# Chapter 18
# Anemia

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## Introduction

Anemia is diagnosed when a patient's hemoglobin is less than 12 mg/dL in women and less than 13 mg/dL in men [1]. Alternatively, one can use the hematocrit to diagnose anemia. The typical ratio between RBC, hemoglobin, and hematocrit is 1:3:9. After determining the patient has anemia, we can look at the reticulocytes to calculate the reticulocyte index. The reticulocyte index adjusts the reticulocyte count based on the degree of anemia. A reticulocyte index >2% indicates hyperproliferation of erythrocytes and that the patient's anemia is from acute blood loss or hemolysis. A reticulocyte index <2% indicates that the anemia is due to hypoproliferation of erythrocytes. Additionally, the MCV will help us to further classify the cause of anemia into microcytic (<80 fL), normocytic (80–100 fL), and macrocytic (>100 fL) [2] (Fig. 18.1).

$$\text{Reticulocyte index} = \text{reticulocyte \%} \times \text{patient's hct} / 45 \div \text{maturation factor } [2]$$

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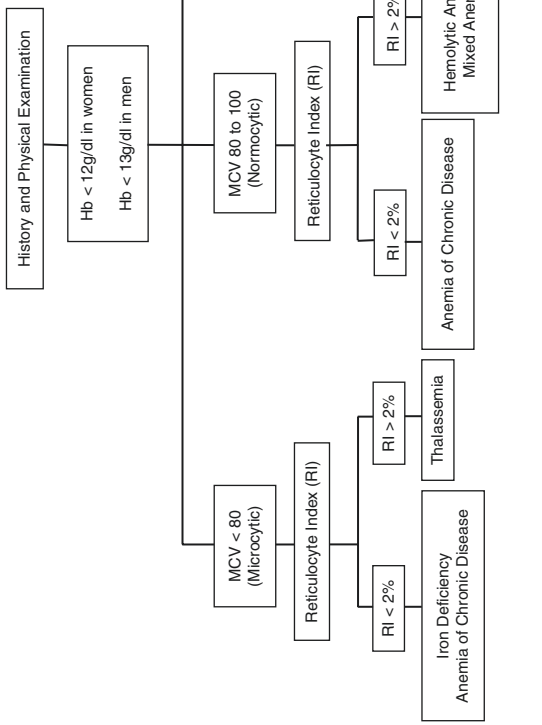


FIGURE 18.1 Anemia Algorithm

| Hematocrit (%) | Maturation factor |
|---|---|
| ≥35 | 1.0 |
| 25–35 | 1.5 |
| 20–25 | 2.0 |
| <20 | 2.5 |

## Key History and Physical Exam

The symptoms of anemia can often be vague and it is important to obtain a thorough history and physical exam to help identify the presence of this condition. When evaluating a patient for anemia, it is essential to identify underlying causes such as a history of bleeding, possibility of infection or malignancy, a history of autoimmune diseases, and adverse medication side effects. Determining the patients cultural and ethnic background along with a detailed family history may cue you into genetic disorders that cause anemia or perhaps a nutritional deficiency. Some common symptoms include fatigue, dizziness, palpitations, lightheadedness, dyspnea, and decreased exercise tolerance. A careful review of systems may also help lead to identifying the underlying cause of the patient's anemia. When assessing the physical exam, it is important to look for the presence of tachycardia or hypotension, as these could be some signs of severe anemia. In addition, the presence of pallor, glossitis, jaundice, and splenomegaly may be some physical exam findings to help with the diagnosis. It is important to note that symptoms may vary depending on the severity and speed at which a patient's anemia progresses. The reason for this is as erythrocytes become fewer in number, there is decreased oxygen delivery to essential tissue and organs [2]. For example, if a patient has an acute blood loss and a hemoglobin of 8 mg/dL, they may have some symptoms such as tachycardia or hypotension, whereas a patient with a chronic anemia from renal disease who also has a hemoglobin of 8 may only have fatigue.

## Laboratory Evaluation

While history and physical exam can lead the provider towards a diagnosis and etiology of anemia, certain laboratory evaluation must be done to complete the evaluation:

- *Complete blood count (CBC)*: The first lab test that should be completed is the CBC. A CBC will reveal the red blood count (RBC), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW). It will also reveal the platelet and white blood cell (WBC) count and differential, which may be high or low in certain types of anemias. It is important to know if the patient has isolated anemia or if there is the presence of other cytopenias.
- *Reticulocyte count*: Reticulocytes are immature red blood cells and can either be increased or decreased depending on the etiology of anemia. This will help to determine if the patient's anemia is due to hypoproliferation or increased destruction of erythrocytes.
- *Peripheral smear*: Obtaining a peripheral smear will show if there are certain characteristics of the red blood cell morphology that may help lead to a diagnosis (e.g., bite cells in G6PD deficiency) or even identify infections such as malaria or babesia.
- *Nutrients*: Iron, total iron binding capacity (TIBC), TIBC %, ferritin, B12, and folic acid.
- *Hemolysis*: Lactate dehydrogenase (LDH) haptoglobin, and indirect bilirubin.
- Sometimes more advanced evaluation needs to be completed and depending on the suspected etiology may include hemoglobin electrophoresis or bone marrow biopsy and analysis.

## Hypoproliferative Anemias

These types of anemias are among the most common anemias and can be further classified based on whether they are microcytic, normocytic, or macrocytic [3].

### *Microcytic Anemia*

Microcytic anemia is characterized by RBCs that are small in size with an MCV <80% [2]. The small size of the RBC is due to a decrease or deficiency in hemoglobin production. The causes of microcytic anemia include iron deficiency, inborn errors of the globin protein production, restriction of iron delivery to the heme group, and defects in the synthesis of the heme group.

#### Differential Diagnosis

- Iron deficiency anemia
- Thalassemia
- Anemia of chronic disease/inflammation
- Sideroblastic anemia

#### Iron Deficiency Anemia

##### *Epidemiology*

Iron deficiency anemia is the most common type of microcytic anemia and most common form of anemia in general, estimated to cause about 50% of all types of anemia. Iron deficiency anemia has a prevalence of 1–2% in the general population. Iron deficiency may be present without anemia in roughly 11% of the adult population [4].

*Pathophysiology*

Iron is an essential component in the creation of hemoglobin, the protein that binds oxygen in the blood. Without this essential component, the body is not able to undergo erythropoiesis [5]. This results in the formation of smaller RBCs.

The most common way to develop iron deficiency is through blood loss. In women, iron deficiency anemia is especially common due to menstrual bleeding. In addition, occult bleeding from a gastrointestinal source is common and can be a sign of malignancy such as colorectal cancer [3]. Since our RBCs contain a large percentage of the body's iron stores, when a patient has blood loss, iron stores are depleted more rapidly [5]. The remaining iron is stored in other iron-containing proteins (e.g., myoglobin, ferritin) or stored as hemosiderin.

Iron deficiency can rarely be caused by poor oral intake of iron-containing foods. The foods highest in iron include red meats, poultry, fish, green leafy vegetables, lentils, beans, and peas.

Aside from poor dietary intake, patient's with malabsorptive disorders may also develop iron deficiency. Iron is primarily absorbed in the duodenum and proximal jejunum [5]. Surgical resection of this area of the gastrointestinal tract or malabsorptive diseases such as Crohn's disease or celiac disease can also lead to insufficient absorption of dietary iron despite diets rich in iron. In certain endemic areas, malabsorptive disorders may also occur due to helminthic infections.

Finally, iron deficiency anemia may be secondary to increased iron requirements. In young children this is especially common since iron is an essential nutrition for growth and development. In the adult population, this is less common but occurs during pregnancy and during lactation.

*Key History*

Some additional features unique to iron deficiency anemia are as follows:

- Pica [3]—this is an abnormal craving of items that are non-food substances such as clay, starch, and chalk. One form

of pica that is common is known as pagophagia, which is a craving and repeated ingestion of ice.
- Restless leg syndrome [3]—This is a condition characterized by significant discomfort of the lower extremities that is relieved with movement. The cause of this is unclear, but may be due to depleted iron stores in the central nervous system.
- Beeturia [3]—this is the reddish discoloration of urine in patients with iron deficiency who ingest beets. This occurs in up to 80% of patients with iron deficiency anemia and is related to increased intestinal absorption of betanin (red pigmentation of beets), which is normally decolorized by ferric ions. This also may occur in up to 10–14% of patients without iron deficiency.
- Brittle integument [3].

*Physical Exam*

Some unique physical exam findings in iron deficiency anemia include evidence of bleeding such as bright red blood or ecchymosis, as well as signs of cheilosis, koilonychia, and glossitis [5].

*Laboratory Evaluation*

The laboratory evaluation is essential for the diagnosis of iron deficiency anemia and includes the following:

- CBC: hemoglobin, RDW, MCV, MCHC.
- Iron studies: ferritin, TIBC, TIBC%, and serum iron.
- Reticulocyte index should show a hypoproliferative anemia.
- Peripheral smear: hypochromic RBCs, microcytosis, and poikilocytosis.
- While no longer routinely done, bone marrow stained with Prussian blue can detect if there is absence of iron stores and help confirm the diagnosis.

*Diagnosis*

The diagnosis of iron deficiency is determined primarily through the laboratory evaluation listed above. The results of the lab evaluation should show a low serum iron concentration, low TIBC%, low ferritin, and high TIBC. In other words, iron concentration needs to be low and the TIBC, or total iron binding capacity, is high (there is a lot of room to bind iron), while the percentage of iron bound (TIBC%) is low (due to low serum iron concentration). The most sensitive test for the diagnosis of iron deficiency anemia is the ferritin, the major protein that binds iron. When ferritin levels drop below 15 μg/L, along with low serum iron concentration, it is diagnostic of iron deficiency [5]. In addition, a low ferritin will help distinguish iron deficiency from anemia of chronic disease. In anemia of chronic disease, ferritin, an acute phase reactant, is elevated and may confound our ability to determine if a patient has iron deficiency. When ferritin is greater than 100 ng/mL, iron deficiency is less likely.

*Treatment*

Understanding the etiology of iron deficiency is essential when choosing the right treatment options. In cases of asymptomatic iron deficiency, patients may be able to be treated by increasing their dietary intake of iron, if the deficiency is minimal. In a majority of cases it is reasonable to prescribe oral iron salts as appropriate iron supplementation. The most common and least expensive option is ferrous sulfate 325 mg. Each tablet contains 65 mg of elemental iron. Since only 10–20% of each iron tablet is absorbed, oral iron supplementation frequently causes gastrointestinal complaints such as nausea, bloating, abdominal cramps, and constipation. The color of the patient's stool may also turn to a black or grey color and must be distinguished from melena.

When providing oral iron supplementation, it is important to identify any antacids or fiber supplementations taken by the patient, as these medications may further impair iron absorption. Furthermore, taking oral iron supplements 2–3

times per day upregulates hepcidin, a protein that decreases iron absorption. Therefore, it is recommended to take oral iron supplementation only once per day, and if a patient has significant gastrointestinal side effects, this can be further decreased to every other day. The response to appropriate iron supplementation may be seen as quickly as after 1 week. Oral iron replacement should show normalization of iron levels and improvement in the anemia after 3–6 months of treatment [3].

When patients do not respond to oral iron supplementation, one must consider that the patient has either a malabsorptive disorder or that oral supplementation is not able to keep up with the rate of iron loss. In these patients it may be necessary to give them intravenous iron infusions. Intravenous iron infusions may improve reliability of iron absorption but does not improve the anemia more quickly than in patients taking oral supplementations. In addition, intravenous iron infusions carry the additional risk of anaphylaxis (particularly with iron dextran) [3, 5].

Finally, in patients with symptomatic anemia, acute anemia, or very low hemoglobin (<7 g/dL), it may be necessary to transfuse pRBCs.

## *Normocytic Anemia*

This anemia is defined by those with an MCV between 80 and 100 [2]. This is typically more challenging to evaluate and there are multiple causes. Many anemias in their early stage present as normocytic anemia but then may later on show microcytosis or macrocytosis.

### Differential Diagnosis [6]

- Early-stage nutritional deficiencies
- Hemolysis
- Anemia of chronic disease/inflammation
- Congestive heart failure

- Hypothyroidism
- Liver disease
- Alcohol use disorder
- Monoclonal gammopathies
- Early blood loss
- Partially treated anemia

## Anemia of Chronic Disease/Inflammation (AOCD)

### *Epidemiology*

AOCD is considered the second most common anemia behind iron deficiency anemia. It is estimated to effect anywhere between 33 and 60% of individuals with some source of chronic systemic inflammation such as rheumatoid arthritis [7]. Common causes of AOCD include infections, malignancy, and autoimmune disorders, advanced CKD, or end-stage kidney disease (ESKD). More recently, ACOD is considered as a cause for anemia in patients with severe trauma and diabetes.

Common infections associated with AOCD are HIV, osteomyelitis, tuberculosis, and endocarditis [7] due to their chronic or subacute nature.

### *Pathophysiology*

AOCD is caused by a variety of disease states associated with systemic inflammation. As mentioned in the previous section, iron is an essential nutrient for many biological processes for humans, but it is also an essential nutrient for microbes. Therefore, it has been proposed that AOCD is an evolutionary mechanism to prevent iron from being accessible during times of infection or other types of chronic inflammation. This occurs via increased production of hepcidin in response to the inflammatory cytokines, including interleukin-6. As hepcidin increases, gut absorption of iron decreases. As hepcidin increases, more iron is kept stored in the reticuloendothelial system. This functional depletion in iron leads to decreased heme and then decreased erythrocyte production.

There is also a simultaneous decrease in erythropoietin (EPO), the hormone that stimulates erythrocyte production, and this process is also mediated by cytokines. Over time, with a decrease in EPO and a functional depletion of iron, patients often initially present with a normocytic anemia. However, as time progresses, if the underlying inflammatory cause is not corrected, it can lead to microcytic anemia [7].

*Key History and Physical Exam*

History taking and physical exam findings should focus on identifying the underlying cause for AOCD, whether that is an infection, malignancy, or rheumatologic condition.

*Laboratory Evaluation*

- CBC: normocytic or microcytic anemia.
- Iron, TIBC, TIBC%, ferritin.
- Reticulocyte count.
- Consider workup for malignancy, infection, or rheumatologic diseases based on your patient's history and physical exam findings: HIV, QuantiFERON, ESR, CRP, ANA, ANCA, colonoscopy, etc.

*Diagnosis*

The diagnosis of AOCD is based on the identification of a source of chronic inflammation as well as specific lab tests. The CBC may show normocytic anemia in early stages of the disease, but may progress to microcytic anemia later. Iron studies are particularly helpful in determining the diagnosis. In particular, these patients have high ferritin levels in conjunction with low TIBC and high TIBC%.

It is important to try and identify if, in addition to AOCD, there is iron deficiency. This may be suspected in patients with ferritin lower than expected and with more severe microcytosis. Laboratory interpretation may be difficult, but if the ratio of the TIBC%/log ferritin is less than 1, then this is suggestive of AOCD. If the ratio is greater than 2, it suggests combined iron deficiency anemia and AOCD [8].

*Treatment*

Treatment for AOCD is focused on treating the underlying cause of the inflammation. Blood transfusions are not typically recommended unless there is evidence of severe anemia. In patients with ESKD who do not make EPO, it is reasonable to consider EPO injections, although it is important to monitor for signs of hypertension and thrombosis if EPO analogs are used.

## *Macrocytic Anemia*

These are the anemias defined as those with an MCV >100 [2]. This type of anemia is caused by abnormalities of RBC production in the bone marrow, or with altered RBC membrane compositions. The most common causes of macrocytic anemia are vitamin B12 and folic acid deficiency [9]. Other etiologies include myelodysplastic syndrome and alcohol-induced anemia. In patients with a reticulocytosis, a macrocytic anemia may also be identified. Reticulocytes are immature erythrocytes and are typically larger than the mature erythrocyte. Therefore, with reticulocytosis, the large number of immature erythrocytes inflates the MCV.

### Differential Diagnosis [2]

- Vitamin B12 deficiency
- Folic acid deficiency
- Drug side effect
- Reticulocytosis
- Aplastic anemia
- Myelodysplastic syndrome
- Sideroblastic anemia
- Liver disease
- Alcohol use disorder
- Multiple myeloma
- Hypothyroidism

## Megaloblastic Anemia

This is a macrocytic anemia that results from impaired DNA synthesis. The most common causes include the nutritional deficiencies of folic acid (B9) or cobalamin (B12) [9]. Additionally, there are many medications that can cause megaloblastic anemia through impaired absorption of either B12 or folic acid.

### Vitamin B12 Deficiency

#### *Epidemiology*

There many causes for vitamin B12 deficiency. Since we cannot synthesize it on our own, it is important that we obtain it in our diet. This essential nutrient is found in animal products such as meats, seafood, dairy products, and eggs. Many foods such as cereals are also fortified with vitamin B12. It has therefore become very uncommon for people to develop vitamin B12 deficiency due to lack of dietary intake, especially in developed countries. The people at most risk include those who adhere to a strict vegan or vegetarian diet. The average person is able to store 2–3 mg of B12, and this is generally considered enough to maintain an individual for 3–4 years [9].

While developing vitamin B12 deficiency solely due to lack of dietary intake is rare, it is more common to develop the deficiency due to a malabsorptive process. Malabsorption can occur anywhere between the stomach and the terminal ileum, the location where it is ultimately absorbed before storing it in the liver. Common causes of malabsorption due to pathology in the stomach include pernicious anemia, atrophic gastritis, chronic H2 blockers or proton pump inhibitor use, and *Helicobacter pylori* infection. In the duodenum, pathologic causes for malabsorbtion include small intenstinal bacterial overgrowth and pancreatic insufficency. In the terminal ileum, malabsorption may be caused by ileal resection, ileitis such as in Chron's disease, chronic metformin use, and fish tapeworm infection from *Diphyllobothrium latum*.

*Pathophysiology*

As mentioned above, vitamin B12 deficiency may arise due to many causes but is mainly due to either poor nutritional intake, or more commonly due to a malabsorptive process. After we eat food and it passes through the stomach into the small intestine, B12 is bound by intrinsic factor, a protein that actively helps with absorption of B12 in the ileum. Alterations to gastric acid secretion from medications such as proton pump inhibitors and H2 blockers prevent B12 bound in food from being bound by intrinsic factor. In the terminal ileum, inflammation from Crohn's disease or infection from *D. latum* leads to poor absorption locally in the terminal ileum and can also lead to deficiency.

*History and Physical Exam*

Vitamin B12 deficiency may manifest with anemia, pancytopenia, jaundice, or neuropsychiatric symptoms, and therefore, a complete history and physical exam are essential when diagnosing and determining the severity of illness associated with B12 deficiency. Vitamin B12 plays a role in myelin basic protein, which is important to maintain the myelin that insulates peripheral nerves, and thus, deficiency can lead to neuropathy. The classic neurologic symptoms associated with vitamin B12 deficiency are known as subacute combined degeneration and occur due to demyelination of the dorsal and lateral column of the spinal cord. As the disease progresses, patients may experience weakness, ataxia, spasticity, and ultimately paraplegia. Other symptoms include depressed mood, irritability, dementia, cognitive slowing, visual disturbances from optic atrophy, abnormal deep tendon reflexes, and glossitis. The neuropsychiatric manifestations of vitamin B12 deficiency may occur without the presence of anemia, and the absence of anemia should not rule out this nutritional deficiency as a cause for any of the above neuropsychiatric symptoms [9].

*Laboratory Evaluation*

- CBC
- Peripheral smear—presence of macrocytes and hypersegmented neutrophils

- Intrinsic factor antibodies, parietal cell antibodies
- B12 and folic acid level
- Methylmalonic acid and homocysteine level
- Schilling test—no longer routinely done [3]

*Diagnosis*

In most cases, B12 levels should be checked when the CBC reveals a macrocytic anemia. For the majority of patients, we do not check folic acid levels, as a deficiency is very uncommon in patients with a routine diet in resource-rich areas and is only of benefit in patients with poor oral intake or frequent alcohol use [3]. The diagnosis of vitamin B12 deficiency can sometimes be obscure. The normal value of vitamin B12 is greater than 300 pg/mL, and when the level is higher than this, it is considered to be 90% sensitive to rule out B12 deficiency. When patients have values less than 200 pg/mL, that is sufficient to diagnose a B12 deficiency. The evaluation can become obscure when values range between 200 and 300 pg/mL. When this occurs, it is appropriate to assess for serum methylmalonic acid (MMA) and serum homocysteine levels, proteins that are intermediaries in the metabolism of vitamin B12. In scenarios where both MMA and homocysteine levels are normal, vitamin B12 deficiency is ruled out. Elevation in both the MMA and homocysteine levels confirms a diagnosis of vitamin B12 deficiency [10] (this would not rule out a simultaneous folic acid deficiency). If MMA is normal and homocysteine levels are elevated, this is more consistent with a folic acid deficiency.

When choosing a treatment option for B12 deficiency, it is important to identify the cause and severity of the deficiency and the anemia. The scenarios where correction is urgent include the patients with symptomatic anemia or Hb <8 g/dL, neuropsychiatric or neurologic symptoms, or in the presence of a malabsorptive process such as pernicious anemia. In these scenarios it is recommended to initiate treatment promptly with intramuscular cyanocobalamin. Treatment should consist of intramuscular injections 1–2 times per week for 2 weeks, followed by weekly injections until clinical improvement is seen, after which repeat CBC and reevaluation of symptoms

should be completed. If results show resolution of macrocytosis, anemia, and/or improvement in the neuropsychiatric or neurologic symptoms, the patient should continue with oral cyanocobalamin 1000 μg daily or monthly intramuscular injections indefinitely. In less severe cases, patients can be started on oral cyanocobalamin 1000 μg daily [10].

### Folic Acid Deficiency

Folic acid deficiency is less common than vitamin B12 deficiency. Folic acid is an essential part of DNA synthesis, and so deficiency leads to megaloblastic anemia. It presents similarly to vitamin B12 deficiency, although the neuropsychiatric symptoms are less common, and it does not cause subacute combined degeneration. The most common cause for folate deficiency is due to medication side effects or alcohol use disorder. In resource-rich countries, many grains are enriched with folic acid, and therefore, it is very difficult to develop a deficiency due to poor intake [2]. In contrast with B12 deficiency, folic acid deficiency can develop quickly after about 3–4 months if a person is cut off from all sources of folate. Deficiency may also arise in the presence of rapid turnover of erythrocytes, such as in hemolytic anemia. Common medications that cause folic acid deficiency include methotrexate, trimethoprim, and phenytoin. Patients taking these medications should also be taking folic acid supplementation [10].

Diagnosis is made on lab testing demonstrating low folic acid levels. Peripheral smear will show similar findings to vitamin B12 deficiency, with macrocytosis and hypersegmented neutrophils. When the diagnosis is uncertain, MMA and homocysteine levels may be evaluated, and lab testing will show normal MMA and elevated homocysteine levels.

Treatment of folic acid deficiency, regardless of cause, is with supplementation of 1 mg of folic acid daily. Patients with alcohol use disorder should be counseled on strategies to decrease or cease their alcohol consumption [10]. In cases of severe deficiency due to medication side effect, the patient should have a discussion with their provider about whether or not to continue the medication or to pursue alternative therapies.

## Hyperproliferative Anemia

This group of anemias is typically more complicated to treat. The causes include sickle cell diseases, hemolytic anemias, and rapid blood loss. In cases other than rapid blood loss, patients should be evaluated by a hematologist.

### *Hemolytic Anemia*

In healthy individuals, an erythrocyte lives for about 90 days, during which about 1% of erythrocytes are destroyed per day. Hemolytic anemia is the process where there is premature destruction of erythrocytes. When erythrocytes are destroyed, the body responds with a reticulocytosis, increasing production of immature RBCs to help replace those that were destroyed. As the erythrocytes are destroyed, lactate dehydrogenase (LDH) is released. In addition, haptoglobin, a protein that binds free hemoglobin, decreases, as it is consumed. The remaining free hemoglobin is also metabolized into unconjugated bilirubin. Thus, the hallmarks of hemolytic anemia are elevated serum LDH and unconjugated bilirubin and decreased or undetectable serum haptoglobin.

Hemolytic anemias are classified as being either intrinsic or extrinsic [11].

#### Intrinsic Hemolytic Anemia

These are the hemolytic anemias that are caused by some acquired, inherited, or congenital hemoglobinopathy, RBC membrane defect, or enzyme deficiency [11]. Causes of intrinsic hemolytic anemia include the following:

- Hemoglobinopathy
  - Sickle cell anemia
  - Thalassemia
- RBC membrane defect
  - Hereditary spherocytosis
  - Hereditary elliptocytosis

- Enzyme deficiency
  - G6PD—glucose-6-phosphate deficiency

Sickle Cell Anemia

*Epidemiology*

Sickle cell anemia is the most common hemoglobinopathy. It is estimated to affect approximately 100,000 Americans each year. It is more prevalent in the African American community and estimated to occur in 1 out of 365 births. Approximately 1 in 13 births in the African American community has sickle cell trait. Finally, it is estimated to occur in 1 out of every 16,300 births in the Hispanic-American community [12]. It is theorized that sickle cell anemia is more common in areas with malaria, as it provided a selective benefit of protecting individuals from becoming infected with the parasite that causes malaria.

*Pathophysiology*

Sickle cell anemia (SCA) is an autosomal recessive disease, and so in order to have the disease, a person must inherit one copy of the defective gene from each parent [13]. In SCA there is a mutation in the beta-globin gene where the hydrophilic nucleotide glutamic acid is replaced with the hydrophobic nucleotide valine at the sixth position of the beta-globin gene, resulting in hemoglobin S (HbS). When the HbS is exposed to deoxygenated environments, polymerization of HbS occurs leading to erythrocyte rigidity and distortion of the erythrocyte membrane, creating the characteristic sickle shape. The rate at which this occurs depends on the concentration of HbS and hemoglobin F (HbF). This sickling leads to premature intravascular hemolysis, which causes vascular injury and endothelial dysfunction. The hemolytic process also depletes available nitric oxide, leading to vasoconstriction. This process also leads to release of inflammatory mediators and overexpression of adhesion molecules, leading to vaso-occlusion [14].

The main pathologies associated with sickle cell anemia are vaso-occlusive crisis, hemolysis, and certain infections [14]:

- *Hemolysis*: The baseline hemoglobin levels are often lower than the average adult due to the shorter life span of their erythrocytes. The rate of hemolysis may increase during times of increased stress such as during an infection, and the patient with SCA will be more prone to vaso-occlusive crisis. The most severe form of this is known as a hyperhemolytic crisis.
- *Vaso-occlusive crisis*: These episodes are often marked by severe pain and can lead to infarction at different locations in the body. The most severe complications of vaso-occlusive crisis include acute chest syndrome, acute papillary necrosis, both ischemic and hemorrhagic stroke, spinal cord infarction, cholecystitis, acute coronary syndrome, and pulmonary embolism. Patients with SCA often develop chronic lifelong pain that may be difficult to treat. Many of these patients may require opioid analgesia and it is important to carefully monitor these patients for signs of opioid addiction and withdrawal. Other complications include osteoporosis, avascular necrosis, pulmonary hypertension, and priapism.
- *Infection*: Patients with sickle cell anemia are at higher risk for osteomyelitis. Patients will develop splenic infarcts and are considered to have functional asplenia, putting them at higher risk for infections from encapsulated bacteria such as *Streptococcus pneumoniae, Neisseria meningitis,* and *Haemophilus influenzae* type B and gram-negative organisms such as *Salmonella* sp., *Enterobacter cloacae, Enterococcus faecium, and Pseudomonas aeruginosa.*

*Key History and Physical Exam*

For patients with SCA it is important to determine the severity of their illness. Each patient may present differently and with a different level of disease burden. History should focus on determining evidence of any complications due to SCA,

and given the widespread effects across all organ systems, it is important that a thorough review of systems is performed for each patient. It is important to determine whether a patient's pain is adequately controlled or not. Understanding what medications have been used and how often blood transfusions have been required may guide your treatment plan.

A full physical exam should be performed to evaluate for any neurologic deficits, conjunctival pallor, tachycardia, jaundice, gait abnormalities, and dactylitis.

*Laboratory Evaluation*

- CBC
- Hemoglobin electrophoresis
- Reticulocyte index
- LDH, haptoglobin, unconjugated bilirubin
- Peripheral blood smear

*Diagnosis*

Diagnosis of sickle cell anemia is based on RBC morphology on a peripheral blood smear along with clinical criteria of hemolysis with a history of ischemic pain. The diagnosis is confirmed with hemoglobin electrophoresis, which will show elevated levels of HbS.

*Treatment*

The overall management of sickle cell anemia varies depending on the severity of the illness and each patient should be managed with the help of a hematologist. For the severe complications such as acute chest syndrome, stroke, transient ischemic attack (TIA), spinal infarcts, hyperhemolytic crisis, and severe infections, or in cases where pain cannot be controlled at home, patients should be referred to their local hospital for further management.

For patients with less severe symptoms, outpatient management is recommended. All patients should be started on folic acid supplementation, as the sickled erythrocytes have a very short life span of 12–16 days and the folic acid is needed

for erythrocytosis and the rapid turnover of RBCs. Pain management should focus on use of NSAIDs for mild to moderate pain control [15].

Studies have found that patients with higher levels of fetal hemoglobin (HbF) have milder courses of disease and decreased hospitalization and may have improved survival [13]. In normal physiology, the fetus has high concentrations of HbF but after birth this gene is turned off and the gene for HbA, or in the case of patients with SCA, HbS, is turned on. The medication hydroxyurea, which is used as a chemotherapy agent by halting the cell cycle between G1 and S phases, also has the advantage of increasing HbF production and is recommended for all patients with sickle cell anemia who have had repeated complications from vaso-occlusive crisis or have had painful crisis more than three times per year [13].

In addition to hydroxyurea, it may sometimes be necessary to give a blood transfusion, particularly if a patient is having any of the more severe complications of a vaso-occlusive crisis.

All patients with sickle cell anemia should receive appropriate pneumococcal and HiB vaccinations to protect against these encapsulated organisms if they have not already received them earlier in life.

## Thalassemia

### *Epidemiology*

Thalassemia is another common hemoglobinopathy. It is estimated that roughly 20% of the world population carries a gene for alpha thalassemia, and 5.2% of the population has a significant form of the disease, either beta-thalassemia or alpha-thalassemia trait. Thalassemia is most prevalent in African and Mediterranean countries, the Middle East, and Southeast Asia. The most common form is the heterozygous form of disease. The homozygous alpha-thalassemia causes intrauterine demise and homozygous beta-thalassemia is associated with a severe anemia diagnosed at early age [16].

*Pathophysiology*

Thalassemia is a group of heterogenous hemoglobinopathies that results in decreased production of either the alpha or beta globin gene on the hemoglobin molecule. Hemoglobin is a tetramer that is composed of two alpha and two beta globin molecules. The beta globin molecule has two genes and the alpha globin gene has four genes. The spectrum of the different thalassemia disorders is dependent on how many genes are lost due to mutation. These mutations leads to a mismatch in the alpha/beta globin ratio, cellular damage, and early hemolysis [2].

*Key History and Physical Exam*

When evaluating for possible thalassemia disorders, it is important to obtain a complete social and family history. The patient's country of origin, or whether their parents have any hemoglobinopathy, can help you determine whether it is likely that a patient could have a thalassemia disorder. Since the laboratory evaluation will show microcytic anemia, it is important that your history and physical exam focus on ruling out causes for iron deficiency anemia.

*Laboratory Evaluation*

- CBC
- Iron studies
- Hemoglobin electrophoresis
- DNA sequencing
- Peripheral blood smear

*Diagnosis and Management*

The evaluation for thalassemia often begins when pursuing causes of microcytic anemia. Therefore, just like with iron deficiency anemia, the evaluation should begin with checking the CBC and iron studies. Patients with thalassemia will often have a mild anemia and a very low MCV. In contrast to iron deficiency anemia, the iron studies in these patients will not

be consistent with iron deficiency anemia. When evaluating the CBC, clinicians can use the Mentzer index to help determine the likelihood of thalassemia over iron deficiency. The Mentzer index is calculated by taking the MCV and dividing by the RBC count on the CBC. When this result is less than 13, it is suggestive of thalassemia.

After ruling out iron deficiency, a hemoglobin electrophoresis can be performed. This is helpful only in the diagnosis of beta-thalassemia disorders. Hemoglobin electrophoresis will show an increased production of HbA2 or HbF, and this is diagnostic of beta-thalassemia [15]. The diagnosis of alpha-thalassemia requires genetic testing to directly sequence the hemoglobin A gene for mutations. Additionally, the peripheral blood smear may show target cells, although this is not specific just to thalassemia [15].

Thalassemia disorders can be classified as follows [2]:

- Beta-thalassemia major: This occurs when both beta globin genes are missing. Patients typically are transfusion dependent. This is usually diagnosed early in childhood.
- Beta-thalassemia minor: This occurs when one of the beta globin genes is missing. Patients are often diagnosed when evaluating for causes of hypochromic and microcytic anemias. The anemia is mild and hemoglobin electrophoresis shows increased production of HbA2 or HbF.
- Alpha-thalassemia minima: This occurs when one out of four alpha globin genes is missing. These patients are asymptomatic. Genetic counseling is recommended if this disorder is identified.
- Alpha-thalassemia minor: This occurs when two out of four alpha globin genes are missing. Patients present similarly to beta-thalassemia minor, with mild and generally asymptomatic anemia. Diagnosis must be made through genetic sequencing of the alpha globin gene.
- Alpha-thalassemia intermedia: This occurs when three out of four alpha globin genes are missing. This typically presents during gestation and later with neonatal jaundice and a chronic hemolytic anemia. As patients reach the second

and third decade of life, patients may become transfusion dependent.
- Alpha-thalassemia major (hemoglobin Bart's disease): This occurs when four out of four alpha globin genes are missing. This causes hydrops fetalis and is often fatal within hours of birth.

For patients who are diagnosed with thalassemia, it is important for them to receive folic acid supplementation, since the turnover of erythrocytes is increased. Iron supplementation should be avoided in these patients [2, 15].

## Hereditary Spherocytosis (HS)

### *Epidemiology*

Hereditary spherocytosis is an autosomal dominant disorder and is the most common type of hemolytic anemia caused by a red cell membrane defect [17]. It is estimated to affect 1 in 5000 people and is more common in people of Northern European descent [11].

### *Pathophysiology*

Hereditary spherocytosis is caused by a defect in genes responsible for the production of the genes ankyrin and spectrin, proteins that are responsible for the structural integrity of the cell membrane. This leads to increased fragility of the cell membrane and increased destruction of erythrocytes as they pass through the spleen.

### *Key History and Physical Exam*

The most important part of the history is the family history. This is an autosomal dominant disease, and so in order for a patient to have hereditary spherocytosis, at least one of the parents must have it as well, although sporadic mutation is still possible. Due to the fragility of the red cell membrane and increased erythrocyte destruction, physical exam may reveal splenomegaly, jaundice, or other common symptoms of anemia.

*Laboratory Evaluation*

- CBC
- Reticulocyte count
- Unconjugated bilirubin
- Lactate dehydrogenase
- Peripheral blood smear
- Flow cytometry with eosin-5-maleimide binding test
- Osmotic fragility test—although flow cytometry is now preferred

*Diagnosis and Management*

Evaluation usually is started when the peripheral blood smear shows spherocytosis and evidence of hemolytic anemia. The CBC will show varying degrees of anemia and is notable for an elevated MCHC >34 and is one of the only disease states where this occurs [11]. The degree of anemia in each individual is variable depending on which genes are affected in this disease and the penetrance. If the disease is suspected, flow cytometry is the preferred test as this test is estimated to have 95% sensitivity and specificity. Classically, the osmotic fragility test was used, but given the success of flow cytometry has become less common for diagnosis [2].

The disease can be classified as based on severity as follows [18]:

- HS trait: normal hemoglobin, bilirubin, reticulocyte count.
- Mild HS is estimated to affect 20–30% of individuals with HS. These patients often have a hemoglobin between 11 and 15 g/dL, bilirubin between 1 and 2 mg/dL, and reticulocytosis of 3–6%.
- Moderate HS is the most common presentation of disease and is estimated to affect 60–75% of individuals with HS. Hemoglobin ranges between 8 and 12 g/dL and reticulocytosis >6% and bilirubin greater than 2 mg/dL.
- Severe HS is present in about 5% of cases and is associated with hemoglobin less than 8 g/dL, reticulocytosis >10%, and bilirubin greater than 3 mg/dL.

It is important to note that patients with moderate or mild HS may often present asymptomatically and with a compensated hemolytic anemia, but medications or infections that cause bone marrow suppression may lead to decompensation and severe anemia.

In scenarios where acute and severe hemolytic anemia occur, it may be necessary to consider splenectomy. These patients should receive adequate vaccinations to protect against encapsulated organisms including vaccination for *Neisseria meningitides, Haemophilus influenzae,* and *Streptococcus pneumoniae* if they had not already received them.

When managing these patients, it is important to note that just like with other types of chronic hemolytic anemias, they should be given folic acid supplementation due to the high turnover of their erythrocytes and increased folic acid requirements.

## Glucose-6-Phosphate Dehydrogenase Deficiency (G6PD Deficiency)

### *Epidemiology*

G6PD deficiency is an X-linked disorder and is the most common enzyme deficiency in human erythrocytes. It is estimated to affect upwards of 400 million people worldwide. It is more common in men than in women and in people of African, Mediterranean, and Asian descent [19].

### *Pathophysiology*

G6PD is an enzyme involved in the pentose phosphate shunt, a biochemical process that converts NADP to its reduced form of NADPH. The role of NADPH in the erythrocyte is to prevent damage to the cell from oxidative stress, by acting as a substrate to glutathione reductase, which converts hydrogen peroxide into water. In G6PD deficiency, the inability to prevent oxidative stress, in the setting of exposure to certain oxidants, initially leads to depletion of glutathione reductase,

which then leads to oxidation of the sulfhydryl group on the hemoglobin molecule, causing hemoglobin to become an insoluble mass that attaches to the red cell membrane, forming the classical Heinz bodies seen on peripheral smear [11]. Since the hemoglobin molecule is no longer free flowing in the cell cytoplasm, erythrocyte loses its shape and becomes the classical bite cells seen on peripheral smear.

Exposures that can lead to oxidative stress include certain medications, foods, or chemical exposures. Common medications that are not safe to use in G6PD deficiency include [11]:

- Dapsone
- Fluoroquinolones
- Nitrofurantoin
- Primaquine
- Rasburicase
- Sulfonylureas
- Sulfa drugs

Common foods and chemicals not considered safe include:

- Fava beans
- Henna compounds
- Naphthalene (mothballs, lavatory deodorants)

*History Physical Exam*

A patient who is being evaluated for G6PD deficiency should focus on possible exposures or triggers, family history of G6PD deficiency, and a social history determining the patient's ethnic background. Physical exam may reveal scleral icterus and jaundice, and during an acute hemolytic crisis, anemia may also be severe and signs of acute anemia may be present.

*Laboratory Evaluation*

- CBC
- Unconjugated bilirubin, LDH, haptoglobin
- Peripheral blood smear
- G6PD activity assay

*Diagnosis and Management*

It is important to identify when it is necessary to screen for GDPD deficiency. Evaluation is typically performed either after a patient had an unexplained hemolytic anemia after exposure to an oxidant, or prior to initiating medications that could cause oxidative injury in patients from the appropriate ethnic background. Laboratory evaluation is the key to diagnosis and begins with a CBC and lab tests used to evaluate for hemolysis. Peripheral smear may reveal bite cells and Heinz bodies. In order to confirm a diagnosis, a G6PD activity assay must be done, but it is important to note that during a hemolytic event, since those cells with low G6PD will hemolyze, the test will only show the activity of RBCs with normal G6PD activity, and thus, there is a high false-negative rate. Therefore, it is recommended to wait 3 months after the hemolytic event to adequately assess G6PD activity. In cases where it is important to quickly know whether a patient has G6PD deficiency, there is a quantitative assay that can be used [20].

Management of G6PD deficiency focuses on avoiding known oxidative triggers. If a hemolytic event occurs, the oxidant that triggered the event should be removed as quickly as possible. Patients should be given IV fluids for hydration, and depending on the severity of the anemia, they may need a blood transfusion.

## Extrinsic Hemolytic Anemia

These types of hemolytic anemia are caused by factors external to the RBC that causes it to be damaged or hemolyze. The causes of extrinsic hemolytic anemia include:

- Autoimmune hemolytic anemia
- Hypersplenism
- Liver disease
- Thrombotic microangiopathies
- Infections: malaria, babesiosis, *Clostridium perfringens*
- Mechanical damage, e.g., through mechanical heart valves

## Autoimmune Hemolytic Anemia

This group of anemias is due to pathologic autoantibodies that bind to and destroy erythrocytes. Depending on the thermal reactivity of the autoantibodies, the disease can be classified as either warm or cold agglutinin disease [11].

### *Warm Autoimmune Hemolytic Anemia (WAHA)*

#### *Epidemiology*

WAHA is the most common type of autoimmune hemolytic anemia. It is estimated to affect approximately 1–3 per 100,000 people every year. It can occur at any age group and is more common in women than in men [21].

#### *Pathophysiology*

WAHA occurs when IgG autoantibodies bind to the red cell membrane and cause hemolysis and consumption by the spleen. In the majority of cases, the inciting trigger is unknown. Whereas most cases thus remain idiopathic in nature, there are some known causes for WAHA. These include [2, 11]:

- Autoimmune diseases: systemic lupus erythematosus and Sjogren's disease
- Malignancies such as CLL
- HIV

#### *History and Physical Exam*

History should focus on identifying any known triggers for hemolytic anemia either from autoimmune disease or from recent infection. Determining if there is the possibility of a previously undiagnosed autoimmune disease is important.

Physical exam should look for signs of autoimmune diseases such as polyarthritis and synovitis, rashes, and lymphadenopathy. An assessment for hepatomegaly or splenomegaly can also help identify a cause.

*Laboratory Evaluation*

- CBC
- Unconjugated bilirubin, haptoglobin, LDH
- Peripheral Smear may show spherocytosis
- Direct Coombs test
- HIV test
- C3, C4, ANA, SSA ab, SSB ab, DSdna ab, and other appropriate antibodies if rheumatologic cause is being suspected or evaluated

*Diagnosis and Management*

Patients who show evidence of hemolysis, as part of their workup, should undergo testing for WAHA. Diagnosis of WAHA is confirmed with a positive direct Coombs test, a test that is able to detect the presence of antibodies attached to the RBC cell membrane. The direct Coombs test is considered to be 95% sensitive [2].

Treatment of WAHA should be performed with the aid of a hematologist. It focuses on immunosuppression with glucocorticoids or biologic agents such as rituximab. It is important to identify the need for blood transfusion, but during an acute hemolytic event, the transfusion may be consumed as well due to circulating IgG autoantibodies. In refractory cases, a splenectomy may be necessary. This would not eradicate the autoantibodies, but rather the location where the tagged erythrocytes are typically destroyed. If a patient required a splenectomy, appropriate vaccination against encapsulated organisms should be administered to the patient [2, 11].

*Cold Autoimmune Hemolytic Anemia*

*Epidemiology*

Cold autoimmune hemolytic anemia, also referred to as cold agglutinin disease (CAD), is less common than WAHA and is seen more often in elderly, compared to young patients [22].

*Pathophysiology*

CAD causes hemolytic anemia and is caused primarily by IgM autoantibodies which fixes complement and leads to complement-mediated intravascular hemolysis. These cases of anemia are typically milder than in WAHA [22].

Just like with WAHA, the majority of cases are idiopathic but some secondary causes are known. These include:

- Lymphoma
- Infections such as *Mycoplasma pneumoniae*, EBV, and CMV
- Waldenström's macroglobulinemia

*History and Physical Exam*

In patients with CAD, the most common presenting symptom is acrocyanosis, which is a dark grey or purplish discoloration of skin in the acral areas, namely, the fingertips, toes, nose, and ears [22]. History should focus on identifying symptoms of anemia and any possible secondary cause. Physical exam may identify acrocyanosis if something cold, such as ice, is applied to acral areas and evidence suggesting certain secondary causes such as lymphadenopathy in a patient with undiagnosed lymphoma.

*Laboratory Assessment*

- CBC
- Unconjugated bilirubin, haptoglobin, LDH
- Peripheral smear may show spherocytosis and also clumping of RBCs
- Direct Coombs test
- C3 DAT
- C3, C4
- Cold agglutinin titer
- Heterophile antibodies
- HIV test

*Diagnosis and Management*

Clinicians should be suspicious of CAD in elderly patients with unexplained chronic anemia or with complaints of acrocyanosis. When labs show evidence of hemolysis, a Coombs test can be performed. If the direct Coombs test is positive, then a more specific C3 direct antibody test should be performed. If this is also positive, a diagnosis of cold agglutinin disease is more likely, and a cold agglutinin titer may be assessed [22].

Management of cold agglutinin disease like with WAHA should be done with the aid of a hematologist. Unlike with WAHA, cold agglutinin disease is more difficult to treat. While patients may be able to reduce symptoms by avoiding cold weather, this is often difficult to achieve all the time. Therefore, glucocorticoids are often used next, but are effective in less than 15% of cases. Most patients will require treatment with biologic agents such as rituximab. Splenectomy has not been shown to be as effective as treating cold agglutinin disease, compared with WAHA, and is typically not performed unless it is determined that the cold agglutinin disease is caused by IgG (~3.5% of patients) [22].

## Conclusion

Anemia is a condition that can manifest from many different causes. Being able to identify the symptoms and diagnosing the exact cause can be difficult, but by classifying the anemia appropriately, a clinician can help to narrow down the differential diagnosis and pursue the correct management.

**Clinical Pearls**

- Iron deficiency anemia in patients older than 50 years may indicate an underlying gastrointestinal malignancy such as colorectal cancer, and all patients should be evaluated through colonoscopy.
- Patients who do not respond to oral iron should be evaluated for malabsorptive disorders such as celiac disease.

- The Mentzer index may help hint towards an undiagnosed thalassemia when the MCV is very low.
- Patients diagnosed with pernicious anemia should be referred to a gastroenterologist for further evaluation and endoscopy given the higher risk of gastric cancer in these patients.
- All patients with sickle cell anemia or who have had a splenectomy should receive appropriate pneumococcal vaccinations to protect against encapsulated organisms, as these patients are particularly susceptible to severe infection.
- All patients with unexplained normocytic anemia should undergo evaluation for hemolytic anemia.

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