How to approach chronic anemia

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We present herein an approach to diagnosing the cause of chronic anemia based on a patient's history and complete blood cell count (CBC). Four patterns that are encountered frequently in CBCs associated with chronic anemias are considered: (1) anemia with abnormal platelet and/or leukocyte counts, (2) anemia with increased reticulocyte counts, (3) life-long history of chronic anemia, and (4) anemia with inappropriately low reticulocytes. The pathophysiologic bases for some chronic anemias with low reticulocyte production are reviewed in terms of the bone marrow (BM) events that reduce normal rates of erythropoiesis. These events include: apoptosis of erythroid progenitor and precursor cells by intrinsic and extrinsic factors, development of macrocytosis when erythroblast DNA replication is impaired, and development of microcytosis due to heme-regulated eIF2α kinase inhibition of protein synthesis in iron-deficient or thalassemic erythroblasts.

Introduction

When a hematologist is consulted about chronic anemia, the referring physician most often has observed the patient for several months or more and a slight or moderate anemia has persisted or worsened. The task of the consulting hematologist is to determine the cause of the anemia, whether it is progressive, and when and how to treat it. This review emphasizes the pathophysiology and diagnosis of chronic anemias based on patient history and complete blood count (CBC). Understanding the pathophysiology of decreased numbers of circulating erythrocytes can help in the diagnosis and treatment of the anemia.

In healthy adults, approximately 1% of erythrocytes turn over daily as 2×10^{11} new erythrocytes (reticulocytes) enter the circulation and the same number of senescent erythrocytes is removed by macrophage phagocytosis. Erythrocytes circulate for approximately 110-120 days until accumulated oxidant stress and other aging events change anion transporter (Band 3) antigenicity and phosphatidylserine externalization, leading to an immune-related phagocytosis.[1-3] Reticulocytes degrade their residual RNA and mitochondria, losing one-fourth to one-third of their cell volumes by exocytosis of vesicles and selective autophagy over the 1-2 days that they mature into erythrocytes in the blood.[4] Chronic anemia results when the life span of circulating erythrocytes is decreased to less than 110 days without a compensatory increase in reticulocyte production, or when fewer reticulocytes are produced than the number of senescent erythrocytes phagocytosed. Bleeding and hemolysis decrease erythrocyte life span, whereas inadequate erythropoietic constituents and factors such as iron, cobalamin, folate, and erythropoietin (EPO) or excess erythropoietic cell apoptosis decrease reticulocyte production. Inadequate reticulocyte production can also be due to intrinsic erythroid progenitor dysfunction as occurs in hypoplasia, dysplasia, or disrupted bone marrow (BM) space from invasive malignancies or fibrosis.

In chronic anemia, the CBC shows a persistently decreased hematocrit and hemoglobin concentration (Hct/Hgb). The CBC also reveals the numbers of reticulocytes, leukocytes, and platelets. Most automated cell counters provide mean corpuscular volume (MCV), mean Hgb content, mean Hgb concentration, variation in erythrocyte sizes termed the RBC distribution width, and a Wright-Giemsa–stained peripheral blood smear. The CBC does not evaluate the BM directly, but most chronic anemias arise from disordered erythropoiesis in the BM. Although a BM aspiration and biopsy are often needed to make a diagnosis, the CBC can provide valuable information about possible erythropoietic abnormalities and thereby help to guide diagnosis and treatment of chronic anemias. Four specific patterns of chronic anemia are emphasized in terms of the underlying erythropoietic defects that give rise to the abnormal CBCs: (1) abnormal platelet and/or leukocyte counts, (2) increased reticulocyte count, (3) life-long persistence of an abnormal CBC, and (4) abnormal erythrocyte size (ie, macrocytosis or microcytosis) when reticulocyte count is decreased.

Erythropoiesis is one component of the process by which hematopoietic stem cells (HSCs) proliferate and differentiate, giving rise to all cell types in the blood. Figure 1 shows the sequential stages of erythropoietic differentiation in the BM, beginning with the HSC and extending through the orthochromatic erythroblast stage. Orthochromatic erythroblasts enucleate, yielding the reticulocytes that enter the circulation. In Figure 1, the stages of erythropoietic differentiation are separated into 4 sequential phases: (1) before EPO dependence, (2) EPO dependent, (3) terminal differentiation when cell size decreases and Hgb accumulates, and (4) reticulocyte maturation in the blood. Figures 2 and 3 provide an algorithm for the sorting of abnormal CBCs in patients with chronic anemia. This binary categorization is a guide to major groupings of patients based on the patterns of the CBC. In clinical practice, some patients will have a combination of these effects on erythroid cells. For example, the patient with the anemia of inflammation due to Crohn's disease may develop iron deficiency from gastrointestinal blood loss or cobalamin deficiency from ileal involvement or resection.

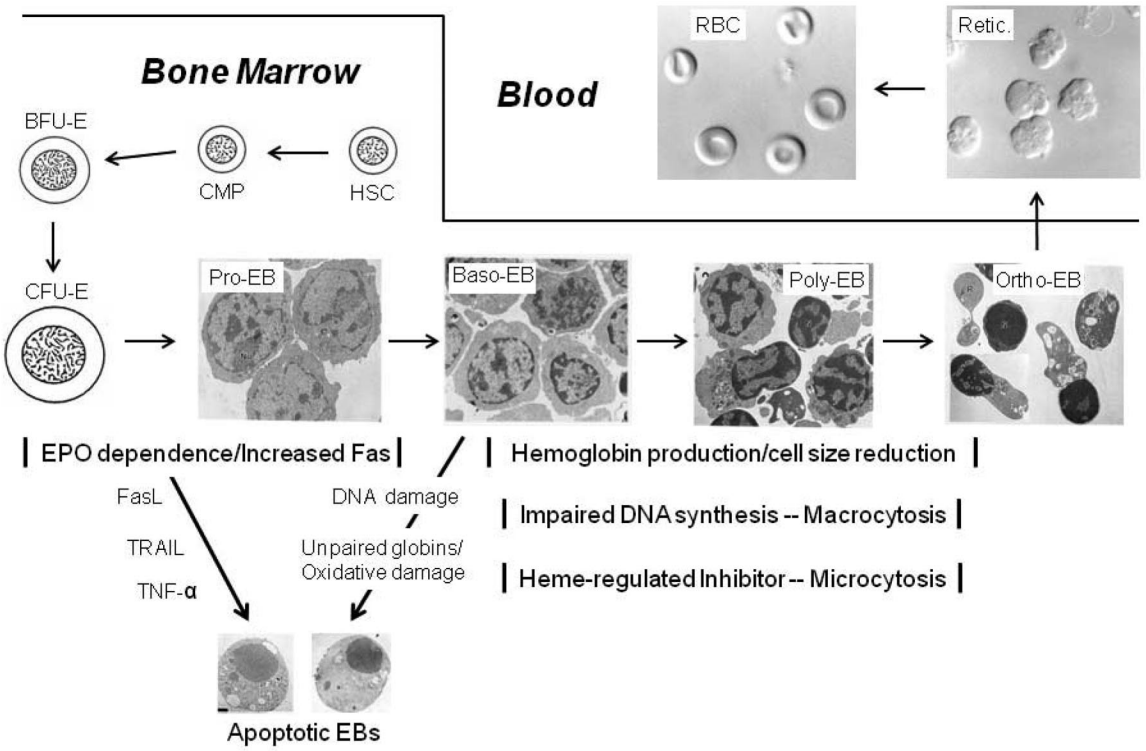

Figure 1. Erythropoietic stages and the development of chronic anemia. The erythropoietic stages in the BM begin with the HSCs, which proliferate and differentiate, giving rise to the common myeloid progenitors (CMPs) and burst-forming unit-erythroid (BFU-Es) before reaching the first stage of EPO dependence, the CFU-E. These progenitors are defined by their growth in culture, but their progeny, the proerythroblast (Pro-EB) through orthochromatic erythroblast (Ortho-EB) stages are recognized by their morphological appearance in stained smears of BM aspirates or ultrastructural studies of purified populations, as shown for mouse erythroblasts. The CFU-E and proerythroblasts express large amounts of the proapoptotic receptor Fas, which can be down-regulated by EPO. Fas mediates apoptosis induced by its binding of FasL on other erythroblasts and multiple myeloma cells. Other apoptosis inducers in the BM include TNF-α and TRAIL. After the Pro-EB stage, the basophilic (Baso-EB) and polychromatophilic (Poly-EB) stages are characterized by Hgb production and progressive deceases in cell size. The relative rates of cell division and protein synthesis during these 2 stages determine the size of the erythrocytes that are produced. Impaired DNA synthesis and DNA damage result in apoptosis of erythroblasts and macrocytic erythrocytes. Excess globin chains that are not assembled into Hgb, as in thalassemia or insufficient heme production from iron deficiency, cause oxidative damage that can lead to apoptosis, but their effects are mitigated by a heme-regulated inhibitor that decreases protein synthesis and leads to microcytosis. Enucleation at the Ortho-EB stage results in extruded nuclei (N) that are rapidly phagocytosed in the BM and reticulocytes (R) that egress into the BM venous sinusoids and circulate in the blood. The circulating reticulocytes (Retics.) mature over 1-2 days by shedding and degrading their internal organelles and assuming their biconcave discoid shape (RBCs). Electron micrograph images of erythroblast stages are modified from Koury et al[41] and Kelley et al[42]; and differential interference contrast microscopy images of reticulocytes and RBCs are modified from Koury et al.[43]

Chronic anemia associated with abnormal platelets and or leukocytes

Any disorder that disrupts the BM space may affect all blood cell lineages. Furthermore, because HSCs and multilineage progenitors between the HSC and BFU-E stages give rise to all blood cell types, a disease affecting these early-stage hematopoietic progenitors will likely affect the platelets and leukocytes as well as erythrocytes. Figure 2 includes the presentation of chronic anemia with decreased platelets and leukocytes. Splenomegaly is a site of trapping of multiple cell lineages, as seen in chronic liver disease with hypersplenism, spleen- and BM-based lymphoid proliferations such as indolent B-cell lymphoma and hairy cell leukemia, and autoimmune cytopenias of systemic lupus erythematosus and Evans syndrome. In chronic liver disease, portal hypertension from cirrhosis enlarges the spleen and traps erythrocytes, platelets, and neutrophils, but the chronic anemia is often multifactorial, with upper gastrointestinal hemorrhage due to varices combined with thrombocytopenia and/or coagulopathy.[5] Discerning the underlying causes of liver disease is important for diagnosing and treating chronic anemia. Some examples are: chronic alcohol consumption can have direct BM toxicity and can be associated with poor intake of folate; antiviral treatments for hepatitis C can suppress erythropoiesis; and hepatitis B, hepatitis C, or autoimmune hepatitis can be complicated by aplastic anemia.

The absence of splenomegaly with multilineage cytopenias usually indicates a primary BM disease, including aplastic anemia, myelodysplasia (MDS), and acute leukemia or BM invasion by a malignancy such as metastatic solid tumors. In addition to physical disruption of the BM, the malignant population can induce chronic anemia by increasing apoptosis or restricting the differentiation of erythroid progenitors. One example is multiple myeloma, a BM-infiltrating disease in which most patients develop chronic anemia. Two members of the TNF-α family, Fas ligand (FasL) and TNF-related apoptosis-inducing ligand (TRAIL), are specific mediators of apoptosis expressed by myeloma cells.[6] Surface FasL and

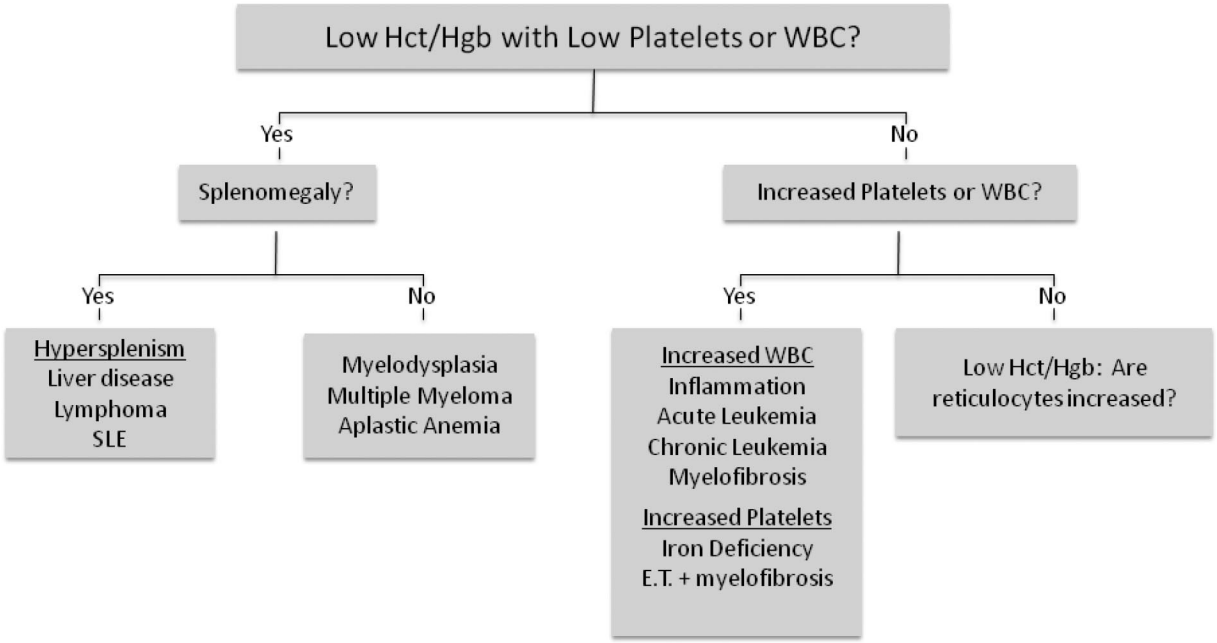

Figure 2. Algorithm for evaluation of chronic anemia based on the CBC. Bottom right: "Low Hct/Hgb: Are reticulocytes increased?" is the same as top box in Figure 3. ET indicates essential thrombocythemia; and SLE, systemic lupus erythematosus.

TRAIL receptors are expressed by proerythroblasts and early basophilic erythroblasts, erythropoietic stages that depend upon EPO to prevent apoptosis (Figure 1). EPO inhibits Fas-mediated erythroblast apoptosis,[7] and down-modulation of Fas and FasL in these early-stage erythroblasts has been shown to be a mechanism of EPO's antiapoptotic effects.[8] Coculture of erythroblasts with myeloma cells expressing FasL and TRAIL triggers the respective receptors in the erythroblasts, thereby activating apoptotic pathways.[6] Multiple myeloma also induces apoptosis indirectly in early erythroblasts when it suppresses renal EPO production. Several types of malignancies reduce EPO levels due to cytokine-mediated suppression of EPO production.[9] In many patients with multiple myeloma, paraprotein-mediated renal insufficiency decreases EPO production further, and the degree of EPO deficiency is correlated with the amount of BM infiltration and the proliferation rate of the myeloma cells.[10,11] Thalidomide chemotherapy[12] and EPO administration[13] in myeloma patients can reverse the inhibited erythropoiesis and improve the anemia. Lastly, hepcidin, the hepatic hormone that restricts iron flux from macrophages to erythropoietic cells by down-regulating ferroportin-1 on macrophages, is induced by bone morphogenetic protein 2 (BMP2), thereby contributing to chronic anemia in multiple myeloma.[14]

Chronic anemia in MDS and aplastic anemia is often associated with low platelets and/or leukocytes. Excessive apoptosis destroys early-stage erythroid progenitors and multilineage hematopoietic cells, and the expressions of receptors for TNF-α, FasL, and TRAIL are increased in these 2 progenitor populations.[15,16] Therefore, intrinsic overexpression of these membrane TNF-α family receptors that mediate apoptosis appears to play a significant role in the development of the anemia in MDS and aplastic anemia.

Frequently, chronic anemia due to iron deficiency is accompanied by increased platelets, and this thrombocytosis resolves with iron repletion. The cause of increased platelet counts is unknown, but increased EPO levels in iron deficiency anemia have been considered as a potential factor in stimulating platelet production. Although EPO and thrombopoietin (TPO) have been shown to have synergist effects on megakaryocytopoiesis, the partial homology of the receptors for EPO and TPO does not appear to play a role, because no cross-competition between EPO and TPO could be detected for receptor binding in cells engineered to express both receptors.[17] In severe iron deficiency anemia, patients may have thrombocytopenia, which also resolves with iron therapy.[18] Chronic anemia can be accompanied by increased leukocytes in patients with chronic inflammation/infection, and the leukocytosis may help to distinguish iron deficiency from the anemia of chronic inflammation. Conversely, chronic anemia is a negative prognostic sign when it is associated with increased platelets due to essential thrombocythemia[19] or with increased leukocytes due to myelofibrosis.[20]

Chronic anemia with increased reticulocytes

Chronic anemia unaccompanied by significant abnormalities of platelets or leukocytes can be separated into anemia with appropriately increased reticulocytes for the degree of anemia and anemia without an appropriate reticulocytosis (Figure 3). Absolute reticulocyte counts and the reticulocyte index indicate whether BM erythrocyte production is appropriately increased, as would be expected in patients with fully responsive erythropoietic capacities, or not appropriately increased, as would be expected in patients with impaired erythropoiesis. In chronic anemia, an increased absolute number of reticulocytes or an increased reticulocyte index indicates low-grade hemolysis or blood loss. Except in patients with liver disease, increased indirect serum bilirubin, increased lactate dehydrogenase, and decreased haptoglobin are characteristic of hemolysis. The blood smear is the most helpful in these cases. After excluding acute disorders such as hemolytic-uremic syndrome, thrombotic thrombocytopenia purpura, and peripartum-related syndromes, chronic intravascular hemolysis with erythrocyte fragmentation is often associated with malignant tumors, large

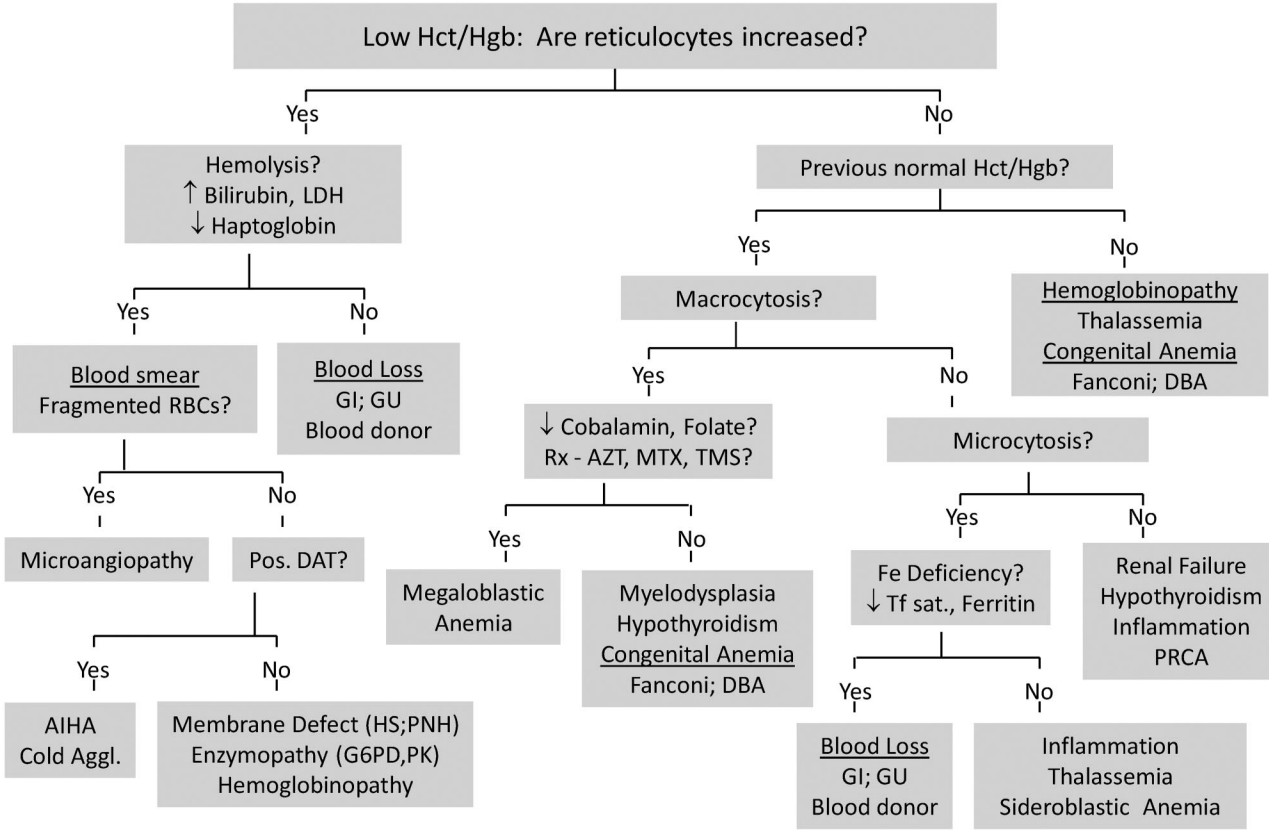

Figure 3. Algorithm for evaluation of chronic anemia based on the CBC (continued). Top box: "Low Hct/Hgb: Are reticulocytes increased?" is the same as the bottom right box in Figure 2. AIHA indicates autoimmune hemolytic anemia; AZT, azidothymidine; Cold Aggl., cold agglutinin disease; DAT pos., direct antiglobulin test (direct Coombs test) positive; DBA, Diamond-Blackfan anemia; GI, gastrointestinal; GU, genitourinary RBC, red blood cell; HS, hereditary spherocytosis; LDH, lactate dehydrogenase; MTX, methotrexate; PNH, paroxysmal nocturnal hemoglobinuria; PK, pyruvate kinase; PRCA, pure red cell aplasia; Tf sat.; transferrin saturation; and TMS, trimethoprim-sulfamethoxazole.

hemangiomas, prosthetic heart valve defects, or direct external trauma (eg, march hemoglobinuria). Spherocytes on the smear suggest hereditary spherocytosis or immune hemolysis; the latter can be diagnosed by surface Abs and/or complement components on erythrocytes in the direct antiglobulin test. Normal erythrocyte morphology and negative direct Ab testing indicate possible enzyme dysfunction. Glucose-6-phosphate dehydrogenase (G6PD), the most common RBC enzyme deficiency, is an exception in that eccentrocytes or blister cells with Hgb "puddled" away from the cell membrane are seen on peripheral smears. Without evidence of hemolysis, chronic anemia with increased reticulocytes can result from bleeding in patients who have not lost sufficient amounts of blood to develop microcytosis from iron deficiency. Patients with intermittent blood losses include many with menorrhagia or upper gastrointestinal lesions such as varices, arteriovenous malformations, and ulcers.

Life-long history of abnormal CBC (no previously normal CBC)

Worldwide, the most common cause of congenital anemia is thalassemia,[21] which is the most frequent cause of a life-long history of undiagnosed chronic anemia (Figure 3). Thalassemia is characterized by deficient globin production with preservation of the RBC number, leading to microcytic cells with decreased Hgb content per cell. The number of cells produced and the degree of microcytosis depend upon the severity of the globin deficiency. Chronic anemia due to α-thalassemia is caused by deletions or abnormalities involving 2 or 3 of the 4 α-globin genes on chromosome 16 and result in ineffective erythropoiesis from early fetal life. Missing 1 of 4 α-globins is asymptomatic, and missing all 4 α-globins usually leads to fetal death. Missing 2 of 4 α-globin genes leads to the α-thalassemia trait, which is characterized by mild microcytic anemia and is often diagnosed on newborn screening by the presence of γ-globin tetramers known as Hgb Barts. If not diagnosed by newborn screening, patients who later have a CBC performed will have a mild microcytic anemia that can be differentiated from iron deficiency anemia by a normal or elevated RBC number and a normal RBC distribution width.[22] If only 2 of the 4 α-globin genes is missing, the Hgb electrophoresis will become normal shortly after birth. Serum ferritin, which reflects total body iron stores, will be normal or elevated, further distinguishing the thalassemia trait from iron deficiency. When a definitive diagnosis of 1 or 2 α-globin gene deletions is desired for treatment or genetic counseling, α-globin gene sequencing can be performed in specialty laboratories. Missing 3 of 4 α-globin genes causes Hgb H disease, which may be symptomatic at birth with anemia and jaundice. The newborn screening in these cases will show greater than 10% Hgb Barts. Because fetal erythropoiesis changes over to adult erythropoiesis during the first several months of life, β-globin tetramers will form and become evident on Hgb electrophoresis as Hgb H. Children with Hgb H disease may be transfusion dependent or have

a moderate microcytic anemia with a component of hemolysis that worsens at times of acute illness.

β-thalassemia is more variable than α-thalassemia, because the mutations may be point mutations or deletions of various sizes in the β-globin gene on chromosome 11. β-Thalassemia major is diagnosed by newborn screening with only fetal Hgb and no evidence of adult Hgb. As γ-globin production decreases, deficient β-globin production for erythropoiesis causes decreased Hgb and RBC number (see "Heme-regulated eIF2α kinase inhibition of protein synthesis in microcytic chronic anemia"). Children with β-thalassemia major typically become transfusion dependent between 3 and 18 months of age. β-Thalassemia intermedia is not diagnosed by newborn screening because some normal adult Hgb is produced. Rather, children develop a microcytic anemia with a relative increase in RBC number, similar to α-thalassemia. Diagnosis is made by Hgb electrophoresis after the child reaches 6-12 months of age, by which time Hgb A2 becomes elevated due to a compensatory increase in δ-globin chains.

Chronic normocytic anemias present from birth are usually due to nonthalassemic hemoglobinopathies, RBC enzyme deficiencies, or RBC membrane defects. Hemoglobinopathies are usually diagnosed by routine newborn screening. Immigrants who have not been screened may present with chronic hemolytic anemia, leukocytosis, thrombocytosis, and symptoms of pain, splenomegaly, swollen joints, and infection. Diagnosis is made by Hgb electrophoresis. One should be cautioned against performing a Sickledex, or sickle screening, because these will be positive in sickle cell trait, which is not responsible for chronic anemia, and will be negative in nonsickling hemoglobinopathies such as homozygous Hgb C or compound heterozygous states such as Hgb C/β-thalassemia, both of which cause chronic mild anemia.

Enzyme deficiencies and membrane defects typically cause nonspecific jaundice in the neonatal period with or without noticeable anemia. These 2 entities are best distinguished by peripheral blood smears, which will demonstrate normal morphology in most enzyme deficiencies and typical morphologic features in hereditary spherocytosis, hereditary elliptocytosis, or pyropoikilocytosis. The most common RBC enzyme deficiency is G6PD deficiency, found mostly as an X-linked mutation in patients of African, Indian, Chinese, or Mediterranean descent. Due to the X-linked inheritance, G6PD deficiency is most common in males, but the gene is sufficiently common that females can also be affected. Diagnosis is made by measuring G6PD levels, but can be missed during periods of reticulocytosis due to the increased levels of G6PD in reticulocytes compared with mature RBCs. The second most common enzyme deficiency, pyruvate kinase deficiency, is typically more severe, often presenting in the neonatal period with hyperbilirubinemia and anemia requiring exchange transfusion. Diagnosis is made by enzyme assay. RBC enzyme panels are performed by a few specialty laboratories and are most helpful in these sick children, who may come to attention later with normocytic anemia, a normal peripheral blood smear, and only a history of jaundice as neonates. The most common membrane disorder is hereditary spherocytosis, inherited as an autosomal-dominant defect in the majority of patients.[23] Children or young adults typically present when they develop an episode of acute hemolysis with a febrile illness and without sufficient reticulocytosis due to BM suppression from viral infection. An almost pathognomonic feature of the CBC is an elevated mean Hgb concentration. Osmotic fragility testing reveals increased lysis of RBCs at higher solute concentrations than controls, because deficiency of ankyrin, spectrin, or anion transporter (Band 3) proteins leads to a loss of membrane surface area, creating a less stable RBC membrane.

Chronic anemia with inappropriately low numbers of reticulocytes

One of the most common presentations of chronic anemia is the patient with inappropriately low number of reticulocytes for the degree of severity of the anemia, indicating a dysfunctional BM. In these patients, who have slight to moderate anemia, the pattern of CBC values over time is often helpful. Even in adults, the patient who has never had a previously normal Hct/Hgb may well have a congenital anemia, such as a hemoglobinopathy, sideroblastic anemia, or Diamond-Blackfan anemia, whereas the history of a previously normal CBC points to an acquired BM dysfunction such as myelodysplasia, fibrosis, or aplastic anemia. For both congenital and acquired anemia, determining whether the chronic anemia is macrocytic, microcytic, or normocytic can be helpful in diagnosing its cause (Figure 3). In adults, macrocytic anemias have MCVs greater than 100 fL and microcytic anemias have MCVs less than 80 fL. In children, these demarcations of macrocytosis and microcytosis are at significantly lower MCVs because children have smaller erythrocytes than adults (except for neonates, who have higher MCVs). At 1 year of age or less, the distribution of MCVs of healthy children between the 3rd and 97th percentiles is in a range of 70-84 fL.[24] This range of normal MCVs increases progressively with age through adolescence until adulthood.[24] Therefore, the determination of an anemia as being macrocytic or microcytic in a child must be based on an age-adjusted range of MCVs.

Impaired DNA replication and chronic macrocytic anemia

In chronic anemia with increased reticulocytes, MCVs are increased due to the transiently enlarged cell volumes of the reticulocytes. Most patients with hypothyroidism have normocytic to slightly macrocytic anemias. Hypothyroid patients with slight to moderate macrocytic anemias who do not have pernicious anemia that can accompany autoimmune thyroid disease resolve their anemia after thyroid hormone treatment. Most of the other chronic macrocytic anemias are characterized by erythroblasts with abnormally prolonged cell cycles. While accumulating Hgb and other specific proteins of the mature erythrocyte, terminally differentiating erythroblasts undergo dramatic size decreases due to a coordinated shortening of the G_1 phase of the cell cycle and reduced protein synthesis rates[25] (Figure 1). Inhibition of erythroblast DNA replication retards cell division, but it does not affect protein synthesis rates, and therefore the relative sizes of the erythroblasts and their daughter cells increase.[26] When the affected cell division is the final one of terminal differentiation (M.J.K., unpublished observation, 1999), the resulting reticulocytes and mature erythrocytes are macrocytic.[27,28] In most macrocytic anemias, the impaired DNA synthesis also triggers increased erythroblast apoptosis, resulting in decreased reticulocyte production, although the individual reticulocytes are larger than normal reticulocytes (Figure 1). Several congenital chronic macrocytic anemias are associated with impaired DNA synthesis. Deficient ribosomal proteins in Diamond-Blackfan anemia trigger p53-mediated cell-cycle delay and apoptosis of erythroid cells.[29] Similarly, impaired DNA synthesis and erythroid progenitor apoptosis are found in the chronic macrocytic anemias of BM failure syndromes related to shortened telomeric DNA[30] and faulty repair of cross-linked DNA.[31] Most acquired macrocytic

anemias also have a similar pattern of impaired DNA synthesis and associated apoptosis. The chronic anemia of the 5q− variant of myelodysplasia with a specific ribosomal protein deficiency requires p53 to mediate the impaired DNA synthesis and apoptosis.[29] In the macrocytic anemia of cobalamin deficiency or folate deficiency, decreased deoxynucleotide synthesis impairs S-phase progression and erythroblasts succumb to apoptosis via a p53-independent mechanism.[32] Medications and excess alcohol consumption account for the majority of hospitalized patients with macrocytosis.[33] The medications that cause a macrocytic anemia are those that inhibit DNA synthesis directly or indirectly and are often prescribed by nonhematologists; these include azidothymidine, azathioprine, trimethoprim-sulfamethoxazole, methotrexate, and anticonvulsants.

Heme-regulated eIF2α kinase inhibition of protein synthesis in microcytic chronic anemia

Chronic microcytic anemia is also related to the relative rates of DNA and protein synthesis during the terminal stages of erythropoiesis. In most microcytic anemias, the rates of erythroblast protein synthesis are decreased while cell division proceeds. Because more than 95% of the proteins synthesized in terminal-stage erythroblasts are globin chains, decreased protein synthesis results in reticulocytes and erythrocytes that contain less Hgb and are smaller than normal (ie, hypochromic and microcytic anemia). During Hgb synthesis, the stoichiometry of 2 α-globins, 2 β-globins, and 4 heme groups per molecule is tightly regulated such that excess heme and globin chains, which are all toxic to the erythroblast, do not accumulate. In terminally differentiating erythroblasts, translation of globin mRNAs and mRNAs coding other proteins is controlled by heme-regulated eIF2α kinase (HRI).[34] HRI phosphorylates the translation initiation factor eIF2, rendering it unable to initiate translation of mRNAs. When heme is in excess relative to globin chains in the erythroblast, it binds and inactivates HRI, thereby allowing globin translation. When intracellular heme is decreased in the erythroblast, HRI is active, and its phosphorylation of eIF2 inhibits protein synthesis such that hypochromic and microcytic reticulocytes are produced (Figure 1).

The HRI-null mouse model has shown that HRI protects the basophilic erythroblasts, the first stage of erythroid differentiation in which Hgb is made, from apoptosis due to iron deficiency, defective heme synthesis (erythropoietic protoporphyria), and β-thalassemia.[34,35] In the HRI-null mouse, each of these diseases is characterized by increased globin chains that are not incorporated into Hgb, but rather precipitate and induce oxidative damage, leading to erythroblast apoptosis. HRI protects the erythroblasts from this potential apoptotic fate by inducing a stress response through the Atf4 pathway that mitigates the oxidative damage, allowing the progression of erythroid differentiation.[35] The other protective effect of HRI is the inhibited protein synthesis that decreases globin chains, reducing the oxidative stress, but the decreased globin chains also reduce Hgb accumulation and cell size. The end result of this HRI-mediated inhibition of protein synthesis is detected clinically as hypochromia and microcytosis in iron deficiency anemia, thalassemia, and sideroblastic anemia (Figure 3).

Acquired normocytic chronic anemia with normal platelets and leukocytes

Patients with acquired normocytic anemia represent one of the largest groups of patients encountered during the evaluation of chronic anemia. Some will have renal failure, which is easily diagnosed by serum creatinine and represents the most common EPO deficiency state because the kidneys produce more than 90% of the body's EPO. High rates of iron deficiency and inflammation also contribute to the anemia of renal failure, but routine administration of EPO and IV iron has made diagnostic questions less frequent in patients with renal disease. The majority of patients with normocytic anemia have the anemia of chronic inflammation, which is also called anemia of chronic disease (ACD), and some of these patients are difficult to diagnose. Especially difficult are elderly patients with rheumatologic disorders such as polymyalgia rheumatica, lupus erythematosus, and inflammatory bowel disease, who have intermittent or low-grade symptoms and signs. Others have an unrecognized bacterial abscess, endocarditis, or HIV or fungal infection. Cytokines produced by inflammatory cells, such as TNF-α and IL-1, suppress EPO production, and TNF-α, IFN-γ, TRAIL, and other cytokines induce apoptosis or suppress erythroid proliferation in the BM.[36] A major mediator of cytokine or microorganism inhibition of erythropoiesis is increased hepcidin, which restricts iron recycling by down-regulating ferroportin expression on macrophages.[37] Increased hepcidin is induced frequently by IL-6 and, in those patients with more significant anemia (Hct/Hgb $< 30\%$ and 10 g/dL, respectively), the erythrocyte sedimentation rate and C-reactive protein are elevated. These patients with moderately severe anemia may also have slightly microcytic MCVs, but microcytosis may be due to iron deficiency complicating ACD. Common examples are blood loss from gastritis due to excessive use of aspirin or nonsteroidal anti-inflammatory medications in arthritic patients and tumor bleeding in patients with occult gastrointestinal malignancies.

Differentiating among patients with early-stage iron deficiency, those with ACD, and those with combined iron deficiency and ACD is very difficult. Each of these 3 situations is associated with borderline or slight microcytosis and low serum iron, but iron deficiency and ACD are associated with opposite and offsetting effects on serum ferritin and total iron binding capacity, which are commonly used to test for iron deficiency. Other laboratory tests have been developed to differentiate iron deficiency, ACD, and ACD with iron deficiency. One test, the serum concentration of soluble transferrin receptor (sTfR), which is increased proportionally in a reciprocal relationship with iron deficiency, has been difficult to standardize. However, an automated sTfR measurement combined with serum ferritin and calculation of the sTfR index (sTfR/log ferritin)[38] was shown to have an 81% sensitivity in detecting patients with iron deficiency with or without ACD and an 83% specificity in detecting patients with ACD but without iron deficiency. Another test is based on the observation that as iron deficiency develops, the most recently produced erythrocytes will have experienced more deficiency than those erythrocytes already in the circulation, which were produced when relatively more iron was available. Therefore, compared with other erythrocytes, the reticulocytes will be more affected by iron deficiency with relatively lower Hgb content than normal reticulocytes. Automated cell counters can determine the Hgb content of reticulocytes, which is useful in detecting iron deficiency in children.[39] In the more complicated situation of trying to differentiate among iron deficiency, ACD, and ACD with iron deficiency, the test for Hgb content of reticulocytes is used in a combined analysis with either the patient's sTfr index or serum hepcidin concentration.[40] However, the generalized use of the sTfr index and serum hepcidin in clinical practice will likely depend upon further automation and standardization of these assays.

Disclosures

Conflict-of-interest disclosure: M.J.K. has consulted for Keryx Biopharmaceuticals Inc and the Pharmaceutical Division, Japan

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