Anemia: Clinical Approach

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Definition of Anemia

The oxygen carrying capacity of red blood cells is provided by hemoglobin. Anemia is present when this value in blood falls below age- and gender-appropriate normal values, which are defined by values two standard deviations below the mean for normal individuals of similar age and gender (i.e., outside the 95% confidence interval for the population). The volume of red blood cells reported as a percentage of the total volume of blood present is the hematocrit. This value is commonly used as an alternative method for defining anemia. In most cases the two values relate to one another roughly by a factor of three (hemoglobin × 3 ≈ hematocrit).

The hemoglobin value in children is lower than that of adults [1]. During puberty, an increase in hemoglobin occurs in males due to androgenic steroids. The normal range for hemoglobin in males is therefore higher than for females (Table 4.1). In maturity, the difference between men and women decreases. In particular, over the two decades after 70 years of age, men's hemoglobin levels drop by about 1 g/dL [2]. Thus, the mean hemoglobin concentration for a 90-year-old man is about 14.1 g/dL compared to about 13.8 g/dL for a 90-year-old woman.

Major categories of anemia are:

- Acute blood loss
- Inadequate production of red blood cells
- Destruction of red blood cells

Acute Blood Loss

Loss of blood acutely may not be associated with an immediate decline in hemoglobin concentration, since this loss consists of an equivalent proportion of cellular elements and plasma. However, after volume repletion a decrease in the hemoglobin concentration or hematocrit proportional to the amount of blood lost may be observed.

Inadequate Production

There are a number of entities commonly associated with an inadequate production of red blood cells. Some of these affect other cell lineages as well.

- *Iron deficiency anemia* is the most common cause of anemia globally (see Chap. 5) [3]. With rare exception, iron deficiency anemia in adults results from chronic blood loss. In women, menstrual blood loss may explain its development (and it is present in about 5% of menstruating females in the United States). In men, the identification of iron deficiency anemia should always provoke a search for blood loss. Even in younger women, consideration of gastrointestinal blood loss may merit consideration, depending on individual circumstance [4].
- *Anemia of inflammation (also known as the anemia of chronic disease)* is commonly encountered in association with a variety of conditions, including serious infections, rheumatologic disease, diabetes mellitus, and malignancy. In this condition the iron regulatory protein hepcidin decreases the ability of the reticuloendothelial system to release stored iron [5]. The lack of bioavailable iron essentially mimics the situation with iron deficiency anemia from blood loss. When combined with the suppressive effect of certain cytokines on red blood cell production, this circumstance leads to a mild to moderate anemia that may share some morphologic features with iron deficiency.
- *Anemia of renal disease* results from erythropoietin deficiency. The synthesis of this hormone is regulated by the oxygen tension in the periglomerular cells of the kidney. Hypoxia drives the synthesis of erythropoietin and its

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Table 4.1 Normal range for red blood cell parameters

Parameter		Adult normal range
Red blood cell number (RBC)	Male	$4.5–5.9 \times 10^{12}$/L
	Female	$4.0–5.0 \times 10^{12}$/L
Hemoglobin (Hgb or Hb)	Male	13.5–17.5 g/dL
	Female	12–16 g/dL
Hematocrit (Hct)	Male	41–53%
	Female	36–46%
Mean corpuscular volume (MCV=Hct/RBC)		80–100 fL
Mean corpuscular hemoglobin (MCH=Hgb/RBC)		26–34 pg
Mean corpuscular hemoglobin concentration (MCHC=Hgb/Hct)		31–37 g/dL
Red cell distribution width (RDW=(SD[a] of MCV/MCV) ×100)		11–15%

[a]*SD* standard deviation

release into the bloodstream, which stimulates the maturation and development of erythrocyte precursors in the bone marrow. These activities result in an increase in red blood cell mass, bringing additional oxygen to the kidney, and ultimately completing the feedback loop by downregulating production of erythropoietin [6]. A reduction in renal function is generally accompanied by a reduction of erythropoietin production.

- *Endocrine anemias* result from deficiencies or excess of hormones that contribute to blood cell development. To provide a few examples, hypothyroidism may be associated with a mild to moderate anemia sometimes associated with macrocytosis; adrenal cortical insufficiency may be accompanied by a normocytic anemia; and decreased levels of serum testosterone may lead to a mild anemia in males [7].
- *Pure red cell aplasia* in children may be the result of heritable disorders, such as congenital hypoplastic anemia (Diamond-Blackfan anemia), or may be the apparent result of infection with a virus (e.g., Parvovirus B19) or an immunologic phenomenon (e.g., as seen in systemic lupus erythematosus) [8]. In contrast to aplastic anemia, in which two or more cell lineages are affected, pure red cell aplasia is characterized by preservation of the white blood cell count and platelet count.
- *Bone marrow replacement* is also known by the term myelophthisis. In this case, the blood forming bone marrow space is taken over by cells or material that should not be there. Causes of bone marrow replacement include hematologic malignancies such as leukemia or lymphoma, metastatic cancer (most commonly breast or prostate), infection with fungi or other microorganisms, and fibrosis such as that which may occur in conjunction with primary myelofibrosis.
- *Folate and vitamin B12 deficiency* are two types of megaloblastic anemia that lead to maturation abnormalities in all three cell lineages. These disorders share in common the pathophysiology of impaired synthesis of DNA [9]. Folate deficiency is generally related to inadequate dietary intake or to increased requirements due to red blood cell hemolysis. The situation for vitamin B12 (also called cobalamin) is more complex. Vitamin B12 is released from food in the acidic environment of the stomach and binds to the intrinsic factor that is secreted by the parietal cells in the stomach (see Chap. 6). The intrinsic factor-vitamin B12 complex then travels to the terminal ileum where it is absorbed. Vitamin B12 deficiency may result from several different causes including inadequate stomach acidity, pernicious anemia (an autoimmune phenomena destroying the parietal cells that synthesize intrinsic factor), structural lesions in the terminal ileum due to conditions such as Crohn's disease, and from surgical resection of portions of the GI tract. Inadequate dietary intake is generally only observed in vegans.
- *Sideroblastic anemia*s represent an uncommon group of hereditary and acquired disorders in which iron is not effectively used in hemoglobin synthesis leading to iron accumulation in the mitochondria of red blood cell precursors [10]. The deposition of iron in mitochondria leads to the morphologic entity of ringed sideroblasts in the bone marrow when it is stained for iron. Exactly as the name implies, ringed sideroblasts are cells in which iron-laden mitochondria encircle at least one-third of the circumference of the erythroblast nucleus. Usually at least five iron-laden mitochondria need to be seen encircling the nucleus to make diagnostic criteria. Hereditary forms of sideroblastic anemia are rare and may be X-linked, autosomal dominant, or recessive. Acquired forms may occur after exposure to drugs (e.g., cyclosporine, vincristine) or toxins (ethanol).

Destruction

Normally red blood cells circulate for about 100 to 120 days before they are cleared by the reticuloendothelial system. Premature red blood cell destruction may result from intrinsic defects such as abnormal hemoglobin molecules, cytoskeletal proteins, or enzymes. It may also result from defects extrinsic to the erythrocyte, including mechanical forces and antibody or complement-mediated red cell breakdown.

- *Hemoglobinopathies* include alpha- and beta-thalassemia, disorders in which there are insufficient production of one of the globin chains, and the structural mutations (See Chap. 7) [11, 12]. Alpha- or beta-thalassemia traits (loss of two alpha genes, or one beta gene) are common causes of microcytosis associated with little or no anemia.

Microcytosis occurs in thalassemia because the deficiency in hemoglobin stimulates additional cell divisions of erythrocyte precursors in order to try to preserve the hemoglobin concentration. Thalassemia trait is particularly common in individuals from Africa, Asia, and the Mediterranean Basin. Among most commonly encountered structural mutations is a glutamine to valine substitution at position 6 of the beta globin gene. This change results in the production of hemoglobin S, which tends to polymerize in its deoxygenated state [13]. Heterozygotes with one copy of hemoglobin S (sickle cell trait) are relatively protected against infection with the malaria parasite. This structural mutation thus provides a survival advantage, and selective pressure has led to persistence of the mutation. Homozygotes with two copies of hemoglobin S have sickle cell anemia, a serious life-defining hematologic disorder. Many other structural mutations exist and can result in changes in the properties of hemoglobin, such as reducing its solubility (e.g., hemoglobin C and hemoglobin D), decreasing its stability, or changing its oxygen affinity.

- *Red blood cell membrane defects* result from a variety of different defects affecting the red blood cell cytoskeleton or the membrane itself (See Chap. 3) [14]. Maintenance of the normal biconcave shape requires intact cytoskeletal architecture. Defects in any of the proteins involved, including ankyrin, spectrin, and band 3, among others, lead to changes that reduce the resiliency of red blood cells as they pass through the narrow passageways in the spleen and other portions of the circulation. This initially leads to the formation of spherocytes and ultimately resulting in hemolysis. The resulting disorder, hereditary spherocytosis, is most common in individuals of Northern European descent. Liver disease is the most common cause of an acquired red cell membrane defect and results in abnormal cells noted on examination of the blood smear (codocytes or target cells). Abnormalities in the lipid composition of the red blood cell membrane result in cells that are abnormally stiff and unable to rebound from deformities that arising from transit of the circulation. Paroxysmal nocturnal hemoglobinuria (PNH) represents a rare type of acquired membrane defect that is derived from a stem cell defect leading to the reduction or absence of phosphatidylinositol glycan-linked membrane proteins [15]. The lack of one such erythrocyte phosphatidylinositol glycan-linked membrane protein, decay accelerating factor, is associated with the hemolysis of red blood cells through the unopposed constitutive activation of components of the complement cascade (See Chap. 9).
- *Red blood cell enzyme defects* are potentially the most common red cell abnormalities globally (See Chap. 8). Glucose-6-phosphate dehydrogenase (G-6-PD) is the enzyme required for function of the hexose monophosphate shunt in the red blood cell. This pathway provides the red blood cell with reduction capacity against oxidant stress. The gene encoding G-6-PD is on the X chromosome. Mutations in G-6-PD are very common and have been preserved in populations because of their relative protection against infection with the malaria parasite, like sickle cell anemia [16]. The two most common mutations cause a reduction in cell enzyme activity in the aging erythrocyte (A-variant) or result in absent function throughout the red cell life span (Mediterranean variant). Those carrying mutations in G-6-PD (especially males, since this is an X-linked trait) have red cells that are susceptible to hemolysis under conditions of oxidant stress. Common causes of oxidant stress include medications such as antimalarials, dapsone, and sulfamethoxazole.
- *Mechanical causes of hemolysis* may result from microscopic or macroscopic forces. Microangiopathic hemolytic anemias (MAHA) include disseminated intravascular coagulation (DIC), thrombotic thrombocytopenic purpura (TTP), and hemolytic uremic syndrome (HUS) [17]. In these conditions abnormalities in the microvasculature result in shearing of the red blood cell and the formation of red cell fragments called schistocytes. Certain uncommon infections, such as those with clostridia or bartonella species, can also be associated with the production of toxins that lead to red blood cell destruction through what is essentially mechanical disruption of the membrane. Accelerated or malignant hypertension and vasculitis are additional etiologies producing mechanical destruction of red blood cells. Malfunctioning mechanical valves, perivalvular leaks, as well as long-distance running can all result in the mechanical destruction of red blood cells by mechanical trauma [18].
- *Autoimmune hemolytic anemia* results from the formation of antibodies that bind to the red blood cell and either fix complement resulting in its destruction in the circulation (intravascular hemolysis) or result in clearance in the reticuloendothelial system and spleen (extravascular hemolysis) (See Chap. 9). Warm autoantibodies are often idiopathic but may be associated with hematologic malignancies such as chronic lymphoid leukemia or rheumatologic disorders such as systemic lupus erythematosus [19]. Similarly, cold autoantibodies may be idiopathic or associated with lymphoproliferative or rheumatologic disorders [20]. Transient cold agglutinins may be associated with infectious mononucleosis and infection with mycoplasma.
- *Alloimmune hemolytic anemia* results from exposure of an individual to foreign red blood cells. In children and adults, this most commonly results from blood transfusion in which there are mismatched minor antigens.

Symptoms and Signs of Anemia

The symptoms and signs of anemia correlate with the reduction in the oxygen carrying capacity of the blood and the ability of the affected individual to compensate for this defect. Rapid loss of an even moderate amount of blood may be associated with shock and collapse of the circulatory system. Blood loss that occurs gradually over time is often reasonably well tolerated until relatively more severe, as adaptive changes can help compensate for the anemia. The corollary to this is that individuals with anemia that has developed gradually often do not require, and in fact can be harmed by aggressive intervention with the administration of the blood (e.g., pulmonary edema may develop with overaggressive transfusion).

The most common symptom of anemia that patients tend to report is fatigue. Decreased exercise tolerance and dyspnea on exertion may be noted by individuals as the extent of the anemia worsens. Certain types of anemia, such as iron deficiency anemia, may be associated with pica (e.g., ice chips, Argo starch), the dietary intake of non-food substances. When anemia is associated with a microangiopathy, bruising or bleeding may be reported due to the accompanying thrombocytopenia or coagulopathy.

Signs of anemia may include conjunctival pallor and a pale complexion. Tachycardia and/or systolic flow murmurs may be present if the degree of anemia is pronounced. Iron deficiency anemia may be associated with cracking of the edges of the lips (angular chelitis) and with spooning of the nails (koilonychia) [21]. Right upper quadrant pain may develop as a result of cholecystitis due to the formation of calcium bilirubin gallstones in the presence of hemolysis. Splenomegaly may result from chronic congenital or acquired hemolysis leading essentially to hypertrophy of the reticuloendothelial system or from extramedullary hematopoiesis (the presence of maturing hematopoietic precursors outside of the bone marrow), which may be associated with some myeloproliferative disorders [22].

Laboratory Diagnosis

A systematic approach to the diagnosis of anemia is essential in order to minimize unnecessary diagnostic testing and to arrive expediently at the correct diagnosis. Despite the availability of sophisticated diagnostic testing, careful consideration of the information provided by the different parameters included in the complete blood count (CBC), and a review of a well-prepared peripheral blood smear often provides a great wealth of diagnostic information. The CBC includes a variety of red blood cell indices. These calculated values are very important to classify anemia and guides one toward the differential diagnosis of anemic patient (Table 4.1) [23, 24].

When evaluating anemia, the two red blood cell indices that provide the greatest diagnostic information are the mean corpuscular volume (MCV) and red cell distribution width (RDW). MCV, which is calculated by the ratio of hematocrit (HCT) to red blood cell count (RBC), denotes red blood cell size in femtoliters (10^{-15} L). It may be small (microcytic), normal (normocytic), or large (macrocytic) depending on where it falls relative to the normal range. The red cell distribution width, which is actually the coefficient of variation of the mean corpuscular volume (mean erythrocyte size) [(standard deviation of MCV/MCV) ×100], may be either normal or elevated. When the MCV and RDW are used in combination, the various types of anemia tend to fall in one of the six possible categories, although overlap obviously exists (Table 4.2).

Reticulocyte Count

The reticulocyte count measures the production and release of newly formed red blood cells. It should be obtained along with the CBC and peripheral blood smear in the evaluation of anemia, as it provides complementary information [25]. Reticulocytes normally contain residual RNA for about the first day that they are present in the circulation. The reticulocyte count is obtained by supravital staining of red cells with dyes that bind to nucleic acid (e.g., new methylene blue or

Table 4.2 MCV and RDW in the categorization of anemia

	Low MCV	Normal MCV	High MCV
Normal RDW	Chronic disease	Acute blood loss	Aplastic anemia
	Thalassemia trait	Inflammation	Chronic liver disease
		Renal disease	Various medications
High RDW	Iron deficiency	Early iron deficiency	B12 deficiency
	Sickle beta-thalassemia	Early B12 deficiency	Folate deficiency
		Early folate efficiency	Immune hemolysis
		Sickle cell anemia	Chronic liver disease
		SC disease	Myelodysplasia
		Chronic liver disease	
		Myelodysplasia	

ethidium bromide) in order to identify the newly released erythrocytes. Although previously determined by manual methods, the reticulocyte count is now often determined by automated methods. Since the normal lifespan of red blood cells is 100–120 days, it follows that in the absence of anemia the normal reticulocyte count is about 1%, which corresponds to an absolute reticulocyte count of 25,000–50,000/μL.

When the reticulocyte count is reported as a percentage, it must be corrected for the degree of anemia. Since the same number of reticulocytes diluted in half the number of red blood cells will double the apparent percentage present, the following correction must be applied:

$$\text{Reticulocyte count}(\%) \times \text{Patient's hematocrit} / \text{Normal hematocrit for age} = \text{Corrected reticulocyte count}(\%) \text{ or Reticulocyte index}$$

In the presence of anemia, the absolute reticulocyte count should be at least 100,000/μL. This value corresponds to a reticulocyte index of at least 2% and represents an appropriate response to blood loss or to red blood cell destruction (hemolysis). Alternatively, a decreased reticulocyte count indicates the presence of a hypoproliferative process or a red blood cell maturation abnormality. Anemias can be classified by whether or not they are associated with an appropriate reticulocyte response (Table 4.3) or by a combination of the observed MCV and reticulocyte count (Table 4.4).

Table 4.3 The reticulocyte count and causes of anemia

Reticulocytes <100,000/μL or reticulocyte index <2%	Reticulocytes ≥100,000/μL or reticulocyte index ≥2%
Hypoproliferative anemias	Appropriate response to blood loss
Iron deficiency anemia	
Anemia of acute inflammation	Hemolytic anemias
Anemia of renal disease	Hemoglobinopathies
Endocrine anemias	Membrane defects
Pure red cell aplasia	Enzyme defects
Bone marrow replacement	Mechanical causes
	Autoimmune hemolytic anemia
Maturation defects	Alloimmune hemolytic anemia
Folate deficiency	
B12 deficiency	
Sideroblastic anemia	

Peripheral Blood Smear

The wealth of information provided by the CBC and reticulocyte count noted above is greatly complimented by review of the peripheral blood smear [26]. Sometimes this may be all that is required in order to reach a diagnosis, and often carefully looking at the peripheral blood smear significantly narrows down the diagnostic entities under consideration. This can help appropriately target further laboratory investigation.

A normal red blood cell is about the size of a lymphocyte nucleus (about 8 μM). The area of central pallor occupies about one-third of the overall diameter. Hypochromic cells have too much and hyperchromic too little central pallor. *Anisocytosis* is the term used to describe variation in cell size, *poikilocytosis* describes the variation in cell shape, and anisopoikilocytosis defines the two combined. Terminology describing the more common morphologic abnormalities of the red blood cell and their associated features are listed in Table 4.5.

Integration of Information from the CBC, Reticulocyte Count, and Peripheral Smear

Because the MCV, RDW, reticulocyte count, and peripheral smear all provide complementary information, integration of

Table 4.4 MCV and reticulocyte count scheme for classification of anemia

	MCV 80–100		
MCV <80	Low reticulocytes	High reticulocytes	MCV >100
Iron deficiency	Anemia of acute inflammation	Acute blood loss	B12 deficiency
Thalassemias	Renal disease	Hemolytic anemias	Folate deficiency
		Hemoglobinopathies	
		Enzyme defects	
		Mechanical anemias	
		Autoimmune hemolytic anemias	
Anemia of acute inflammation	Endocrine anemias	Alloimmune hemolytic anemias	Liver disease
Sideroblastic anemias	Aplastic anemia		Thyroid disease
Lead poisoning	Pure red cell aplasia		
	Bone marrow failure		
	Leukemia		
	Myelodysplastic syndromes		
	Myeloproliferative syndromes		

Table 4.5 Morphologic features of the erythrocyte

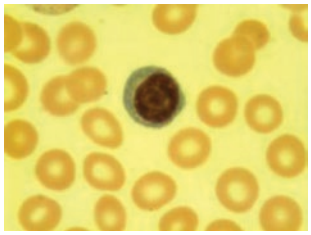
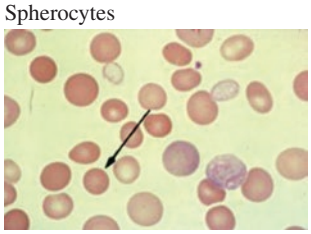
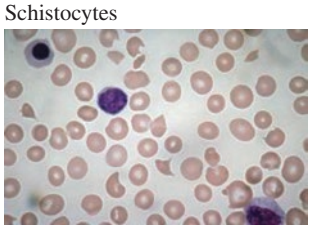
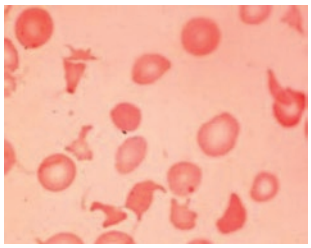
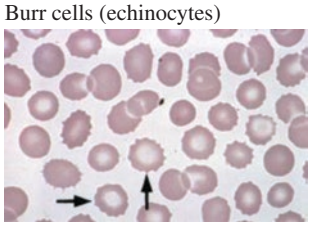
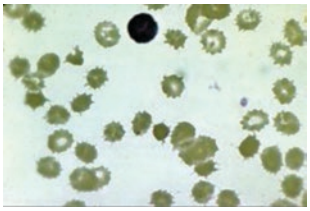
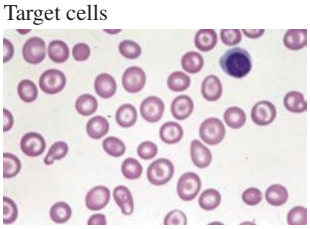
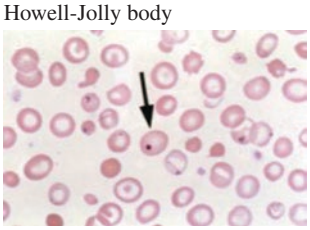
Normal erythrocyte 	Biconcave disk about 8 μM in diameter (about the size of the nucleus of a normal lymphocyte) Area of central pallor about 1/3 of the overall diameter
Spherocytes 	Loss of central pallor of the RBC DDx: immune hemolysis, hereditary spherocytosis
Schistocytes 	RBC fragmentation DDx: DIC, TTP, HUS, mechanical hemolysis
Bite cells 	Bites taken out of the RBC membrane DDx: hemolysis w/G-6-PD deficiency, unstable hemoglobins
Burr cells (echinocytes) 	Undulations of the RBC surface on blood smear DDx: uremia

Table 4.5 (continued)

Spur cells (acanthocytes) 	Spikes off of the RBC surface with loss of central pallor DDx: liver disease, abetalipoproteinemia
Target cells 	RBC that look like bull's eye targets DDx: liver disease, hemoglobin C
Howell-Jolly body 	Single purple inclusion in the RBC Represent small nuclear remnants DDx: asplenia or functional asplenia, very brisk hemolysis

these parameters leads to the correct diagnosis or provides significant insight as to the differential diagnosis (Fig. 4.1). For example, an anemia presenting with a low MCV and high RDW in which the reticulocyte count is low is almost always iron deficiency anemia. The finding on peripheral blood smear of hypochromic, microcytic cells along with wide variation in cell shape and size makes the diagnosis very likely. Iron studies and a ferritin level can then be obtained. At the other end of the spectrum, a newly occurring anemia that presents with a high MCV and high RDW in which the reticulocyte count is high is most likely to be associated with autoimmune hemolysis. A finding of spherocytes on examination of the peripheral blood smear would be highly suggestive of this diagnosis and would provide further laboratory investigation such as obtaining a direct antiglobulin test.

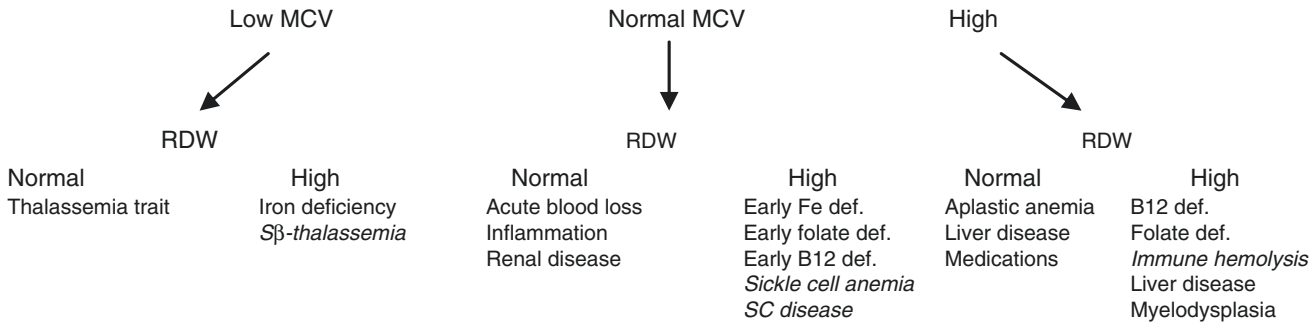


Fig. 4.1 Integration of information from the mean corpuscular volume (MCV) and red cell distribution width (RDW)

Summary

Anemia is the most commonly encountered hematologic abnormality in clinical practice. Careful consideration of the information provided by the complete blood count and reticulocyte count in conjunction with review of the peripheral blood smear often provides significant insight into the differential diagnosis and by guiding further testing expedites appropriate diagnosis with a minimum number of tests.

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