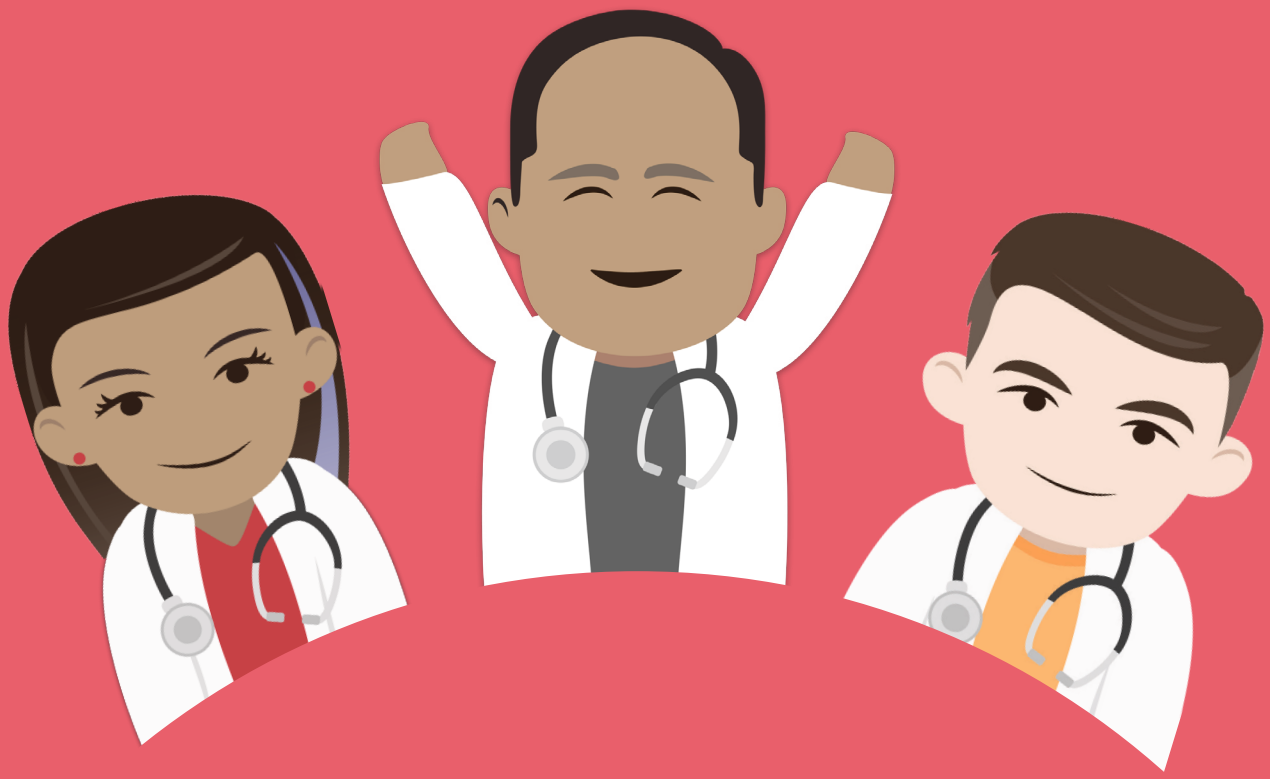
Hematology and Coagulation Essentials
Chapter 2

# CLASSIFYING AND DIAGNOSING ANEMIA



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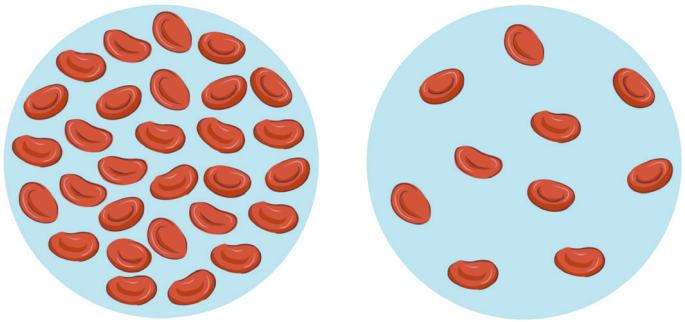
## Classifying and diagnosing anemia

# CATEGORIZING ANEMIAS



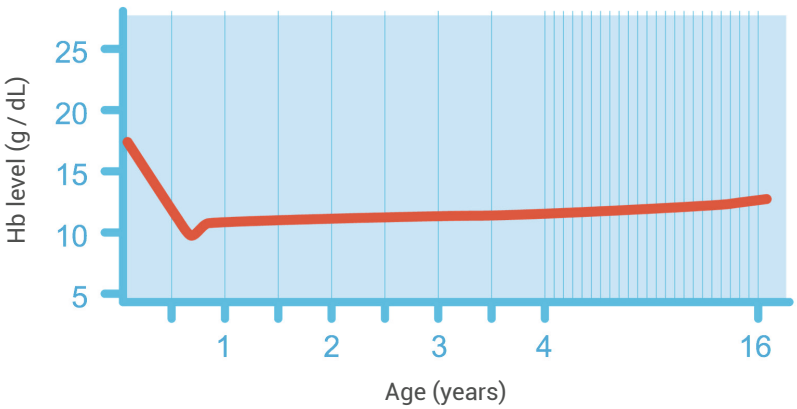
Normal Anemia

## *Anemia*

Anemia results when there is a reduction in the concentration of hemoglobin (Hb). A decrease in the hematocrit (Hct) and red blood cell (RBC) count accompanies a fall in the Hb level.

An individual's age and sex can affect their hemoglobin levels. Females have a lower level of Hb compared to males. This is due to the stimulatory effect of androgens.

When a baby is born, the Hb is at its highest (ranging from 16 to 20). The Hb level drops to its lowest (9–11) around 6–9 months of age. In fact all complete blood count (CBC) values are higher in newborns compared to adults, with the exception of platelets.



## *Classification of anemias*

Anemia can be classified based on the morphology of the red cells, as well as based on the etiology.

### Morphologic classification

**The morphologic classification of anemia traditionally divides anemia into three major types.**

**Microcytic hypochromic**
- Low mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH)
- Under the microscope, RBCs appear smaller than normal and the central pallor encompasses more than one third of the cell

**Normocytic normochromic**
- Normal MCV and MCH
- Under the microscope, RBCs are normal in size and central pallor encompasses less than one third of the cell

**Macrocytic**
- High MCV
- Under the microscope, RBCs appear enlarged

### Etiologic classification

**Etiologic classifications of anemia**
- Blood loss
- Deficiency of erythropoietic factors (e.g., protein, iron, folate, and B12 deficiency)
- Bone marrow failure
- Increased red cell destruction (hemolytic anemia)

## Classifying and diagnosing anemia

# DIAGNOSING MICROCYTIC HYPOCHROMIC ANEMIAS

### *Important causes of microcytic hypochromic anemias*

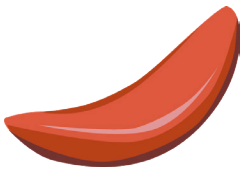
**Hemoglobinopathies and thalassemias**
Hemoglobin is a protein made up of two alpha and two beta chains—each of which contains a heme group that is able to bind iron. Hemoglobinopathy occurs when the hemoglobin molecule is structurally defective. Thalassemia occurs when the quantity of globin chains is reduced. In alpha thalassemia, synthesis of the alpha chains is reduced and in beta thalassemias, synthesis of the beta chains is reduced.

**Iron deficiency**
Iron deficiency may result from decreased iron intake, poor absorption or when the body's iron requirement is increased. An example of this is hemolytic anemia, where the iron requirement exceeds normal because the bone marrow must compensate for the hemolysis by producing additional red cells, a process which requires higher levels of iron.

**Chronic disease**
In anemia of chronic disease, iron cannot be made available to red cells to be incorporated in the hemoglobin molecule. This is known as reticulo-endothelial blockade of iron.

**Sideroblastic anemia**
This is a group of disorders characterized by impaired utilization of iron resulting in diminished heme synthesis. The diminished heme synthesis results in a continued stimulus for iron absorption despite an adequate or increased level of intracellular iron. Excess iron is deposited in the mitochondria, forming ring sideroblasts.

The cause of sideroblastic anemia may be hereditary (either X-linked or autosomal), idiopathic (usually as a part of myelodysplastic syndrome), or secondary to toxic insult (drugs, lead, alcohol).




## *What tests should be ordered if we suspect microcytic hypochromic anemia?*

**Red cell distribution width (RDW)**
The RDW is a measure of anisocytosis (range of variation in RBC volume). If the RDW is normal or close to normal, this suggests beta thalassemia. High RDW suggests iron deficiency.

**Iron panel**
An iron panel measures four parameters:

- Serum iron
- Serum ferritin
- % transferrin saturation
- Total iron binding capacity (TIBC)

*In iron deficiency*—serum iron, serum ferritin, and % transferring saturation are low and TIBC is high.

*In anemia of chronic disease*—serum iron and % saturation are low, serum ferritin is variable, and TIBC is not high.

**Hemoglobin electrophoresis**
To make a definitive diagnosis of hemoglobinopathy or thalassemia, a hemoglobin electrophoresis may be required. This is especially true when we have microcytic hypochromic anemia with normal or near normal RDW or when we see abundant target cells in the peripheral smear.

**Bone marrow exam**
A bone marrow exam may be considered when sideroblastic anemia is a possibility. This will help us to identify the ring sideroblasts.

Since sideroblastic anemia is hereditary or also part of myelodysplastic syndrome (MDS), additional testing for these conditions may also be performed from the bone marrow biopsy.

## Classifying and diagnosing anemia

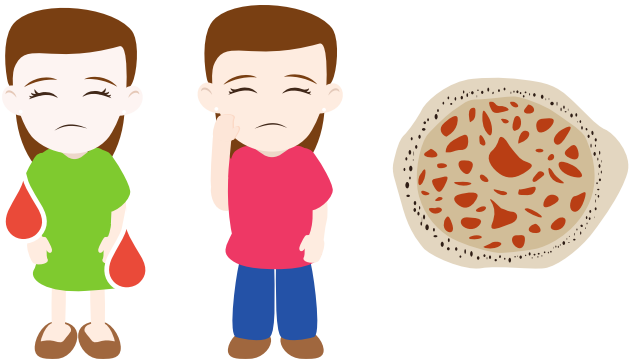
# RECOGNIZING NORMOCYTIC NORMOCHROMIC ANEMIAS

### *Important causes of normocytic normochromic anemia*

**Blood loss**
Blood loss may be acute or chronic. In acute blood loss, replacement of plasma occurs within 24 hours and thus there is hemodilution. Anemia is normocytic normochromic. The maximum decline in hemoglobin (Hb) / hematocrit (Hct) is usually observed within three days. There may be transient leukocytosis and thrombocytosis. With chronic blood loss, iron deficiency may occur resulting in microcytic hypochromic anemia.

**Chronic disease**
Anemia of chronic disease is seen in the setting of chronic infection, inflammation or malignancy. It is characterized by low serum iron, reduced transferrin saturation, reduced TIBC, and normal or raised serum ferritin. It is thought to result from the release of cytokines from inflammatory cells, which lead to reduced release of iron from reticuloendothelial cells. There is also reduced red cell survival and inadequate erythropoietin response.

**Bone marrow failure**
Bone marrow failure may be due to bone marrow aplasia or due to bone marrow infiltration. This will be discussed in the next lesson.



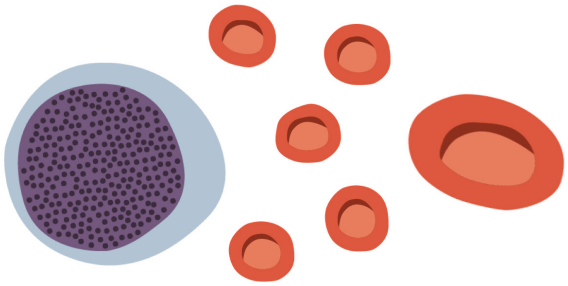
*Remember, to determine the cause of normocytic normochromic anemias*

- *We need to inquire about the presence of chronic diseases.*
- *The history of blood loss should be obvious.*
- *The peripheral smear should be reviewed.*
- *A bone marrow exam is indicated for anemias due to bone marrow failure.*

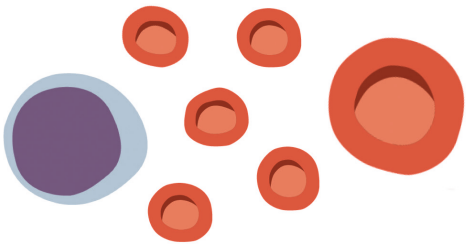
## Classifying and diagnosing anemia

# IDENTIFYING MACROCYTIC ANEMIAS

**Macrocytic anemias can be divided into two broad groups**



Megaloblastic macrocytic anemia



Normoblastic macrocytic anemia

### *Megaloblastic macrocytic anemia*

In megaloblastic macrocytic anemia the red cells are large in the peripheral blood and the red cell precursors in the bone marrow are also large.

**The most important causes of megaloblastic macrocytic anemia are**

- Folate deficiency
- B12 deficiency

It is also important to note that B12 or folate deficiency can also cause pancytopenia.

### *Normoblastic macrocytic anemia*

In normoblastic macrocytic anemia the red cells are large in the peripheral blood but red cell precursors in the bone marrow are not large.

**Causes of normoblastic macrocytic anemia include**

- Alcohol
- Liver disease
- Pregnancy
- Hypothyroidism

## *How do we differentiate?*

To differentiate between the two broad categories, analysis of the complete blood count (CBC) values and review of the peripheral smear are helpful.

**In megaloblastic macrocytic anemia**

- Red cells are significantly enlarged
- Mean corpuscular volume (MCV) is typically > 120
- Morphologically, the enlarged RBCs are described as oval macrocytes
- Polymorphonuclear leukocytes (PMNs) are hypersegmented, meaning the neutrophils have more than five nuclear segments

If the clinical picture suggests megaloblastic macrocytic anemia, then ordering folate and B12 levels are the next appropriate steps.

**In normoblastic macrocytic anemia**

- Red cells are enlarged, but not to the same extent as in megaloblastic macrocytic anemia
- MCV is usually < 110
- Red cells are not oval
- Hypersegmented PMNs are not seen

If the clinical picture suggests normoblastic macrocytic anemia, then ordering a liver function test, TSH level, checking for alcohol consumption, and pregnancy is the next appropriate step.

*To distinguish between megaloblastic and normoblastic macrocytic anemia*

- *A CBC*
- *A peripheral blood smear*

Classifying and diagnosing anemias

# DIAGNOSING ANEMIAS DUE TO BONE MARROW FAILURE

## *Bone marrow failure*

Bone marrow failure typically presents as pancytopenia.

**Bone marrow failure may be due to**
- Aplastic anemia
- Bone marrow infiltration due to disease (e.g., leukemias, fibrosis, infections)
- B12 or folate deficiency
- Myelodysplastic syndrome (MDS)

## *Aplastic anemia*

About 80% of cases of aplastic anemia are due to idiopathic or unknown mechanisms.

**Known causes of aplastic anemia**
- Inherited (e.g., Fanconi anemia)
- Paroxysmal nocturnal hemoglobinuria (PNH)
- Viral infections (e.g., hepatitis)
- Drugs
- Radiation

**Diagnostic approach**
A detailed patient history should be taken, being certain to ascertain whether the patient has a history of any of the following:
- Drugs
- Occupational exposure
- Radiation exposure
- Family history—Fanconi anemia
- Medical history—hepatitis

## *Investigations*

**CBC and peripheral blood smear**
Initial investigations include a CBC with reticulocyte count and a peripheral smear examination.

CBC should demonstrate pancytopenia. The corrected reticulocyte count should be low. Large platelets are not expected.

If the bone marrow is truly failing, then immature red cells (reticulocytes) and platelets (large platelets) should not be increased. If the patient has MDS, then dysplasia may be picked up in the peripheral smear.

## *Bone marrow exam*

A bone marrow exam is essential.

It should demonstrate a hypocellular marrow. It would also help to rule out marrow failure due to bone marrow infiltration. If MDS is in the differential, the bone marrow should demonstrate dysplasia. Cytogenetic studies performed from the bone marrow aspirate will be very helpful for MDS cases.

Additional testing, such as cytogenetics and flow cytometry, can also be performed from bone marrow aspirate. Flow cytometry can be used to help diagnose PNH. Diagnosis of Fanocni anemia requires cytogenetic studies.

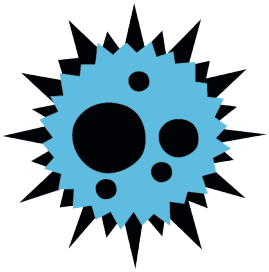
**Additional tests**
Additional tests can help to determine the cause of bone marrow failure:

- B12 and folate level
- Viral hepatitis markers

B12 and folate deficiency are a well recognized cause of pancytopenia and bone marrow failure. This is because these are required for DNA synthesis. Without DNA synthesis cells cannot multiply, which result in bone marrow failure.


B12 level

Folate level



Virus

## Classifying and diagnosing anemia

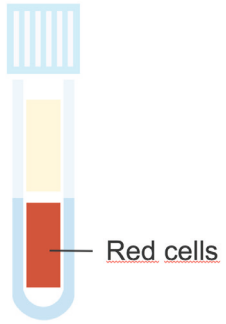
# RECOGNIZING HEMOLYTIC ANEMIAS

Hemolytic anemias result from a shortened red blood cell (RBC) life span.

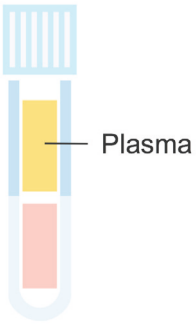
A normal RBC life span is 120 days. When red cells are destroyed before this time, the bone marrow will attempt to compensate by producing red cells at a faster than normal rate. With time, the bone marrow may not be able to keep up with the rate of destruction.

## *Hemolytic anemias are classified into two broad groups*

**Corpuscular defects**




**Extracorpuscular defects**




## *Corpuscular defects*

**Membrane defects**
- Hereditary spherocytosis, hereditary elliptocytosis

**Enzyme defects**
- Glucose-6-phosphate dehydrogenase (G6PD) deficiency, pyruate kinase deficiency

**Hemoglobinopathies and thalassemias**

**Paroxysomal Nocturnal Hemoglobinemia (PNH)**

## *Extracorpuscular defects*

**Immune mechanisms**
- Hemolytic disease of newborn, hemolysis due to mismatch transfusion, and autoimmune hemolytic anemia

**Non-immune mediated**
- Infections (e.g., malaria)
- Microangiopathic hemolysis (e.g., disseminated intravascular coagulation [DIC])
- Trauma (e.g., karate injury)

Hemolysis can also be classified based on its location—in other words, whether the site of hemolysis is within or outside of the blood vessels.

In intravascular hemolysis, enzymes in the red cells, such as lactate dehydrogenase (LDH), are released into the circulation resulting in high levels of LDH in the blood. Free hemoglobin is also released and this is split into heme and globin. The heme binds to haptoglobin and the heme-haptoglobin complex is cleared from the circulation, resulting in lowered levels of haptoglobin in the blood.

Thus, features of intravascular hemolysis are high LDH and low haptoglobin.

## *Features of hemolytic anemia*

**High reticulocyte count**
This occurs because the bone marrow responds to the anemia by producing and releasing immature red cells into the circulation.

**Jaundice**
The hemoglobin released from red cells results in high levels of unconjugated bilirubin, often leading the patient to appear jaundiced.

**Splenomegaly**
This occurs in some patients. An abdominal ultrasound will confirm this.

**High intravascular LDH and low haptoglobin levels**
This indicates intravascular hemolysis.

## *Investigations*

- CBC with reticulocyte count
- Peripheral smear examination
- Bilirubin levels

Based on the morphology of red cells seen in the peripheral smear we should be able to narrow down the differential diagnosis.

Further confirmatory tests can be ordered after this.

## Classifying and diagnosing anemia

# SCREENING FOR MEMBRANE DEFECTS

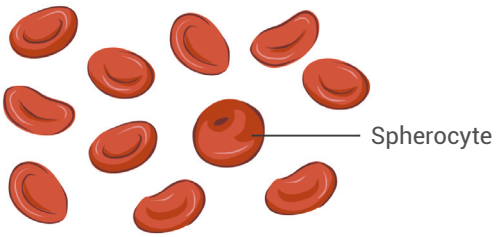
Multiple proteins are involved in the formation of normal red cell membranes. Defects in any of these proteins, or their interactions with other proteins, can result in early red cell destruction, leading to anemia. This group of diseases is referred to as hemolytic anemias due to red cell membrane defects.

**Hereditary spherocytosis** is the most common example of hemolytic anemia due to membrane defect. It is transmitted as an autosomal dominant disorder; however, in about 25% of cases the disease is due to a new mutation.

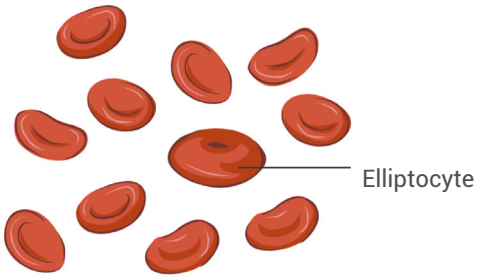
In **hereditary spherocytosis** we will see spherocytes in the peripheral smear. Normal red cells have a central pallor, but spherocytes do not. Spherocytes can also be seen when there is antibody-mediated membrane damage to red cells. Membrane damage results in water leaking into red cells, causing the cells to become spherical instead of their normal discoid shape central pallor is lost. Thus spherocytes are also seen in autoimmune hemolytic anemia.

**Hereditary elliptocytosis** and **hereditary stomatocytosis** are two important, but less common, examples of hemolytic anemias due to membrane defects. Both of these conditions are also transmitted as autosomal dominant.

In all three cases, peripheral blood smear is very helpful.

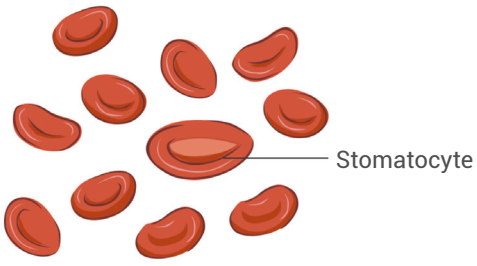



Hereditary spherocytosis




Hereditary elliptocytosis

In **hereditary elliptocytosis** > 20% of red cells will be elliptocytes.




Hereditary stomatocytosis

In **hereditary stomatocytosis** > 35% of red cells will be stomatocytes.

For all of the three conditions, we expect to see the common features of hemolytic anemia: anemia with high reticulocyte count, increased serum unconjugated bilirubin, and typical morphology on the peripheral smear.

The presence of spherocytes in the peripheral smear suggests autoimmune hemolytic anemia. In this case, we would expect to be able to document antibodies on red cell surface. This can be done by ordering a direct antiglobulin test (DAT). This test will be positive in autoimmune hemolytic anemia and negative in hereditary spherocytosis.

**Two additional tests are available for hereditary spherocytosis.** These are osmotic fragility test and flow cytometry.

Spherocytes cannot take in as much water when compared to normal red cells. This is because normal red cells are discoid and when water enters the cells they start to become spherical and finally they swell to an extent that they rupture. Spherocytes are already swollen and rupture much more easily. This is the basis of the osmotic fragility test.

Genetic testing is also available for hereditary spherocytosis, hereditary elliptocytosis, and hereditary stomatocytosis. This is not commonly required to reach a definitive diagnosis.

## Classifying and diagnosing anemia

# TESTING FOR ENZYME DEFECTS

The mature RBC is totally dependent on glucose as a source of energy. RBCs do not have mitochondria, therefore the tricarboxylic acid (TCA) cycle cannot take place. RBCs are entirely dependent on the glycolytic pathway for adenosine triphosphate (ATP) production. The ATP is required to maintain the sodium potassium pump ($Na^+$ / $K^+$ ATPase). This pump ensures that sodium and water are constantly pumped out of the RBC and thus, its biconcave shape is maintained.

## *Pyruvate kinase deficiency*

Pyruvate kinase deficiency is the most common enzyme deficiency of the glycolytic pathway in red cells. It is transmitted as autosomal recessive.

Severity of the disease varies from patient to patient.

**Clinical features include**

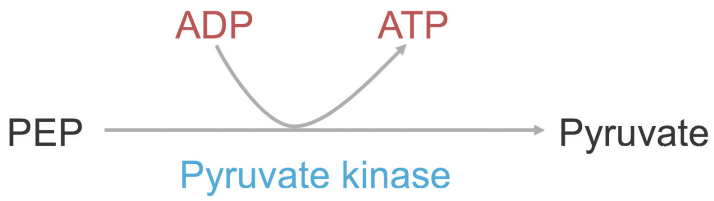
- Anemia
- Jaundice
- Splenomegaly
- Gallstones

**Diagnosis**

Like all hemolytic anemias, diagnosis starts with establishing the general features of hemolytic anemias by ordering the following tests:

- CBC with reticulocyte count
- High unconjugated bilirubin

A peripheral smear is generally not helpful in diagnosing pyruvate kinase deficiency, since the findings are generally nonspecific. Fluorescent screen tests are available. Confirmation requires enzyme assay or genetic studies.

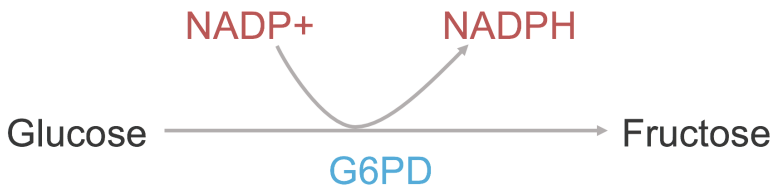


## *Glucose-6-phosphate dehydrogenase (G6PD) deficiency*

The pentose phosphate pathway, which is also known as the hexose monophosphate shunt, is another important metabolic pathway in red cells. Here glucose is converted to fructose and NADPH is produced. NADPH donates the hydrogen radical to glutathione to ensure glutathione remains in the reduced state. If this does not happen, globin will precipitate to form inclusions known as Heinz bodies.

The most common enzyme deficiency in the pentose phosphate pathway is G6PD deficiency, which is transmitted in an X-linked recessive manner.

The Heinz bodies are removed by splenic macrophages causing red cells to assume a different morphology: bite cells or blister cells.




**Diagnosis**
Like all hemolytic anemias, diagnosis starts with establishing general features of hemolytic anemias by ordering the following tests:

- CBC with reticulocyte count
- High unconjugated bilirubin
- Peripheral smear—to look for bite or blister cells

Fluorescent screen tests are available. Confirmation requires enzyme assay.

## Classifying and diagnosing anemia

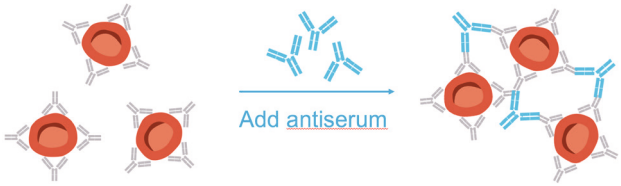
# IDENTIFYING EXTRACORPUSCULAR DEFECTS

### *extracorpuscular defects*

**Immune mechanisms**
- Hemolytic disease of newborn, hemolysis due to mismatch transfusion, and autoimmune hemolytic anemia

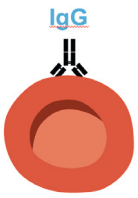
**Non-immune mediated mechanisms**
- Infections (e.g., malaria)
- Microangiopathic hemolysis (e.g., DIC)
- Trauma (e.g., karate-induced hemoglobinuria)

Immune-mediated hemolysis should always result in the presence of antibodies or complement on the red cell surface. If the red cell membrane is damaged by antibodies, spherocytes should be detectable on the peripheral smear. The antibodies or complement on the red cell surface can be detected using a direct antiglobulin (DAT) test.

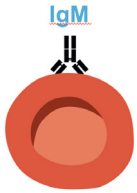



Patient's erythrocytes — Agglutination

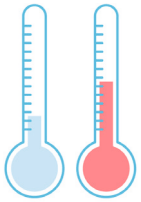
### *The three types of autoimmune hemolytic anemia*




Warm autoimmune hemolytic anemia (WAHA)




Paroxysmal cold hemoglobinuria (PCH)



Cold hemagglutinin disease (CHAD)

**Warm autoimmune hemolytic anemia (WAHA)**
This is due to the presence of IgG antibodies on the red cell surface. Red cells become spherocytic. The red cells are removed in the spleen with resultant splenomegaly. Hemolysis is thus extravascular.

**Paroxysmal cold hemoglobinuria (PCH)**
On exposure to cold temperature, IgG antibodies bind to red cells and, once moved into warmer temperatures, complement is activated which destroys red cells. The IgG antibody moves away from red cells. Again, a DAT test can identify complement on the red cell surface, and hemolysis is intravascular.

**Cold hemagglutinin disease (CHAD)**
This is due to the presence of IgM antibodies on red cell surface. IgM activates complement which destroys red cells within blood vessels. Hemolysis is therefore intravascular. DAT cannot detect IgM antibodies, rather DAT detects the complement present on the red cell surface.

A peripheral smear demonstrates red cell clumping (agglutination).

## *Investigations*

- CBC with reticulocyte count (expect to see anemia with high reticulocyte count)
- DAT
- Peripheral smear

**Features of intravascular hemolysis**
- High lactate dehydrogenase (LDH)
- Low haptoglobin

Microangiopathic hemolysis refers to hemolysis which occurs when red cells pass through clots in small vessels and are mechanically damaged and lysed.

Examples of microangiopathic hemolysis are disseminated intravascular coagulation (DIC), thrombotic thrombocytopenic purpura (TTP), and hemolytic-uremic syndrome (HUS).

A peripheral smear of patients with microangiopathic hemolysis will reveal fragmented red cells (schistocytes) and thrombocytopenia. Thrombocytopenia occurs because platelets are consumed in widespread clot formation.

***IN DIC THE PATIENT IS COAGULOPATHIC:***
*The prothrombin time (PT) and partial thromboplastin time (PTT) are abnormal.*

***IN TTP / HUS PATIENT IS NOT COAGULOPATHIC:***
*The prothrombin time (PT) and partial thromboplastin time (PTT) are normal.*

## Classifying and diagnosing anemia

# ASSESSING FOR HEMOGLOBINOPATHIES AND THALASSEMIAS

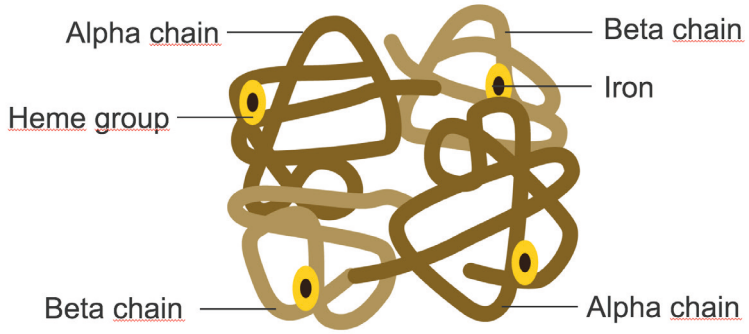
### *Hemoglobinopathies*

Hemoglobinopathies are structural defects in the hemoglobin molecule. Over a thousand hemoglobinopathies have been identified, but fortunately most of them are clinically insignificant. Worldwide, the most common hemoglobinopathies are Hb S and Hb E.

Most clinically significant hemoglobinopathies result from a defect in the beta gene.

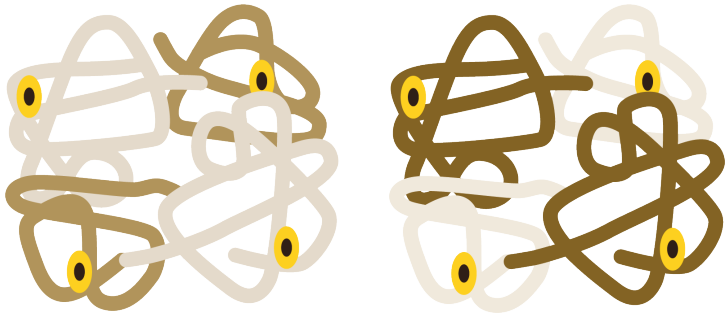
If one copy of the beta gene is defective, the patient is said to have a trait (e.g., Hb S trait). In Hb S trait, the majority (50–60%) of the Hb will be Hb A (normal) and most of the remaining Hb will be Hb S.

If both genes are defective then most of the Hb will be the abnormal Hb and no normal Hb A will be present (e.g., Hb SS disease).




Thalassemias result from a reduced rate of globin chain synthesis. Thus, in alpha thalassemia synthesis of the alpha chain is reduced and in beta thalassemia, production of the beta chain is reduced.

Like hemoglobinopathies, patients with thalassemias may be thalassemia trait / minor where most of the Hb produced is normal (Hb A) and clinical features are mild / minimal, or the patient may have thalassemia disease, where Hb A production is seriously impaired and the patient has significant clinical features.



Alpha thalassemia

Beta thalassemia

## Clinical features

Hemoglobinopathies and thalassemias are actually examples of hemolytic anemias. Like all hemolytic anemias, patients will be anemic with an increased reticulocyte count and high levels of unconjugated bilirubin. Almost all cases of hemoglobinopathies and thalassemias exhibit microcytic hypochromic red cells. A notable exception is sickle cell disease, which produces normocytic normochromic red cells.

In thalassemias, a variable reduction in the hemoglobin level is present; however, the RBC value is disproportionally high.

For example, a patient with the beta thalassemia trait may have a slightly reduced Hb level but still show a normal or slightly elevated RBC value.

Patients with hemoglobinopathies or thalassemia may also exhibit a significant number of target cells in the peripheral smear.

## Investigations

When hemoglobinopathy or thalassemia is suspected, Hb electrophoresis should be ordered. The laboratory should be able to detect any of the major hemoglonipathies or thalassemias with confidence. However, a notable exception is the alpha thalassemia trait which cannot be diagnosed by this method.

For the alpha thalassemia trait, and sometimes for unusual hemoglobinopathies, genetic diagnosis may be required. This situation is quite rare.