Aplastic and Hypoplastic Anemias

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Education Gaps

1. Anemia is a common finding in general pediatrics, but knowing when to refer a patient can be challenging.
2. Pediatricians do not always order reticulocyte counts as part of the evaluation of anemia. The reticulocyte count, interpreted in the context of the mean corpuscular volume, is very useful in the development of differential diagnoses for anemia and determination of urgency of referral.
3. Pediatricians (and subspecialists) may not realize that diagnosis of malignancy, particularly head and neck cancers, at an unexpectedly early age may be associated with an inherited bone marrow failure.

Objectives

After completing this article, readers should be able to:

1. Assess the need for referring a patient with anemia based on whether anemia is isolated or accompanied by other hematologic or physical anomalies.
2. Recognize that anemia in the setting of congenital anomalies may signify an inherited bone marrow failure disorder.
3. Understand that inherited bone marrow failure disorders confer a higher risk of malignancy.

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ABBREVIATIONS

AA	aplastic anemia
AML	acute myeloid leukemia
ANC	absolute neutrophil count
ARC	absolute reticulocyte count
CBC	complete blood cell
DBA	Diamond-Blackfan anemia
DC	dyskeratosis congenita
FA	Fanconi anemia
GPI	glycosylphosphatidylinositol
HSCT	hematopoietic stem cell transplant
MCV	mean corpuscular volume
MSD	matched sibling donor
MUD	matched unrelated donor
OS	overall survival
PNH	paroxysmal nocturnal hemoglobinuria
PRCA	pure red cell aplasia
SDS	Shwachman-Diamond syndrome
TEC	transient erythroblastopenia of childhood

INTRODUCTION

Children with anemias often initially present to their pediatricians. Anemia may result from blood loss, a destructive process (ie, hemolysis), nutritional deficiency, or poor production (eg, ineffective erythropoiesis or hypoplastic or aplastic marrow) (Table). Hemolytic and nutritional anemias have been discussed elsewhere and are referenced briefly in this article. (1)(2)(3)(4) This review focuses on the broad differential diagnosis for anemia associated with poor production. These anemias can lead to chronic morbidity and require subspecialty care, whereas others may be managed effectively in primary care. This review offers a framework to guide evaluation and the need for subspecialty investigation.

TABLE. **Classification of Anemias by Mechanism and Notable Associated Laboratory Data**

MECHANISM OF ANEMIA	EXAMPLES	CHANGES IN LABORATORY DATA
Loss or destruction	Bleeding	↑ Reticulocyte count
	Autoimmune hemolytic anemia Hereditary spherocytosis Sickle cell anemia	↑ Reticulocyte count ↑ LDH ↑ Indirect bilirubin ↓ Haptoglobin
Nutritional deficiency	Iron deficiency	↓ MCV ↓ Reticulocyte count ↓ Ferritin ↓ Iron saturation ↑ TIBC
	Folate deficiency Vitamin B_{12} deficiency	↑ MCV ↓ Reticulocyte count
Poor production	Anemia of chronic inflammation	Normal or ↓ MCV ↓ Reticulocyte count
	Pure red cell aplasia	↑ MCV ↓ Reticulocyte count
	Aplastic anemia	↑ MCV ↓ Reticulocyte count +/– Neutropenia +/– Thrombocytopenia

LDH=lactate dehydrogenase; MCV=mean corpuscular volume; TIBC=total iron binding capacity.

PATHOGENESIS

Aplastic and hypoplastic anemias are characterized by impaired erythropoiesis due to decreased hematopoietic cells in the bone marrow. Impairment of erythropoiesis can result from an acquired condition or an inherited defect in marrow function. Acquired conditions may include parvovirus infection of erythrocyte precursors or T-cell–mediated destruction of hematopoietic cells in acquired aplastic anemia. Inherited defects in marrow function can present at any point throughout the life span with progressive anemia.

Impaired erythropoiesis can also result from abnormal metabolism of iron, which is a critical component of hemoglobin. Although this class of disorders is not associated with marrow hypoplasia, it should still be considered as part of the differential diagnosis for anemia with reticulocytopenia (Fig).

CLINICAL FINDINGS

A thorough history and physical examination will provide important clues to the presence and potential causes of anemia. Patients with anemia may be asymptomatic or may present with pallor or fatigue. Infants and children with anemia may display irritability, poor feeding, or growth failure. A detailed dietary history may reveal a nutritional deficiency. Fever or symptoms of a viral illness preceding new-onset anemia could imply an infectious etiology. Anemia of chronic disease may be accompanied by symptoms related to the underlying condition. Inherited causes of anemia can be associated with short stature, skeletal abnormalities, or other congenital anomalies. Early-onset head and neck tumors can be the first presentation of Fanconi anemia. (5) The most common inherited bone marrow failure syndromes are considered cancer predisposition syndromes.

LABORATORY FINDINGS

The most helpful initial tests for the evaluation of any form of anemia include the complete blood cell (CBC) count, differential count, and reticulocyte count with indices. A normal or decreased reticulocyte count in the setting of anemia generally represents an inappropriate compensatory response and may be an indication of impaired erythropoiesis or marrow hypoplasia. An elevated reticulocyte count reflects a response to blood loss or hemolytic anemia. Mean corpuscular volume (MCV), reported as part of the CBC count, can guide the clinician in developing a differential diagnosis. In children older than 1 year, MCV less than 80 μm^3 (<80 fL) is considered microcytic, and 80 to 100 μm^3 (80–100 fL) is normocytic. Macrocytosis is defined as MCV greater than 100 μm^3 (>100 fL). Microcytosis could reflect iron deficiency, whereas macrocytosis may be concerning for vitamin B_{12}

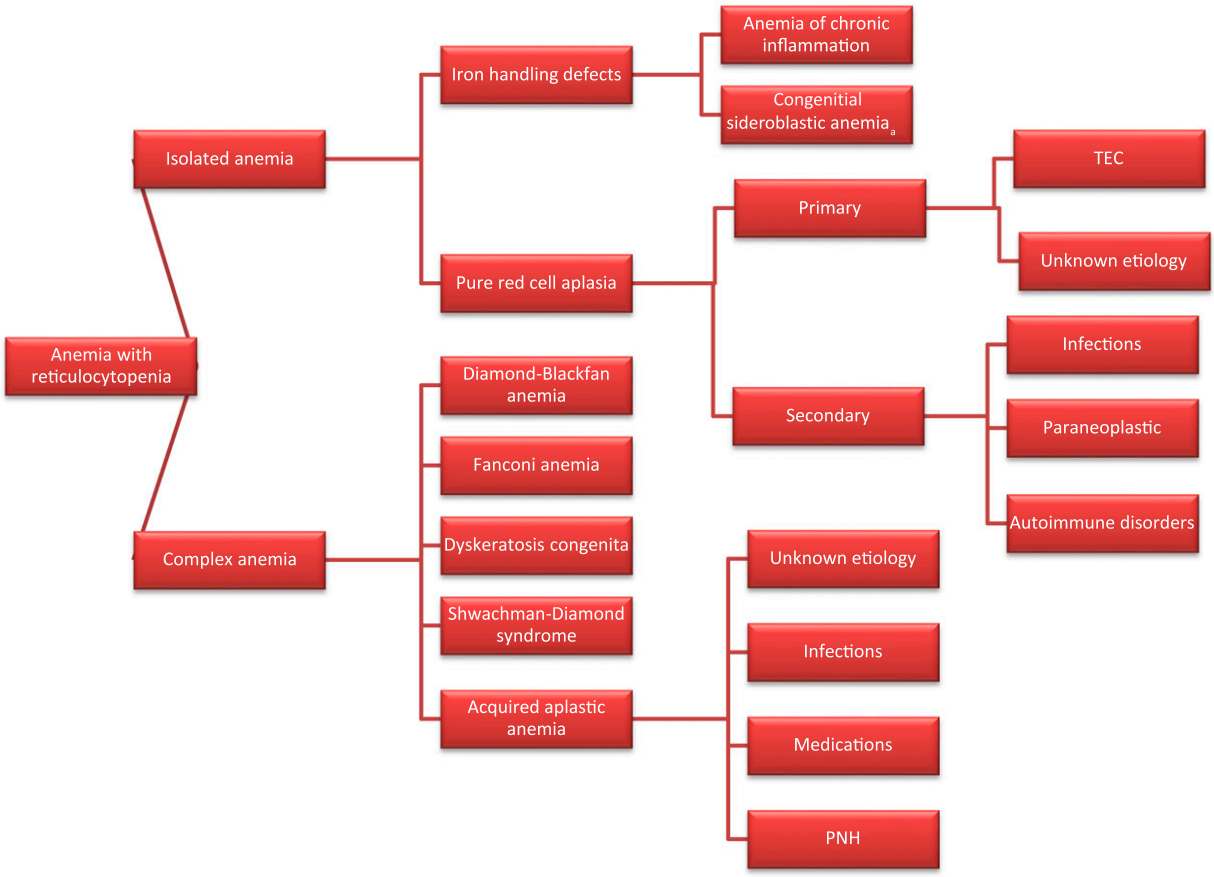

Figure. Classification of anemia with reticulocytopenia based on association with additional hematologic or physical anomalies.[a] There are rare syndromic forms of sideroblastic anemia associated with physical anomalies.
TEC=transient erythroblastopenia of childhood.

or folate deficiency or marrow failure. Concomitant neutropenia and/or thrombocytopenia are worrisome for more severe pathology, such as aplastic anemia or leukemia.

Additional blood work may be ordered based on clues from the initial evaluation. A peripheral blood smear may reveal microcytosis and hypochromia in an iron-deficient patient. Iron deficiency may also be accompanied by low ferritin level, increased iron-binding capacity, and low transferrin saturation. Ferritin, an acute phase reactant, can be within the reference range if iron deficiency and inflammation are both present. Vitamin B_{12} and folate levels should be measured if macrocytosis is observed. Hemoglobin electrophoresis provides an analysis of the types of hemoglobin expressed by an individual. Increased fetal hemoglobin level, in conjunction with progressive macrocytosis, may be signs of stress erythropoiesis and can be a harbinger of acquired or inherited bone marrow failure. (6)(7)

ANEMIA IN GENERAL PEDIATRICS

When confronted with newly discovered anemia with reticulocytopenia, the general pediatrician should consider the clinical context in conjunction with the blood work to determine the need for referral. In a well child without organomegaly or significant lymphadenopathy, it may be reasonable to monitor isolated normocytic anemia until the hemoglobin level normalizes. The frequency of monitoring depends on the severity of anemia. Surveillance blood work every 1 to 4 weeks may be sufficient for mild to moderate anemia. The development of severe anemia possibly requiring transfusion, constitutional symptoms (eg, fatigue, weight loss, night sweats, unexplained fevers, increased bleeding, or bruising), or other cytopenias represents a complex clinical picture and should prompt urgent referral to the pediatric hematologist/oncologist. Persistent macrocytosis or anemia in a child with congenital anomalies may raise concern for an inherited bone marrow failure syndrome and requires evaluation and close monitoring by pediatric hematology/oncology.

Classification of aplastic and hypoplastic anemias based on clinical complexity may be of practical utility for clinicians. For the purposes of this review, clinical complexity is defined by whether the anemia is isolated or associated with physical or other hematologic anomalies (Fig).

ISOLATED ANEMIAS

Usually microcytic or normocytic, anemia due to chronic inflammation is extremely common and is the end product of several processes. Inflammation increases expression of hepcidin, a key regulator of iron metabolism, and interleukin-6, a proinflammatory cytokine. (8) Through its effect on ferroportin, hepcidin activity leads to decreased gastrointestinal iron absorption and increased iron sequestration in macrophages, reducing its availability for erythropoiesis. (9) Interleukin-6 promotes iron sequestration by upregulation of hepcidin via the *JAK/Stat* pathway. (10) Other proinflammatory cytokines, such as interleukin-1, tumor necrosis factor α, and interferon-γ, seem to inhibit erythropoiesis by impairing erythropoietin sensitivity and erythroid lineage differentiation. (11)(12) Currently, the most effective intervention for anemia of chronic inflammation is to treat the underlying disease, but experimental treatments are being studied. (8)

Congenital sideroblastic anemias comprise a group of uncommon inherited anemias defined by ringed sideroblasts in the bone marrow. This morphologic finding represents iron deposition in the mitochondria of erythroid precursors, a result of impaired mitochondrial iron handling and abnormal heme synthesis. Sideroblastic anemias may be microcytic, normocytic, or macrocytic. A variety of mutations have been implicated, but the most commonly affected gene is *ALAS2*, which encodes a critical enzyme involved in the production of heme. *ALAS2* deficiency is X-linked but can also affect girls and women due to lyonization. Patients with congenital sideroblastic anemia can have mild to severe forms of anemia with concomitant iron overload, even in the absence of transfusion dependence. Anemia with elevated ferritin and transferrin saturation should prompt referral to a pediatric hematologist to further investigate whether this disorder may be present. Pyridoxine (vitamin B_6) has historically been used to treat anemia. Iron overload may be addressed by chelation therapy or phlebotomy. (13)(14)

Acquired pure red cell aplasia (PRCA) encompasses a heterogeneous set of disorders causing normocytic, normochromic anemia and a selective decrease in erythroid progenitors. Pure red cell aplasia has been associated with infections, medications, (15)(16) malignancies, and underlying autoimmune disorders. (17) When an etiology cannot be identified, it is designated as primary PRCA. Most cases of primary and secondary PRCA are thought to be immune mediated. This is supported by reports of association with autoimmune diseases, such as rheumatoid arthritis and lupus. Pure red cell aplasia can also be paraneoplastic, preceding or in association with several malignant processes, such as thymoma, (18) Hodgkin (19) and non-Hodgkin lymphoma, (20) and large granular leukemia. (21) Immunoglobulin G and T- and natural killer–cell–mediated PRCA have been described. (22) The incidence of PRCA after ABO-mismatched allogeneic hematopoietic stem cell transplant (HSCT) was 7.5% in one case series. (23)

Pure red cell aplasia may be suspected in patients with persistent normocytic, normochromic anemia and reticulocytopenia. The absolute reticulocyte count (ARC) is uniformly less than $10\times10^3/\mu$L (10×10^9/L). If clinically warranted, secondary causes of PRCA should be ruled out. A bone marrow examination is necessary for diagnosis. Aside from decreased or absent erythroid progenitors, the bone marrow should be normal, without dysplasia or cytogenetic abnormalities. (17)

Primary PRCA is treated with immunosuppression, with cyclosporine with or without corticosteroids achieving the most durable responses. (17) Treatment of secondary PRCA is usually directed at the underlying etiology.

Parvovirus B19 infection is a classic cause of PRCA. A marker of past infection, parvovirus immunoglobulin G is present in 2% to 15% of children aged 1 to 5 years and 15% to 60% of children aged 6 to 19 years. (24) The virus preferentially infects red blood cell precursors by binding to the blood group P antigen on these cells. (25) Individuals who do not express P antigen are resistant to parvovirus infection. The resulting death of erythroid progenitors temporarily halts erythropoiesis, causing a transient anemia. Immunocompetent patients whose circulating red blood cells live long enough until the viral infection is cleared generally experience no sequelae. In contrast, patients with a chronic hemolytic process such as sickle cell disease, in which erythrocyte life span is abbreviated, can develop life-threatening anemia. Immunocompromised patients may develop chronic parvovirus infection, most often manifesting as PRCA. Bone marrow examination may reveal unusually large pronormoblasts, which are the earliest red blood cell precursor. Polymerase chain reaction may be used to detect parvovirus DNA, although there is a risk of both false-positive and false-negative results. Pure red cell aplasia due to chronic parvovirus infection can be treated with intravenous immunoglobulin for several days. (26)

New-onset isolated normocytic anemia with reticulocytopenia in a healthy toddler may raise suspicion of transient erythroblastopenia of childhood (TEC), a type of PRCA. From 1987 to 1989, the incidence of TEC in Sweden was estimated to be 4.3 per 100,000 children younger than 3 years. (27) This is likely an underestimate because cases of mild to moderate anemia may have resolved untreated and

were not reported. TEC has been reported less frequently in recent years. (28) An infectious etiology has been suggested, but no consistent pathogens have been identified. (29)(30)(31)

TEC primarily affects children aged 1 to 4 years, although infants can be affected. The anemia is often found incidentally in the setting of an illness or as part of an investigation of pallor in an otherwise vigorous child. A history of nonspecific viral symptoms may or may not precede the onset of anemia. The presenting hemoglobin level may sometimes be low enough to affect hemodynamic stability and thus warrant transfusion, but TEC is ultimately defined by spontaneous recovery starting within an average of 2 weeks after diagnosis. The recovery phase will be characterized by reticulocytosis and elevated fetal hemoglobin level. The bone marrow is not necessary for diagnosis but would show markedly decreased red blood cell precursors. A bone marrow examination is needed only if recovery of erythropoiesis does not occur as expected after 3 to 4 weeks or there are red flags in the clinical presentation. (28)

COMPLEX ANEMIAS

Diamond-Blackfan Anemia

TEC sometimes must be differentiated from Diamond-Blackfan anemia (DBA), especially in young children and infants. A rare inherited red cell aplasia, DBA is diagnosed in approximately 30 patients per year in the United States. (32) This is consistent with reports from other countries, where the annual incidence rate ranges from 4 to 7 cases per million births. (33)(34)(35)(36) Most patients with classical DBA are diagnosed in infancy, when they present with an unexplained macrocytic anemia with reticulocytopenia. Additional cytopenias can be seen but should prompt a search for other bone marrow failure disorders. Persistence of the hematologic findings warrants referral to the pediatric hematologist. Almost 50% of patients exhibit congenital anomalies, such as abnormal thumbs, short stature, facial dysmorphism, genitourinary anomalies, or cardiac defects. (37) Further investigation, ideally performed before transfusion, would reveal elevated erythrocyte adenosine deaminase and fetal hemoglobin levels in 80% to 85% of patients. In contrast, 90% of patients with TEC express normal levels of erythrocyte adenosine deaminase. Adenosine deaminase is a critical ubiquitous enzyme catalyzing purine residue metabolism, but its role in DBA is unknown. (38) The bone marrow examination, which is mandatory for diagnosis, demonstrates decreased or absent erythroid progenitor cells but otherwise normal cellularity. Alternative causes of red cell aplasia, such as viral infection, malignancy, and other bone marrow failure conditions, should be ruled out. (39)(40)

Genetics of DBA. Manifestations of DBA arise from autosomal dominant mutations in ribosomal genes, which increase vulnerability to apoptosis in ways that continue to be elucidated. The first gene to be identified, *RPS19*, is mutated in 25% of patients. *RPS19* plus several other ribosomal genes (*RPS24, RPS17, RPL35A, RPL5, RPL11, RPS7, RPS10, RPS26,* and *RPL26*) account for 54% of patients with DBA. (41) Discovery of these mutations has led to the diagnosis of many "nonclassical" cases, even in adulthood. Thus, DBA represents a spectrum of disease that can encompass minimal anemia to transfusion dependence, isolated macrocytosis, only congenital anomalies, or no medical abnormalities at all. (40)

Management of DBA. To maintain appropriate growth and cognitive development, regular red blood cell transfusions are initiated in infancy to keep pretransfusion hemoglobin levels higher than 8 g/dL (80 g/L). Chronic transfusions are associated with alloimmunization and iron overload, so after 1 year of age, prednisone is used at a dose of 2 mg/kg per day for no longer than 4 weeks. Clinicians have used glucocorticoids in patients with DBA for more than 50 years, but the mechanism by which they improve erythropoiesis is still under investigation. In a mouse model of DBA, glucocorticoids inhibited apoptosis and delayed differentiation, leading to a greater pool of erythroid progenitors. (42) Clinically, 80% of children respond to corticosteroids within 4 weeks, defined as maintaining a hemoglobin level of at least 9 g/dL (90 g/L) without transfusion. The remainder do not respond or are unable to tolerate dose tapering. A second trial of prednisone can be attempted a year later because some children become steroid responsive. Once a response is seen, the dose should be tapered slowly to a target dose of 0.5 mg/kg per day or 1 mg/kg every other day. (40) Those taking long-term corticosteroids require periodic evaluations of growth, cataract formation, and bone health. Patients who fail a taper require resumption of chronic transfusions.

Patients receiving transfusions require management of iron overload with appropriate chelation and organ function evaluation. Matched sibling donor (MSD) allogeneic HSCT can be considered during childhood if the patient remains transfusion dependent. Five-year overall survival (OS) after MSD HSCT reached 87.5% in a small series from the Diamond Blackfan Anemia Registry of North America. (43) Donors must be screened carefully to ensure that they are not asymptomatic carriers of DBA.

Ominously, DBA is now considered a cancer predisposition syndrome, with a cumulative incidence of solid tumor

or acute myeloid leukemia (AML) of 20% by 46 years of age. (44) Guidelines on cancer surveillance in this population are not available yet, but clinicians should be aware of patients' vulnerability and have a low threshold to consider malignancy evaluation in the appropriate clinical setting.

Fanconi Anemia

Although rare, affecting 0.1 to 0.5 per 100,000 live births, Fanconi anemia (FA) is the most common cause of inherited bone marrow failure syndrome and may present with macrocytic anemia and reticulocytopenia, often accompanied by other cytopenias. Mild to moderate cytopenias, first emerging between 5 and 10 years of age, are present in more than 70% of patients at diagnosis. (45)(46) Congenital anomalies, including radial or thumb abnormalities, genitourinary malformations, abnormal skin pigmentation, gastrointestinal anomalies, microphthalmia, short stature, cardiac defects, and developmental delay are present with variable severity in 90% of patients at diagnosis. (47) Furthermore, the VACTERL (vertebral anomalies, anal atresia, cardiac defects, tracheoesophageal fistula with esophageal atresia, renal and limb anomalies) and VACTERL with hydrocephalus syndromes are frequently associated with certain mutations in the FA pathway: *FANCI*, (48) *FANCB*, (49) and *FANCD1/BRCA2*. (50)

Genetics of FA. To date, 20 FA or FA-like genes have been identified through mutation analysis, including *BRCA1*. Fanconi anemia is transmitted in an autosomal recessive manner, except *FANCB* mutations, which are X-linked. The FA pathway encompasses critical proteins involved in DNA crosslinking repair and genomic stability. (51)(52)(53) Defects in the FA pathway significantly increase patients' vulnerability to ionizing radiation and chemotherapy. (5) Diagnostic assays for FA capitalize on this by using chemicals that induce DNA crosslinking. If left unrepaired due to mutations in an FA gene, DNA replication is impaired and chromosomal aberrations develop. Chromosomal breakage studies using mitomycin C or diepoxybutane are the gold standard method of diagnosis for FA. If mosaicism is suspected (ie, clinical suspicion is high but chromosome fragility test results are negative), skin fibroblasts should be collected for testing. Next-generation sequencing is commercially available to identify pathogenic mutations. (45) Full siblings of patients with FA, even if asymptomatic, should be tested as well.

Management of FA. Once the diagnosis is established, surveillance for progressive health problems is imperative. Bone marrow failure ultimately develops in 50% of patients by age 40 years. Remarkably, patients with *FANCD1/BRCA2* exhibit a cumulative incidence of malignancy of 97% by 7 years of age. The risk of developing AML or solid tumors (predominantly head, neck, and gynecologic cancers, but other solid tumors also occur) conferred by other FA mutations is 20% or 30%, respectively, by age 40 years. (54) Regular examinations to detect head and neck cancers should start at age 10 years, accompanied by counseling about smoking and alcohol avoidance and good dental hygiene. Annual screening for gynecologic epithelial cancers should begin by age 13 years, with examination of external genitalia. Full gynecologic examinations should begin when women become sexually active or by age 18 years if not sexually active. Vaccination against human papillomavirus should be strongly considered. (55)

Matched sibling donor HSCT before the onset of AML achieves the best hematologic outcomes, so careful monitoring of CBC count with differential count and annual bone marrow morphology, cellularity, and cytogenetic testing is recommended. (56) The development of moderate to severe cytopenias due to bone marrow failure or the acquisition of clonal abnormalities such as monosomy 7, –7q, +3q, complex karyotype, or *RUNX1* abnormalities confers poor prognosis. Such patients should proceed to HSCT if a suitable match is available. (45) The addition of fludarabine as part of reduced-intensity preparative regimens has improved survival in this population, which had historically experienced excess treatment toxicity due to irradiation and alkylating agents. In a retrospective, multicenter trial, patients undergoing MSD HSCT since 2000 achieved OS of 76% at 5 years and 64% in matched unrelated donor (MUD) HSCT. This is in contrast to 68% and 43%, respectively, for transplants performed before 2000. (57) Second malignancies pose a high risk after transplant, with recipients carrying a 4.4-fold higher risk of head and neck cancer compared with patients who did not undergo transplant. (58) A recent prospective, multicenter trial using radiation-free conditioning regimens for MUD HSCT reported an OS of 80% at 3 years. (59) It remains to be seen whether elimination of radiation from the preparative region will ameliorate the long-term risks of posttransplant malignancy.

Patients without appropriate HSCT donors who develop bone marrow failure may be treated with androgens. The mechanism of androgen effect on hematopoiesis remains unclear but perhaps relates to effects on regulation of stem cell cycling. (60) Danazol, oxymetholone, and oxandrolone can improve erythropoiesis, with smaller improvements seen in platelet or leukocyte counts. Virilization, liver adenomas, and elevated transaminase levels are common sequelae of androgens. Androgens do not delay onset of myelodysplasia or AML.

OTHER INHERITED BONE MARROW FAILURE DISORDERS

Dyskeratosis congenita (DC) is another rare bone marrow failure syndrome that can present with macrocytic anemia and other cytopenias. The classic triad of DC includes lacy, reticulated rash, leukoplakia, and dysplastic nails, but these features may not all be present at the same time. Patients with DC are at higher risk for pulmonary fibrosis, bone marrow failure, myelodysplasia, AML, and solid tumors. (56) It is caused by several mutations in genes regulating telomerase stability and function, such as *DKC1*, (61) *TERT*, (62) and *TERC*. (63) As with FA, patients with DC require regular monitoring of CBC counts and marrow function. Nonmyeloablative HSCT is indicated if myelodysplasia or severe bone marrow failure is detected. Androgens may be used as a bridge or in lieu of HSCT if no suitable donor can be found. A cancer predisposition syndrome, DC is associated with malignancy in 40% to 50% of patients by age 50 years. Head and neck cancers are the most common, followed by squamous cell carcinoma of the skin and anus. (64)

If anemia is accompanied by neutropenia, Shwachman-Diamond syndrome (SDS) should be considered. Associated with biallelic mutations in the *SBDS* gene, SDS more commonly presents with neutropenia and exocrine pancreatic insufficiency. However, SDS can present with a variety of clinical findings, including transfusion-dependent anemia with other cytopenias, isolated macrocytosis, failure to thrive, and/or skeletal anomalies. Bone marrow failure or myelodysplasia may develop, which may progress to AML. Hematopoietic stem cell transplant can be curative, but outcomes are better if performed before the development of AML. (65)

ACQUIRED APLASTIC ANEMIA

Most patients with marrow failure do not harbor any known inherited genetic lesions and are ultimately diagnosed as having acquired aplastic anemia (AA). Acquired AA is estimated to affect 2 to 3 per million people per year, according to European registries. Asian countries have reported higher incidence rates, ranging from 3.9 per million in Bangkok, Thailand, to 7.4 per million in China. The age distribution is bimodal, affecting young adults and the elderly most frequently. (66) An immune-mediated process, the etiology is understood to be multifactorial, attributed to medications, infections, or environmental exposures in genetically susceptible individuals. Nevertheless, the etiology is usually not evident at diagnosis.

Patients may present with symptoms due to cytopenias, including fatigue or pallor due to anemia, increased bruising or bleeding due to thrombocytopenia, or mucosal ulcers or infections due to neutropenia. If left untreated, severe AA is usually fatal.

Diagnosis of AA

Aplastic anemia should be considered in a patient with 2 or more persistent cytopenias, with early referral made to a pediatric hematologist. Anemia is usually normocytic or macrocytic, accompanied by reticulocytopenia. Thrombocytopenia and/or neutropenia are also seen. Bone marrow biopsy examination is mandatory and would show decreased cellularity of all lineages. There is some variability, but most centers define severe AA as an absolute neutrophil count (ANC) less than 500/μL, ARC less than $20\times10^3/\mu$L (20×10^9/L), platelet count less than $20\times10^3/\mu$L (20×10^9/L), and bone marrow cellularity less than 25% for age. Criteria for very severe AA is the same except ANC less than 200/μL. Moderate AA is typically defined by a hemoglobin level less than 10 g/dL (100 g/L), ANC less than 1500/μL, platelet count less than $50\times10^3/\mu$L (50×10^9/L), ARC less than $40\times10^3/\mu$L (40×10^9/L), and bone marrow cellularity 25% to 50%. (67) Other causes of pancytopenia should be ruled out, including viral infections such as hepatitis, myelodysplasia, leukemia, FA, DC, SDS, and paroxysmal nocturnal hemoglobinuria. Human leukocyte antigen (HLA) typing should be undertaken for the patient and any full siblings as soon as the diagnosis of AA is made to facilitate timely HSCT if the sibling is a match.

Management of AA

Patients may become transfusion dependent before definitive therapy proves effective. Stringent criteria for transfusion are necessary to avoid alloimmunization and graft rejection if the patient proceeds to HSCT. Transfusions are administered to alleviate symptoms rather than to correct laboratory values. Directed donations of blood products from family members are contraindicated because they may precipitate alloimmunization against potential hematopoietic stem cell donors. Prolonged severe neutropenia increases the risk of opportunistic infections. The development of fever in this setting requires collection of blood cultures and institution of broad spectrum empirical antibiotics at a minimum.

If the patient has an HLA MSD, first-line therapy for severe AA would be allogeneic HSCT. Bone marrow is preferred over peripheral blood stem cells because it confers a lower risk of graft-versus-host disease. Overall

survival 3 to 5 years after upfront MSD HSCT is excellent, reaching greater than 90% in several series. (68)(69)(70) Ten-year OS for patients in a large series from Japan was 89.7%. (71)

Immunosuppression with horse antithymocyte globulin (72) and cyclosporine is used in severe AA if HSCT is not an option or in patients with moderate AA who are transfusion dependent. After immunosuppression, OS ranges from 75% to 87%, but event-free survival is only 33%. (68)(73) Patients who have shortened leukocyte telomeres at diagnosis of AA are at higher risk for relapse after immunosuppressive therapy as well as for clonal evolution, (74) but it is not standard practice to check telomere length before starting treatment. Published guidelines recommend treatment for 4 months before starting to slowly taper cyclosporine if there is a response or pursuing second-line treatment in the absence of hematologic recovery. Most clinicians continue cyclosporine therapy for at least 6 months if not longer. If there is no response to immunosuppression, MUD HSCT can be pursued. (73)(75)(76) With improvements in conditioning and supportive care, outcomes after MUD HSCT are almost as good as those after MSD HSCT. (77)(78)(79)

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

Paroxysmal nocturnal hemoglobinuria (PNH) is an underlying cause of AA in children. A small Italian multicenter trial diagnosed only 2.5% of children with AA with concomitant PNH, (80) whereas a retrospective series of pediatric AA reported a 20% prevalence of PNH. (81) Accounting for less than 10% of patients with PNH, childhood PNH is a clonal hematopoietic disorder that can be primarily associated with AA or hemolysis. The latter is classified as classical PNH. Some patients may also exhibit subclinical disease that does not require treatment. Thrombosis occurs more commonly in patients with hemolytic anemia. Patients with classical PNH may also experience nonspecific symptoms, such as abdominal pain, esophageal spasm, or erectile dysfunction. These are sequelae of ongoing intravascular hemolysis ultimately causing nitric oxide deficiency and dysregulation of smooth muscle tone. Aplastic anemia is a more common presenting condition in children (58%–83%) than in adults (16%–23%), (81)(82)(83)(84) whereas hemoglobinuria is relatively rare in children.

Pathophysiology of PNH

Manifestations of PNH result from a global deficiency of glycosylphosphatidylinositol (GPI) anchor proteins due to an acquired mutation in the *PIGA* gene. The GPI-anchored proteins are an important class of proteins and include blood group antigens, adhesion molecules, and complement regulatory proteins. Most of the clinical manifestations of PNH are specifically due to loss of CD55 and CD59 on the red blood cell surface. (85) CD59 normally prevents lysis of host cells by inhibiting formation of the membrane attack complex. CD55 inhibits C3 convertase, which leads to reduction in the amount of C3 cleaved. The absence of these GPI-anchored molecules leads to uncontrolled complement-mediated lysis and intermittent hemoglobinuria. The link between AA and PNH remains unclear, but the favored hypothesis is that PNH clones have a survival advantage in the setting of T-cell–mediated destruction of hematopoietic stem cells. (85)

Diagnosis of PNH

All patients diagnosed as having AA and those with unexplained nonimmune hemolytic anemia, hemoglobinuria, or thrombosis with unexplained cytopenias or hemolysis should be evaluated for PNH. The gold standard method of diagnosis uses peripheral blood flow cytometry with fluorescent aerolysin, which binds to the glycan segment of GPI anchor proteins. Paroxysmal nocturnal hemoglobinuria is diagnosed if there is severe deficiency of GPI-anchored proteins in at least 2 cell lines.

Management of PNH

Patients with classical PNH may be treated with eculizumab, a humanized monoclonal antibody directed against C5, thereby blocking the terminal complement pathway. A favorable safety profile has been reported in pediatric patients, (86) but eculizumab does not address AA, which is the presenting symptom for most children. Furthermore, eculizumab is expensive, is needed indefinitely, and is associated with increased risk of *Neisseria* infection.

There are few data on comparative outcomes of immunosuppression versus HSCT for AA associated with PNH, particularly in pediatrics, so the management approach is similar to that of acquired AA. Allogeneic HSCT can also be considered for recurrent severe thromboembolic disease or transfusion-dependent hemolytic anemia despite treatment with eculizumab.

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Summary

- Based on some research evidence as well as consensus, (28)(37)(39)(45)(65)(67)(81) a broad differential diagnosis exists for hypoplastic anemias but can be developed rationally by accounting for concomitant congenital anomalies, mean corpuscular volume, reticulocyte count, and other affected hematopoietic cell lines.
- Based primarily on consensus due to lack of studies examining this question, the severity of anemia or the complexity of the clinical context can help clinicians decide on the need for referral.
- Based on strong research evidence, (16)(17)(20)(23)(26)(27)(32)(51)(66)(84) hypoplastic or aplastic anemias may be acquired or congenital.
- Based on strong research evidence, (5)(44)(54)(64) the inherited bone marrow failure syndromes are also cancer predisposition syndromes. Early presentation of malignancy may suggest an underlying bone marrow failure syndrome.

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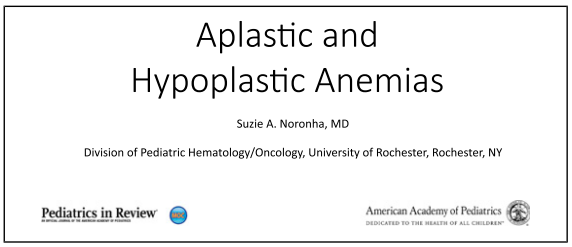

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1. A 2-year-old girl is brought to the clinic because of pallor after a febrile illness 2 weeks ago. Her vital signs and physical examination findings are normal. The complete blood cell (CBC) count reveals a white blood cell (WBC) count of 9000/μL (9.0×10^9/L), absolute neutrophil count (ANC) of 2800/μL, hemoglobin level of 5.5 g/dL (55 g/L), mean corpuscular volume (MCV) of 85 μm^3 (85 fL), platelet count of $420\times10^3/\mu$L (420×10^9/L), reticulocyte count of 8%, and total bilirubin level of 1.0 mg/dL (17.1 μmol/L). Which of the following is the most likely clinical course of this patient's anemia?
   A. Iron deposition in the mitochondria of erythroblasts.
   B. Progression to aplastic anemia.
   C. Progression to leukemia.
   D. Resolution in 4 weeks.
   E. Transfusion dependence.
2. A 9-month-old boy is brought to the clinic with pallor and poor feeding. On examination he is noted to have hypospadias and a grade III/VI systolic ejection murmur. No lymphadenopathy or hepatosplenomegaly are seen on physical examination. The CBC count reveals a WBC count of 9000/μL (9.0×10^9/L), ANC of 2500/μL, hemoglobin level of 6.6 g/dL (66 g/L), MCV of 102 μm^3 (102 fL), platelet count of $155\times10^3/\mu$L (155×10^9/L), and reticulocyte count of 0.5%. Which of the following is the most likely diagnosis at this time in this patient?
   A. Diamond-Blackfan anemia.
   B. Infant leukemia.
   C. Severe aplastic anemia.
   D. Shwachman-Diamond syndrome.
   E. Transient erythroblastopenia of childhood.
3. A 7-year-old girl is brought to the clinic with petechiae and easy bruising. On physical examination she is noted to have short stature and multiple café-au-lait spots. The CBC count reveals a WBC count of 3000/μL (3.0×10^9/L), hemoglobin level of 9.0 g/dL (90 g/L), MCV of 101 μm^3 (101 fL), platelet count of $35\times10^3/\mu$L (35×10^9/L), and reticulocyte count of 0.5%. Which of the following mechanisms is the most likely cause of anemia in this patient?
   A. Autoimmune destruction.
   B. Bone marrow failure.
   C. Chronic inflammation.
   D. Complement-mediated hemolysis.
   E. Viral infection of erythroblasts.
4. A 3-year-old girl is brought to the clinic with fever and oral ulcers. On physical examination, she is noted to have leukoplakia, dysplastic fingernails, and a diffuse reticulated rash. The CBC count reveals a WBC count of 5000/μL (5.0×10^9/L), ANC of 700/μL, hemoglobin level of 9.8 g/dL (98 g/L), MCV of 103 μm^3 (103 fL), and platelet count of $150\times10^3/\mu$L (150×10^9/L). Which of the following best describes the characteristics of this patient's hematologic condition?
   A. Is likely to be associated with thrombosis and hemolysis.
   B. Should be monitored for progression to bone marrow failure and myelodysplasia.
   C. Should be treated with danazol to prevent progression to leukemia.
   D. Should be treated with glucocorticoids to decrease transfusion requirement.
   E. Will likely resolve once the cause of inflammation is treated.

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5. A 16-year-old girl has recently been diagnosed as having acquired severe aplastic anemia. Her mother has questions regarding other family members. Which of the following is the most appropriate counseling for this family?
    A. Distant family members should not be bone marrow donors because they may be silent carriers of disease.
    B. First-degree relatives should be screened annually because they have an increased risk of acute myeloid leukemia.
    C. First-degree relatives should be directed donors of blood products because the patient will likely be transfusion dependent.
    D. Matched sibling donor transplant is indicated only if the patient fails immunosuppressive therapy.
    E. Siblings should have human leukocyte antigen (HLA) testing to be potential bone marrow donors.

Aplastic and Hypoplastic Anemias

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