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A practical approach to the evaluation of the anemic child

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Introduction

Anemia can be defined as a reduction in the hemoglobin concentration, hematocrit, or number of red blood cells (RBC) per cubic millimeter. Conventionally, the lower limit of the normal range is set at two standard deviations below the mean for the normal population. Thus, 2.5% of the normal population will be mistakenly classified as anemic [1]. Conversely, relying strictly on a numerical definition of anemia may obscure significant pathological features [2]. The primary function of red blood cells is to deliver adequate quantities of oxygen to meet the body's metabolic demands. Thus, a measure of oxygen metabolism and accompanying cardiovascular compensation should be considered in defining anemia. Individuals with a predilection for hemoglobin in the upper portion of the normal range may be hemoglobin-deficient with values in the low normal range. For example, children with cyanotic congenital heart disease, respiratory insufficiency, or hemoglobinopathy that alters oxygen affinity can be functionally anemic with hemoglobin in the normal range.

A normal erythrocyte lives approximately 120 days and is then removed from the circulation as it passes through the reticuloendothelial system. Under normal steady state conditions, this daily loss of RBCs is balanced by effective erythropoiesis. Anemia results whenever the homeostatic balance between cell production and loss is disrupted. Traditionally, the physiological etiology of

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Table 1
Physiologic classification of anemia

Disorders of effective red cell production
1. Marrow failure
   a. Aplastic anemia
      Congenital
      Acquired
   b. Pure red cell aplasia
      Congenital: Diamond–Blackfan Syndrome
      Acquired: transient erythroblastopenia of childhood
   c. Marrow replacement
      Malignancies
   Osteopetrosis
   Myelofibrosis
      Chronic renal disease
      Vitamin D deficiency
   Infection
      Tuberculosis
   d. Pancreatic insufficiency-marrow hypoplasia syndrome
2. Impaired erythropoietin production
   a. Chronic renal disease
   b. Hypothyroidism, hypopituitarism
   c. Chronic inflammation
   d. Protein malnutrition
   e. Hemoglobin mutants with decreased affinity for oxygen
3. Abnormalities of cytoplasmic maturation
   a. Iron deficiency
   b. Thalassemia syndromes
   c. Sideroblastic anemias
   d. Lead poisoning
4. Abnormalities of nuclear maturation
   a. Vitamin B_{12} deficiency
   b. Folic acid deficiency
   c. Thiamine-responsive megaloblastic anemia
   d. Hereditary abnormalities in folate metabolism
   e. Orotic aciduria
5. Primary dyserythropoieitc anemias
6. Erythropoiétic protoporphyria
7. Refractory sideroblastic anemia

Disorders of increased red cell destruction or loss
1. Defects of hemoglobin
   a. Structural mutants (eg. HbSS, HbSC)
   b. Diminished globin production (eg. Thalassemias)
2. Defects of the red cell membrane
3. Defects of red cell metabolism
4. Antibody-mediated
5. Mechanical injury to the erythrocyte
   a. Hemolytic uremic syndrome
   b. Thrombotic thrombocytopenic purpura
   c. Disseminated intravascular coagulation
6. Thermal injury to the erythrocyte
7. Oxidant-induced red cell injury

(continued on next page)

Table 1 (*continued*)

Disorders of increased red cell destruction or loss
8. Paroxysmal nocturnal hemoglobinuria
9. Plasma-lipid-induced abnormalities of the red cell membrane
10. Acute/Chronic blood loss
11. Hypersplenism

Adapted from Oski FA, Brugnara C, Nathan DG. A Diagnostic Approach to the Anemic Patient. In: Nathan G, Orkin SH, eds. Nathan and Oski's Hematology of Infancy and Childhood, 5th edition. Philadelphia: WB Saunders; 1998 p. 376; with permission.

anemia is divided into two categories: (1) ineffective red cell production and (2) accelerated destruction or loss of red blood cells. Because anemia is a sign of disease and not a final diagnosis, the goal of the diagnostic evaluation of the anemic infant or child is to determine the cause of perturbed erythrocyte homeostasis. In Table 1, the major childhood anemias are classified according to physiologic disturbance. Distinguishing between these causes can be daunting and requires the use of the reticulocyte count, which is often not obtained during the initial evaluation of most patients. Most commonly, anemia is an incidental finding during routine screening of an otherwise well child [3], or an unanticipated observation during the evaluation of an acute or chronically ill child. Here, we describe a focused approach, integrating clues from the complete blood count (CBC) and peripheral blood smear (PBS), a careful history and physical examination, and the judicious ordering of additional laboratory tests as indicated to aid in defining the underlying cause of the anemia and guide in further treatment plans.

Developing a differential diagnosis of anemia: use of the CBC, blood smear, history, and physical examination

The CBC and the peripheral blood smear (PBS)

Careful evaluation of the CBC, red blood cell indices, and PBS is invaluable for narrowing the differential diagnosis. Before embarking on an extensive and/or expensive evaluation, it is important to make sure a child is truly anemic. Normal values for hemoglobin (Hb) and hematocrit (Hct) vary with age and sex (Table 2) [1]. Thus, the first step in evaluating the anemic child is to compare the patient's hemoglobin and hematocrit with normal values for children of the same age and sex. Spurious Hb or Hct results can occur due to sampling or laboratory errors. It is imperative that care is taken in obtaining the sample. Venous samples are preferred. When capillary samples are obtained, it is important that the extremity is warm and that a free flow of blood is obtained. To ensure accurate results, an adequate volume of blood should be obtained to avoid excessive dilution by the anticoagulant. Analysis of the hemoglobin concentration is preferred as it is determined by direct spectrophotometry [4]. In contrast, the hematocrit is determined indirectly by calculations using the red count and MCV [1,5].

Table 2
Normal hematologic values by age

	Hemoglobin (g/dL)		Hematocrit (%)		MCV (μ^3)	
Age (y)	Mean	Lower limit	Mean	Lower limit	Mean	Lower limit
0.5–1.9	12.5	11.0	37	33	77	70
2–4	12.5	11.0	38	34	79	73
5–7	13.0	11.5	39	35	81	75
8–11	13.5	12.0	40	36	83	76
12–14						
Female	13.5	12.0	41	36	85	78
Male	14.0	12.5	43	37	84	77
15–17						
Female	14.0	12.0	41	36	87	79
Male	15.0	13.0	46	38	86	78
18–49						
Female	14.0	12.0	42	37	90	80
Male	16.0	14.0	47	40	90	80

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Once it is determined that a child is anemic, the next step is to evaluate the red cell indices. Of these, the mean corpuscular volume (MCV) is the most useful. It is the only red cell index directly measured by the electronic counter and enables the classification of anemia by red blood cell size as microcytic, normocytic, or macrocytic. While this classification is arbitrary and categories are not mutually exclusive, it provides a useful starting point for directing further evaluation. In children less than age10 years, the lower limit for the MCV is approximately 70 fL + age in years. After 6 months of age, the approximate upper limit for the MCV is 84 + 0.6 fL per year until the upper limit of 96 fL in adults is reached [6]. The mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) are calculated values and generally less diagnostic. The MCH usually parallels the MCV. Both the MCV and MCH have small measurement errors and biological variation. The MCHC is a measure of cellular hydration status. It remains relatively constant throughout development and in most clinical settings. A high value (>35 g/dl) is characteristic of spherocytosis, while a low value is most commonly associated with iron deficiency [7]. The red cell volume distribution width (RDW) reflects the variability in cell size and can be used as a measure of anisocytosis [8]. The use of the RDW and the total RBC count to aid in further differentiating between specific etiologies of microcytic, normocytic, and macrocytic anemia is discussed below.

The next step is to access the white blood cell (WBC) and platelet counts. Is the anemia isolated or are other cell lines affected? Pancytopenia results from disorders that are distinct from those causing simple anemia and generally mandate analysis of the bone marrow. A leukoerythroblastic blood picture (nucleated red cells, reticulocytosis, a shift to the left in the neutrophil cell line, tear drop red cells) is characteristic of diseases in which the normal bone marrow is replaced by tumor or other diseases. Elevated WBC and/or platelet counts in children are most often due to reactive processes. While infection is the most common cause, other etiologies including iron deficiency anemia, autoimmune disorders such as collagen vascular disease, inflammatory bowel diseases, or hemolytic anemia, vitamin E deficiency, and post-operative states are possible.

Microscopic examination of the PBS can aid in further focusing the differential. Assess the size, color, and shape of the red cells. The normal red blood cell is about the size of the nucleus of a small lymphocyte. On a well-stained blood smear the area of central pallor in a normal erythrocyte has a diameter about one third of that of the entire cell. Cells with excessive central pallor are hypochromic. Absence of central pallor is seen in spherocytosis. Polychromasia with large cells is indicative of reticulocytosis. Distinctive abnormalities in shape are suggestive of red cell membrane disorders (eg spherocytosis, stomatocytosis, or elliptocytosis) or hemoglobinopathies (eg sickle cell disease, thalassemia). Presence of inclusions such as basophilic stippling (as seen in thalassemia, lead poisoning) should be noted. Nucleated red blood cells are never normal, except in the newborn, and are indicative of a stressed marrow. The number and morphology of WBCs and platelets should also be assessed. Toxic granulation suggests an acute inflammatory state while hypersegmented neutrophils are characteristic of vitamin B_{12} and folate deficiency.

History and physical examination

A detailed history and a careful physical examination are helpful in further defining the cause of anemia. In addition to the patient's history, a maternal history should be included in evaluation of anemic infants from birth to six months of age. Because many anemias have a hereditary basis, it is essential to include a thorough family history in the evaluation of any anemic child. Key features of the history are summarized in Table 3 [1].

In obtaining the history, the relative frequency of the various causes of anemia with age should be considered. For example, iron deficiency anemia is the most common anemia of childhood [9]. However, iron deficiency is never responsible for anemia in healthy term infants prior to six months of age [10]. In the absence of blood loss, the term neonate is born with adequate iron stores for the first six months of life. The premature infant has lower total iron stores and can become anemic from nutritional iron deficiency at a younger age. More likely causes of anemia in the neonatal period include recent blood loss, isoimmunization, congenital infection, or the initial manifestation of a congenital hemolytic anemia [11,12]. β-chain hemoglobinopathies such as sickle cell disease or β-thalassemia are generally not apparent until 3 to 6 months of age when synthesis of the β globin chain increases, whereas α-chain hemoglobinopathies are evident during fetal life and at birth [13]. Iron deficiency anemia is unusual in school age children unless ongoing blood loss, malabsorption, or a very poor diet is concomitant [14].

Table 3
Important features in the history of the anemic child

1. Maternal History
a. Pregnancy/delivery complications
b. Drug ingestion
c. Pica, nonfood product ingestion
d. Anemic during pregnancy
2. Family History
a. Ethinicity
b. Anemia
c. Jaundice
d. Splenomegally
e. Gallstones
f. Bleeding disorders
g. Cancer
h. Transfusions
3. Patient History
a. Hyperbilirubinemia
b. Prematurity
c. Diet history
Type/quantity of milk
Ingestion of non-food items
d. Medications
e. Activity level
f. Acute or recent infection
g. Evidence of chronic infection/disease
h. Evidence of endocrinopathy
i. Evidence of liver disease
j. Easy bruising/blood loss

While relying on physical examination alone to diagnosis anemia has often been unreliable, the physical examination can provide several clues to the etiology of anemia [15,16]. Tachycardia suggests an acute process with poor compensation necessitating prompt intervention. A normal heart rate suggests a more chronic process. Jaundice points to a hemolytic process. Splenomegaly may be seen in inherited hemolytic anemia, malignancy, acute infection, or hypersplenism secondary to portal hypertension. Petechiae indicate multiple cell lineages are involved. Additional characteristic clinical signs are discussed below.

Refining the differential diagnosis of anemia: use of the CBC, blood smear, history and physical examination to guide selection of further diagnostic tests

Microcytic anemia

Microcytic anemias reflect a quantitative defect in the production of hemoglobin during erythrocyte maturation. This may be due to a defect in heme synthesis (due to inadequate quantity of substrate or an inability to use substrate) or to a defect in globin synthesis (due to an inherited hemoglobinopathy). In

pediatrics, the differential diagnosis is generally limited to one of four diagnoses: iron deficiency, lead poisoning, anemia of inflammation, or thalassemia (or other more rare hemoglobinopathies). Sideroblastic anemias also cause microcytosis but are rare in children.

Iron deficiency anemia is the most common of these [9,14,17]. As suggested above, age of the patient and diet history [18–20] raise the index of suspicion for this diagnosis. Peak prevalence occurs during late infancy and early childhood when there is a combination of rapid body growth, low levels of dietary iron, and exhaustion of the iron stores accumulated during gestation. A second peak of iron deficiency is seen during adolescence when rapid growth and suboptimal diet are again culprits. This is amplified in females who experience monthly menstrual blood loss. In these populations, a therapeutic trial of oral iron is an appropriate initial diagnostic test. Further investigation is unnecessary unless there is no response. A dose of 6 mg/kg/day of elemental iron divide bid to tid is indicated. Ferrous sulfate is the most bioavailable preparation, but ferrous gluconate may be more palatable for some children. The reticulocyte count should rise in five to ten days and the serum hemoglobin should increase by 1 gm/dl/week thereafter. Poor compliance, poor absorption, an incorrect diagnosis, or ongoing blood loss can account for an inadequate response.

Further laboratory analysis should be pursued in children who do not have a history suspicious for iron deficiency anemia, who have severe anemia or atypical hematologic findings, who are less than age 6 months or more than age 18 months, or who do not respond to an initial trial of iron therapy. Several clues from the initial CBC, PBS, history, and physical examination can be used to direct this testing. The erythrocyte count is elevated (usually to >5 million/uL) in the child with thalassemia trait and depressed in the child with iron deficiency. Conversely, the RDW is high in iron deficiency and normal in thalassemia trait [8]. Indices such as the MCV/RBC ratio that are derived from the original CBC results may guide further testing. A value less than 13 favors thalassemia trait while a value greater than 14 points to iron deficiency [21]. Although helpful in forming an initial diagnostic impression, these indices lack the sensitivity and specificity to reach a definitive diagnosis without additional confirmatory studies [22]. The smear is sometimes helpful. Microcytic, hypochromic cells are seen in both thalassemia and iron deficiency. However, for the same level of anemia, there is often greater poikilocytosis (variation in shape) as well as target cells and basophilic stippling in the patient with thalassemia trait. The morphologic aberrations in thalassemia trait are out of proportion to the mildness of the anemia [1]. In iron deficiency anemia, however, the smear shows marked anisocytosis (variation in size) and the severity of anemia is proportional to the decline in the MCV. Ethnic background can also be enlightening (reviewed in [13]). The α-thalassemias are most prevalent in populations of Southeast Asian descent. The β-thalassemias are most common in individuals of Southeastern Asian, Middle Eastern, or Mediterranean descent. Onset of symptoms at 3 to 6 months of age, a positive family history of anemia, miscarriage, jaundice, gallstones, anemia, or splenomegaly further raise the index of suspicion.

Evidence of chronic infection or inflammatory disease increases the likelihood of anemia of inflammation [23,24].

The reticulocyte count is the most valuable tool for further narrowing of the differential diagnosis. Because the reticulocyte count reflects the rate at which new red cells are being produced, it can be used to classify anemia in kinetic terms. When production and destruction are balanced, the reticulocyte count reflects the rate of RBC destruction. During acute red blood cell destruction or loss, the reticulocyte response will not be evident until three to five days after the onset of the event. Maximal reticulocytosis is seen seven to 10 days after the onset of hemolysis or initiation of therapy for iron-deficiency anemia. In severely anemic patients, the reticulocyte lifespan increases from one to two days reflecting release of immature reticulocytes into the circulation [5]. The absolute reticulocyte count or reticulocyte index more accurately reflects the rate of erythropoiesis. This is determined by multiplying the reported reticulocyte percentage by the RBC count divided by 100. Normally, the absolute reticulocyte count is 50,000 to 100,000/mm^3. The reticulocyte count will be depressed in disorders of heme synthesis (ie; iron deficiency anemia, lead poisoning, and anemia of acute or chronic inflammation) and elevated in inherited disorders of globin synthesis that result in unstable hemoglobin (ie; α-β-thalassemia, hemoglobin E disease). However, in many of the disorders of β-globin synthesis, the reticulocyte count will be less than expected for the degree of anemia, reflecting destruction of erythroid precursors in the bone marrow prior to their emergence in the peripheral blood.

Additional tests should be ordered on the basis of clinical suspicion. Ferritin, free erythrocyte protoporphyrin (FEP), serum iron, and iron binding capacity are traditionally used to screen for iron deficiency anemia. However, ferritin is often the only test needed because it is low in iron deficiency (< 10 μg/dl), normal in thalassemia, normal to high in lead poisoning, and high in anemia of chronic inflammation [25]. Note that the serum ferritin can be elevated in infection, inflammation, or malignancy [26]. The combined use of the soluble transferring receptor (sTfR) and the sTfR/log ferretin index is useful in these instances [27]. The sTfR is increased in instances of hyperplasia of erythroid precursors such as iron deficiency anemia and thalassemia and low or normal in inflammation and malignancy [28–30]. The sTfR/log ferritin index is elevated in iron deficiency but not thalassemia, further differentiating these disorders [29,31]. The reticulocyte hemoglobin content, while not widely available, may represent an additional test for detecting iron deficiency anemia in children [32]. A lead level should be obtained to rule out lead toxicity as a cause of microscopic anemia. Since lead intoxication is exacerbated in iron deficiency anemia, lead levels should always be assessed in young children with suspected or confirmed iron deficiency anemia [33].

FEP accumulates in red blood cells when iron is unavailable or unable to combine with protoporphyrin to form heme. It is thus also useful in distinguishing iron deficiency and lead poisoning (elevated FEP) from thalassemia minor (normal FEP) [34]. If the rate of hemolysis in the patient with thalassemia

is significant, serum lactate dehydrogenase and total and indirect bilirubin will also be elevated, further differentiating this disorder from other causes of microcytic anemia. An erythrocyte sedimentation rate (ESR), while non-specific, can help confirm anemia of inflammation. Urinalysis and stool guaiac to rule out ongoing blood loss are also prudent.

Hb electrophoresis (or its more modern descendents, thin layer isoelectric focussing and/or high pressure liquid chromatography) [35] should (only) be obtained if there is a strong clinical suspicion of a hemoglobinopathy and/or an elevated reticulocyte count. The patient should not be iron deficient at the time of electrophoresis as iron deficiency depresses δ-globin synthesis, obscuring a rise in Hb A_2. β-thalassemia minor results from mutations affecting a single β globin locus. The mutations generally reduce the output of β-globin mRNA and therefore the synthesis of β-globin but do not produce structurally abnormal hemoglobins. These patients have microcytosis with numerous target cells, normal or borderline low hemoglobin, and elevated levels of HbA_2 and/or HbF. Thalassemic hemoglobinopathies may also reduce the output of β-globin and lead to a clinical picture of β thalassemia minor [36]. Such patients also make structurally abnormal hemoglobin that can often be detected by electrophoresis. A common example in southeastern Asian populations is hemoglobin E [13]. Mutations affecting both β globin loci lead to β-thalassemia major. These children present during the first few months of life with a severe hemolytic anemia, jaundice, and splenomegaly. RBC morphology is markedly abnormal. Electrophoresis shows reduced or absent HbA with elevated percentages of HbF and Hb A_2.

α-Thalassemia trait (deletion/mutation of two of the four α-globin genes) usually causes microcytosis with or without a mild anemia (reviewed in [13] and elsewhere in this volume). Clinically, patients are asymptomatic. Hb electrophoresis may reveal Hb Barts (γ_4 homotetramers) in the neonatal period but is subsequently normal in older patients. Diagnosis is important for genetic counseling and involves family history, elimination of β-thalassemia as a diagnosis by hemoglobin electrophoresis, and subsequent confirmation of α-globin loci mutations by molecular approaches. Hemoglobin H disease (deletion of three α-globin genes) causes microcytosis with a moderately severe hemolytic anemia. Electrophoresis reveals HbH (β_4 homotetramers), large amounts (about 25%) of hemoglobin Bart's in the neonatal period, and low-normal levels of Hb A_2. Incubation of peripheral blood with brilliant cresyl blue will reveal typical Hemoglobin H inclusions. Deletion of all four α-globin results in hydrops fetalis and fetal or perinatal death. Individuals with single α-globin gene deletions are silent carriers and asymptomatic.

Macrocytic anemia

Macrocytic anemias are defined by a MCV more than two standard deviations above the normal mean for age and sex. The first step in evaluating macrocytic anemia is to determine if the elevated MCV is due to increased reticulocytosis. In

this case, evaluation of the PBS will reveal large, polychromatic reticulocytes in a background of normocytic, normochromic cells. The RDW will correspondingly be elevated. An elevated MCV due to brisk reticulocytosis is seen in response to anemia caused by acute red cell loss due to hemorrhage, hemolysis, hypersplenism, or during the recovery from transient erythroblastopenia of childhood (TEC) [37,38] or aplastic crisis in the patient with G6PD deficiency [39]. Ancillary measures of erythrocyte destruction such as serum bilirubin, LDH, and/or a positive Coombs' test are useful in demonstrating hemolysis. Physical examination can confirm sources of hemorrhage or provide evidence of hypersplenism.

A PBS with a paucity of reticulocytes suggests macrocytic anemia due to a relative decrease in DNA synthesis during erythropoiesis [40,41]. This may be due to deficiency or disordered metabolism of folate and/or vitamin B_{12} or to ineffective erythropoiesis or marrow failure (eg Fanconi's Anemia, Diamond-Blackfan syndrome, severe aplastic anemia, or myelodysplasia). While more commonly associated with normocytic anemia, liver disease and hypothyroidism can occasionally cause macrocytosis. Clues from the CBC, peripheral blood smear, history, and physical examination can help differentiate between these etiologies. Megaloblastic changes on the PBS such as oval macrocytes, hypersegmented polymorphonuclear (PMN) leukocytes, and sometimes giant platelets suggest folate or vitamin B_{12} deficiency or disorders of DNA metabolism (eg, inborn errors of folate metabolism). The absence of hypersegmented PMNs and round macrocytes are more consistent with myelodysplasia and bone marrow failure.

A careful history highlighting diet, current medications, past surgical history, family history, and GI function is helpful. The common dietary sources of folate are green leafy vegetables. Thus, dietary deficiencies can certainly occur in children. Breast milk and infant formulas provide adequate folate. However, folate deficiency can be found in infants and children who primarily consume goat's milk or health food milk alternatives [19]. Other causes of folate deficiency include malabsorption, increased utilization of folate as in chronic hemolytic anemia, or genetic or acquired impairment of folate metabolism. Drugs that impair folate metabolism include antimetabolites (eg, methotrexate and mercaptupurine, anticonvulsants phenytoin (Dilantin), and antibiotics (trimethoprim-sulfa) [40]. Vitamin B_{12} is found in foods derived from animals. Because only very small quantities are needed and liver stores are generally adequate for many years, nutritional deficiency of vitamin B_{12} is extremely rare except in strict vegans. More commonly, deficiency is due to malabsorption of the absorption site in the terminal ileum. Infrequently, pernicious anemia (autoimmune destruction of parietal cells, the source of intrinsic factor, a cofactor in vitamin B_{12} absorption) and inherited disorders of transport or metabolism of vitamin B_{12} are responsible. Finally, the age of the patient can be helpful. Nutritional deficiency is extremely rare in infants less than a year who are fed commercial formula or breastfed by mothers with an adequate diet and/or taking supplements. DBA should be considered in this population as 80% of cases occur during the first 6 months of life [38,42]. Excessive alcohol intake should be considered in adolescents. Alcohol can have a direct toxic effect on the bone marrow. Heavy ingestion of alcohol of just one week's duration can cause vacuolization of erythroid precursors [43].

Physical examination is also revealing. The typical facies of DBA [42] or Fanconi's anemia [44] increase suspicion for myelodysplasia or bone marrow failure. Glossitis and evidence of mucosal atrophy (indigestion, anorexia, diarrhea) suggest nutritional deficiency. Neurologic disorders secondary to dorsal and lateral column degeneration in the spinal cord and resulting paresthesias, ataxia, and spastic weakness of the legs greater than the arms point to vitamin B_{12} deficiency.

Integration of the above findings should dictate further testing. A low reticulocyte count will help confirm the diagnosis. Vitamin B_{12} and serum folate levels should be obtained if the clinical scenario dictates. Serum folate levels decrease within one to two weeks following poor folate intake and before true deficiency develops. RBC folate levels are set during formation of the red cell and persist throughout the cell's life span. Consequently, two to three months of poor intake are needed before low values are observed. Measurement of methylmalonic acid and homocysteine may serve as more sensitive indicators of vitamin B_{12} and folate deficiency, respectively, at the tissue level [45]. If empiric therapy of megaloblastic anemia is attempted as a diagnostic test, both vitamin B_{12} and folate should be given since administration of folic acid alone can exacerbate the neurologic symptoms of vitamin B_{12} deficiency [46]. Liver disease and hypothyroidism are generally evident from the history and physical examination and should be screened for only when indicated or when other work-up is negative. A bone marrow biopsy and aspirate are indicated when myelodysplasia or bone marrow failure is suspected or other diagnostic tests are negative.

Normocytic anemia

The first step in evaluating normocytic anemia is to assess the CBC for evidence of pancytopenia. The presence of pancytopenia suggests ineffective hematopoeisis affecting all cell lineages. The peripheral blood smear will often show evidence of disordered erythropoiesis with red cells that vary significantly in size and shape, nucleated red cells, immature white cells, and a decreased number of platelets. A bone marrow biopsy and aspirate are indicated to rule out severe aplastic anemia, leukemia or infiltration by metastatic malignant cells, myelodysplastic syndromes (the latter often present with macrocytic anemia), osteopetrosis, or evidence of a storage disease (eg, Gaucher's disease). The presence of a hypercellular marrow in the presence of pancytopenia indicates adequate marrow function with peripheral sequestration or destruction of normal blood cells. Hypersplenism is the most common cause. Spherocytes may be present on the peripheral smear.

If pancytopenia is absent, the next step is to assess the bone marrow response to the anemia. In children, an inappropriately low reticulocyte count is most commonly associated with anemia of acute or chronic inflammation, which can

also be microcytic (reviewed in [23, 24]). Transient depression of erythrocyte production is common during and following acute viral illness. It may be appropriate to carefully watch a child with borderline isolated anemia in this setting and to do further evaluation only if there is a continued drop in the Hb/Hct or some other hematologic abnormality. Generally, the Hb is greater than 8, RBC morphology is normal, serum ferritin and ESR are elevated, and clinical history often points to a likely cause of inflammation which should be treated. Patients may be monitored for resolution of the anemia, which can take 1 to 3 months, without embarking on further evaluation. Iron therapy has been shown to be unhelpful [47]. Evaluation of adult patients with chronic inflammation suggests erythropoeitin may be helpful in selected cases [47]. Careful clinical trials need to be performed in children to address this issue. Liver disease, renal insufficiency (Hb almost always in the range of 8 to 10 g/dL), and endocrinopathy are other potential causes. Clues from the clinical picture often aid in these diagnoses. Balanced microcytic and macrocytic anemias, such as iron deficiency concomitant folate and/or B_{12} deficiency can present as a normocytic anemia. Assessing the RDW, which should be greatly increased, is helpful.

Normocytic anemia with an elevated reticulocyte count suggests premature disappearance of RBCs due to blood loss or hemolysis. Tachycardia suggests acute loss while an inappropriately low heart rate for the level of anemia suggests chronic loss or destruction. The source of blood loss may be overt or occult. Repeated guaiac tests are indicated, particularly in the cow's milk fed infant. More uncommon sources of hemorrhage include beneath the scalp, intrabdominal, urinary tract, and pulmonary hemorrhage.

Hemolysis is defined as an abnormally increased rate of red blood cell destruction [12,48]. It may be acute or chronic, congenital or acquired, and intrinsic or extrinsic to the RBC. With chronic hemolysis, anemia may or may not be present depending on the rate of red cell destruction and the degree of bone marrow compensation. Moreover, the clinical signs and laboratory findings in hemolysis depend on both the rate and site of red cell destruction. If red cells are destroyed extravascularly in the reticuloendothelial system, the normal site of red cell catabolism, Hb is degraded to iron, bilirubin metabolites, and amino acids. Because hepatic clearance of bilirubin can increase substantially, a normal serum bilirubin does not exclude hemolysis. Unconjugated (indirect) bilirubin will be increased in more severe hemolysis resulting in jaundice. When hemolysis occurs intravascularly, free hemoglobin is released into the plasma where it is bound by haptoglobin and subsequently cleared in the liver or lost in the urine. An increase in plasma hemoglobin, a decrease in serum haptoglobin, and the presence of hemoglobinuria suggest intravascular hemolysis.

Congenital hemolytic anemias include disorders of the red cell membrane (eg hereditary spherocytosis, stomatocytosis, or elliptocytosis), hemoglobinopathies (eg, hemoglobin SS), and red cell enzyme deficiencies (eg glucose-6-phosphate dehydrogenase (G6PD) deficiency, pyruvate kinase deficiency). A family history positive for anemia, splenomegaly, jaundice, and/or gallstones supports a congenital hemolytic anemia. Ethnic background can also be helpful.

Hereditary spherocytosis can occur in any ethnicity, but is most common in whites [49]. An elevated MCHC and spherocytes on the PBS supports this diagnosis. An MCHC greater than 35.4 coupled with an RDW >14 is almost always diagnostic of hereditary spherocytosis [7]. The osmotic fragility test or ektacytometry are confirmatory. Family members should also be screened. G6PD deficiency is most common in those of African or Mediterranean descent (with the latter tending to have more severe disease) [39]. Individuals with G6PD deficiency often present with acute hemolysis after an infection or after encountering an oxidant stress. Signs of acute intravascular hemolysis with tachycardia, jaundice, and hemoglobinuria will be observed. The PBS reveals schistocytes and spherocytes initially, then becomes normal after the enzyme deficient cells are hemolyzed. The sickle cell syndromes are usually detected by newborn screening in the United States and the pediatrician becomes aware of the diagnosis before anemia or other clinical manifestations occur. More detailed information about these syndromes can be found elsewhere in this volume. In any patient with congenital hemolytic anemia, an aplastic episode is the exception to the rule that hemolytic anemias are associated with reticulocytosis. While many viruses have been implicated, human parvovirus B19 is the most frequent cause [50]. Complete suppression of erythropoiesis can last for 7 to 10 days after infection. Prompt intervention with red blood cell transfusions as needed can be life saving.

Acquired hemolytic anemias can be immune-mediated or secondary to factors that cause mechanical damage to the red cells such as toxins, mechanical or abnormal heart valves, and fibrin strands in disseminated intravascular coagulation (DIC) or hemolytic uremic syndrome (HUS). Immune-mediated destruction is confirmed by a positive direct and indirect Coombs' test. Spherocytes may be seen on the PBS. Antibody-mediated hemolytic anemia can occur as part of a more generalized autoimmune process (such as lupus) or after exposure to a drug. However, in children it is most often a self-limited disease following a viral illness. In neonates, immune-mediated hemolysis is due to placentally-transferred maternal antibodies (reviewed in [12]). Historically, this was most commonly due to sensitization to the Rh antigen in Rh negative mothers. The advent of anti-D serum in women who are a potential setup for sensitization has caused a dramatic decrease in the incidence of this disease. Currently, immune-mediated hemolysis in the infant most often reflects a maternal response to other blood group antigens. Mechanical damage is usually intravascular and associated with red cell fragments on the PBS. The history and/or physical examination can often identify the source of damage.

Summary

Anemia is a sign of disease and not a final diagnosis. The clinician's goal is to define the underlying cause. The anemia may be due to decreased production or increased destruction or loss of red blood cells. Integration of the results of the initial CBC, particularly the RBC indices, the peripheral blood smear, the history

and the physical examination can help organize the focus of further evaluations and, ultimately, minimize the number of tests needed to make a firm diagnosis.

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