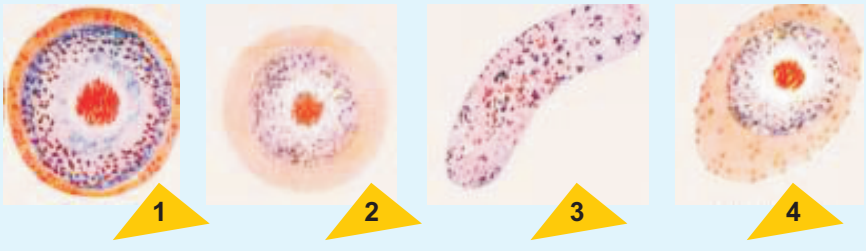
Always Welcome Your Problems, Because Problems Gives You Dual Advice, Firstly, You Can learn How To Solve Them, Secondly, You Learn How To Avoid Them In Future, Have Faith In GOD And Yourself…!

Reflection Cannot Be Seen In Boiling Water, In The Same Way,
Truth Cannot Be Seen In A State Of Anger! Analyze Before You Finalize.

Success Is Like A Beautiful Lover It Will Leave Us At Anytime,
But Failure Is Like A Mother It Will Teach Us Some Important Lessons Of Life!

Brain Teasers

Identify the species of the malarial parasites by looking at the images of their micro-gametocytes



ANSWERS. 1. P. vivax 2. P. malariae 3. P. falciparum 4 P. ovale

TROUBLESHOOTING

ISSUES FACED WITH AUTOMATED CELL COUNTERS

Measurement of complete blood cell count is one of the essential laboratory tests. Electronic blood cell counters simplify and speed up the performance of blood counts and the calculation of red cell indexes. Because of their high precision, physicians tend to accept their results as accurate. However, these counters can be "fooled" by changes in cell size with platelet clumping, agglutination of erythrocytes, or precipitation of abnormal proteins. Failure of the physician to recognize these errors may lead to patients being subjected to unnecessary procedures and therapy. In this issue of Crux, we present four patients in whom the results of red blood cell indexes measured by an automated counter were incompatible and unreasonable. These patients had cold agglutinins: two of them had a respiratory infection, one had cytomegalovirus mononucleosis, and the fourth had a B cell lymphoproliferative disease. One of the patients also had a clinically overt autoimmune hemolytic anemia. Cold agglutinins are polyclonal or monoclonal autoantibodies, usually of immunoglobulin M subtype, directed against I or i antigens and preferentially binding erythrocytes at cold temperatures. These autoantibodies may be associated with malignant or benign disorders (e.g., B cell neoplasm, post-infection, collagen vascular disease) and can be manifested by transient laboratory abnormalities up to severe autoimmune hemolytic anemia. With automated analyzers, cold agglutinin laboratory abnormalities typically present as a discrepancy between the red blood cell indexes. The agglutinated erythrocytes may be recognized as single cells or may be too large to be counted as RBC; therefore, measured mean corpuscular volume is falsely elevated and the red blood cell count is disproportionately low. While the measured hemoglobin is correct, the calculated indexes are incorrect: the hematocrit (red cell count x MCV) is low, while the mean corpuscular hemoglobin (hemoglobin/red cell count) and the mean corpuscular hemoglobin concentration (hemoglobin/hematocrit) are elevated. By rewarming the blood sample to 37 degrees C, the erythrocyte agglutination is abolished and correct values will be read. In the blood sample, hemagglutination may be visible to the unaided eye and examination of the peripheral blood smear may reveal erythrocyte clumping. Another problem might appear with the presence of cryoglobulins. Cryoglobulins are immunoglobulins that precipitate at temperatures below 37 degrees C, producing high molecular weight aggregates. The first clue to a diagnosis of cryoglobulinemia could be laboratory artifacts detected in the automated blood cell counts. The precipitated cryoglobulin particles of various sizes may falsely be recognized as leukocytes or platelets causing pseudoleukocytosis and pseudothrombocytosis. At the same time, the RBC indexes are generally unaffected. Reliable automated counts can be obtained by warming the blood to 37 degrees C or by keeping the blood at 37 degrees C from the time of venipuncture to analysis. May-Grunwald-Giemsa-stained blood films are usually normal, extracellular material is occasionally seen, and leukocyte cytoplasmic inclusion is rarely found. Another important laboratory artifact seen with the automated analyzers is pseudothrombocytopenia. This condition is caused by diverse mechanisms, including: anticoagulant-induced pseudothrombocytopenia, platelet satellitism, giant platelets, and cold agglutinin-induced platelet agglutination. The anticoagulant-induced pseudothrombocytopenia is an in vitro platelet agglutination generally seen in specimens collected into EDTA. It has been reported both in healthy subjects and in patients with various diseases (e.g., collagen vascular disease, neoplasm, and in severely ill patients) and has an overall incidence of approximately 0.1% Although the agglutination is most pronounced with EDTA, it may occur with other anticoagulants as well, such as heparin, citrate or oxalate. Because the generated platelet aggregates are large, the automated counters do not recognize them as platelets, leading to lower platelet counts. In some cases the aggregates are large enough to be counted as leukocytes, causing a concomitant pseudoleukocytosis. The aggregation in pseudothrombocytopenia is time-dependent and usually temperature-sensitive, with maximal activity at room temperature. The EDTA-induced pseudothrombocytopenia is mediated by autoantibodies of IgG, IgM and IgA subclasses directed at an epitope on glycoprotein IIb. This epitope is normally hidden in the membrane GP IIb/IIIa. Ionized calcium has an important role in maintaining the heterodimeric structure of the GP IIIb/IIa complex. The EDTA, through its chelating effect, dissociates the GP IIIb/IIa complex with epitope exposure. In Glanzmann's thrombasthenia, a disorder characterized by the quantitative and/or qualitative abnormality of glycoprotein IIb/IIIa, pseudothrombocytopenia does not occur. Interestingly, in recent years, Abciximab-a GP IIb/IIIa antagonist has been associated with pseudothrombocytopenia. If anticoagulant-induced pseudothrombocytopenia is suspected a peripheral blood smear should be examined for platelet clumping. Platelet satellitism is similar to anticoagulant pseudothrombocytopenia. In the presence of EDTA, platelets bind to leukocytes and form rosettes. The binding is usually to neutrophils but binding to other white blood cells has been reported. The automated analyzers do not correctly recognize platelet-neutrophil clumping, resulting in pseudothrombocytopenia. Platelet satellitism is mediated by autoantibodies of IgG type. These autoantibodies are directed at GP IIIb/IIa on the platelet membrane and to an Fc gamma receptor III on the neutrophil membrane. Pseudothrombocytopenia occurs with giant platelets. Because of their size, the giant platelets are excluded from electronic platelet counting. Platelet cold agglutinin-induced pseudothrombocytopenia is a rare condition. The platelet agglutination is anticoagulant-independent, occurs at maximal activity at 48 degrees C, and is mediated by IgM autoantibody directed against GP IIb/IIIa. Because this autoantibody has little activity at temperatures above 30 degrees C, no clinical complication occurs. Other technical problems and less known situations may cause abnormal cell counts and indexes with automated analyzers. These include clots or overfilling of tubes, hypertriglyceridemia, hyperbilirubinemia, and extreme high white blood count ± any of which may interfere with cell counting and cell indexes. Severe microcytosis, microorganisms, and cytoplasmic fragments of leukocytes may cause spurious elevation of the platelet counts. These conditions are characterized by small particles that are wrongly counted as platelets. Larger particles may be recognized as leukocytes, e.g., circulating normoblasts, giant platelets, and erythrocytes with more resistance to lysis. The latter occurs in automated analyzers when leukocyte counting is based on prior erythrocyte lysis. Erythrocyte resistance to lysis, causing interference with leukocyte counting, was reported in hemoglobinopathies (e.g., hemoglobin C trait, CC, SC, and SS) and fetal (cord) red cells. EDTA-dependent leukoagglutination (similar to platelet satellitism) and cold-induced leukoagglutination uncommonly cause pseudoleukopenia. Rarely does severe hyperglycemia cause spurious macrocytosis. The hyperosmolar glucose-"loaded" erythrocytes become swollen when they are diluted into a relatively hypotonic counting medium, but after hyperglycemia is corrected the MCV returns to normal. To conclude, in our modern era, automated analyzers are able to increasingly recognize pathologic conditions and artifacts. First, the results are presented in numbers, histograms, and scatter plots with or without flags for internal laboratory review. The results are then transferred to the clinician, usually as numbers only. Still, undetected artifacts occur and go unnoticed. The clinician should be alert to those artifacts, thus avoiding unnecessary investigations and therapies.

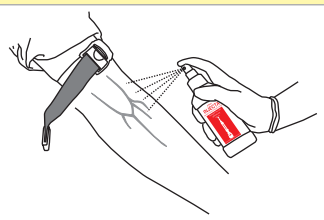
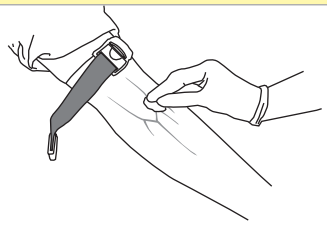
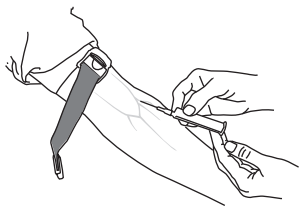
TULIP NEWS

INTRODUCING

INJECTA™

SPRAY

SIMPLIFIES PHLEBOTOMY IN STYLE

SPRAY	CLEAN	DRAW
		

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- Benefits :

- Dual acting antiseptic effective against wide spectrum of microbes.
- Facilitates adequate wetting of phlebotomy site for effective antiseptic activity.
- Convenient to handle in laboratory settings, home sample collection and medical camps.
- Convenient for distribution within departments in hospitals and collection centres.

...Inspires Safe Phlebotomy !

* The images reflect skin preparation technique only & not the complete Phlebotomy protocol.

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Fax: (0832) 2458544. E-mail: sales@tulipgroup.com Website: www.tulipgroup.com