Anemia

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Anemia can be defined as a reduction in red blood cell mass below normal levels for age, gender and race. This can lead to a reduction in blood's oxygen carrying capacity and consequently oxygen delivery to tissues. To measure the red blood cell mass, we can look at one of 2 parameters: hemoglobin concentration (usually measured in grams of hemoglobin per dL of blood volume) or hematocrit, which is the fractional volume of the whole blood occupied by red blood cells represented as a percentage. Since the red blood cell hemoglobin content can be variable, these 2 parameters don't have an exact linear relationship with each other. For practical reasons, anemia is usually defined as hemoglobin concentration values more than 2 standard deviations below population mean. The problem with this statistical definition is that it may not go hand in hand with the physiologic definition or meaning of anemia. For example, while a hemoglobin value of 13 is normal in a 1-year old male child, this boy is considered anemic if he has a cyanotic congenital heart disease. On the other hand, children with hypothyroidism may have lower than statistically normal hemoglobin values due to decreased metabolic demands. The point to take home here is to look at the patient's pathophysiologic status and not only the absolute test value.

Many factors determine hemoglobin values for a specific individual under normal physiologic conditions. Blood hemoglobin concentration is at its highest at birth since it consists mainly of high affinity fetal hemoglobin. This adaptation is vital for the fetus to get enough oxygen supply in the hypoxic intrauterine environment. After birth, hemoglobin production switches to adult type hemoglobin and under the influence of higher oxygen tension, its concentration drops steadily until it reaches its nadir of about 9-10 gm/dL around the age of 12 weeks for term infants. Nadir is lower and occurs earlier for preterm infants. Post pubertal males have a significantly higher hemoglobin values compared to females. While menses may play a part in this, the difference is attributed mainly due to androgen effects in males. This means that hemoglobin concentration in males will correlate with their tanner stage. Normal hemoglobin values are also race and heredity dependent. For example, parents with higher hemoglobin values tend to have offspring with higher hemoglobin levels. The higher the altitude an individual lives at, the higher their hemoglobin concentration will be due to relative hypoxia.

Having a classification system can be very helpful approaching and diagnosing an individual with anemia. If we base our classification system on the pathophysiology of anemia it can be due to decreased production, blood loss or hemolysis. According to the cause of anemia, it can be due to a nutritional deficit, a defect in the bone marrow (can be either congenital or acquired), a hemoglobinopathy or hemolysis. Another way is to look at the red blood cell morphology (red blood cell size based on MCV, RBC indices and peripheral smear). It's vital to realize that many times we need to look at the three classifications at the same time to help arrive at the diagnosis. The simplest approach is to start by looking at the MCV and reticulocyte count and go down the diagnostic tree from there. Diagnosing anemia can be incidental on a CBC obtained for baseline values or other reasons like pre- op evaluation or to work up an infection, or it can be triggered by history (diet, blood loss, etc.), physical findings (pallor, jaundice) or symptoms (exercise intolerance, dizziness).

Iron deficiency anemia is the most prevalent type of anemia worldwide. And to have a better understanding of this entity, it's vital to quickly review the basics of iron absorption, metabolism and systemic economy. Iron is a trace element that contributes less than 60 per million of the human body weight. None the less, our life would be impossible without it. While most of the body iron (about 2 thirds) is contained in red blood cells and is essential for transporting oxygen to various tissues, iron is central to almost all major metabolic pathways (it's part of the structure of many enzymes) from nucleic acid synthesis to energy production. It's also important for normal CNS growth and functioning.

On the other hand, the accumulation of too much iron in tissues can lead to oxygen radical formation resulting in lipid peroxidation, DNA damage and ultimately tissue fibrosis, a clinical entity known as hemochromatosis.

Dietary iron comes in 2 forms: heme and non heme iron. Heme iron is found in animal sources especially red meat and is highly absorbable (around 70%), while non heme iron (ferrous Fe+2 or ferric Fe+3) is found in plant sources and is poorly absorbed (5%). It's not very well understood yet how heme iron is absorbed. The overall poor absorption of dietary iron can be explained by the fact that its absorbed exclusively in the short duodenum and most dietary iron is present in the ferric (Fe+3) form that must be first converted to ferrous (Fe+2) iron before it can be absorbed through the divalent metal transferase transport protein on the luminal surface of duodenal enterocytes. This transport protein is not specific to iron and can transport any other divalent metals as Ca+2 or Pb+2 which explains decreased iron absorption in the presence of calcium rich foods or lead poisoning. To facilitate the absorption of ferric iron, its reduced to the ferrous form through the action of a reductase enzyme present on the luminal side of enterocytes. This can be further enhanced by consuming vitamin C (orange or lemon juice for example) along with dietary iron since these anti-oxidant agents facilitate the reduction process to the ferrous form. Once inside the enterocyte, iron can be either stored in the form of ferritin, which will be removed later from the body when these enterocytes are sloughed at the end of their lives, or transported to the blood stream through ferroportin, a transport protein of the basolateral side of duodenal intestinal cells. Of note, duodenal intestinal cell sloughing is the main mechanism (in addition to menses in females) through which the human body disposes of extra iron. This process occurs at a steady rate that can't be regulated as opposed to absorption that can be. To be transported in the blood stream, iron must be converted back to the ferric form so that it can be carried on transferrin to target tissues. This happens through the action of an oxidase enzyme.

Ferroportin can be up or downregulated by the action of Hepcidin, a peptide synthesized by the liver and excreted by the kidneys, it acts as a feedback inhibitor to ferroportin, or in other words, cellular iron export. This is the main mechanism through which iron absorption is regulated. Hepcidin plasma levels are decreased in response to iron deficiency states and increased erythropoietic demand and are elevated in response to iron overload and inflammation.

We need to differentiate between iron deficiency and iron deficiency anemia, with the former being a disorder with impact on multiple body systems and the latter being a late manifestation of this disorder. More than a billion people are iron deficient globally and only a small fraction of these present with anemia. Given the prevalence of iron deficiency and the lack of significant symptoms before the anemia stage, we can appreciate the large scale and alarming nature of this problem especially that some of the systemic effects, such as neurodevelopmental delay can be irreversible. Populations at higher risk for iron deficiency include teenage girls, vegetarians, individuals of lower socioeconomic status with less access to red meat products and infants and toddlers beyond the first 6 months of life who are predominantly on an only cow milk diet. Cow milk iron has a much lower bioavailability than breast milk iron while both types of milk have comparable iron content. For this reason, cow milk based infant formulas are fortified with iron to make up for the gap in absorption and cereals are fortified with iron for infants and toddlers. Excessive intake of whole cow milk in toddlers can result in iron deficiency due to induction of satiety limiting intake of iron rich food compared to the low content, poorly absorbed cow milk iron. In addition, whole cow milk intake during infancy may lead to micro GI bleeds exacerbating iron deficiency.

Iron deficiency anemia generally presents with microcytic red blood cells secondary to the decreased red blood cell content of hemoglobin. As iron stores run out, the bone marrow will start producing variable sizes of red blood cells (anisocytosis) depending on the amount of iron available for erythropoiesis at that time. This drives an increase in the red cell distribution width (RDW), which is the coefficient of variation of red blood cell volumes. Diagnosis can be supported by historical findings, such as poor intake of iron rich foods and menorrhagia. While in most cases this can be sufficient for diagnosis (coupled with complete response to iron replacement therapy), further laboratory studies can be obtained to help with confirmation such as serum ferritin reflecting total body iron content, serum iron, and total iron binding capacity (serum transferrin) levels.

Before proceeding with treatment, it's vital to have the etiology identified as iron deficiency can be the result of an underlying disorder. In addition to dietary factors, excessive blood loss as in GI or urinary tract bleeds or underlying malignancy need to be considered although less common. In addition to treating underlying causes and correcting dietary problems, treatment consists of oral iron replacement therapy given usually at a dose of 3-6 mg/kg/day of elemental iron. We need to note that there are numerous commercially available forms of oral iron supplements with multiple different chemical formulations thus requiring the dose to be based on the elemental iron content rather than the chemical compound dose. Packed red blood cell transfusions maybe considered but are usually reserved to symptomatic patients and those with brisk bleeding that can't be kept up with through oral replacement therapy. Parenteral formulations of iron replacement are also available but not widely used in children and are generally preserved for the rare patients with iron malabsorption. Once therapy is initiated, the first parameter to show signs of recovery is reticulocyte iron content (if measured) followed by reticulocytosis. This is followed by a gradual increase in hemoglobin values with the time for normalization depending on pretreatment hemoglobin concentration levels and the dose of iron used but is generally from 2-3 months. Iron replacement therapy needs to continue for 1-2 months beyond normalization of hemoglobin levels to replenish the exhausted iron stores as these are the latest to normalize and the ultimate goal of therapy is to cure iron deficiency rather than anemia itself. Early on treating iron deficiency, RDW will increase significantly before trending down again due to the production of normal size red blood cells once serum iron rises while the older microcytic cells are still around leading to increased variation in red blood cell sizes. As with many other conditions, the most common cause for oral iron therapy failure is non-compliance followed by other causes like incorrect diagnosis (usually second most common), in adequate iron dosage, ongoing underlying problem (GI and renal losses) and malabsorption.

Occasionally, iron deficiency can be secondary to malabsorption. This can be due to multiple causes as consumption of poorly absorbed iron food sources such as whole milk as outlined earlier, competitive inhibition by other divalent metals as in lead poisoning or absorptive surface issues such as post duodenectomy, short gut syndrome and gluten-sensitive enteropathy. This can be screened for with the oral iron challenge test. A very rare form of iron deficiency anemia due to malabsorption is iron refractory iron deficiency anemia, characterized by congenital onset of severe microcytic anemia (MCV 50-60 fL) with no response to oral iron therapy and sluggish response to intravenous iron therapy. This condition is the result of a mutation in the TMPRSS6 gene that leads to very high blood hepcidin levels.

One of the effects of iron deficiency on the central nervous system is PICA in which an individual develops an appetite to non-nutritive items such as dirt, cardboard, paper, ice cubes (pagophagia) etc. this is of special historic interest in older built houses with lead based wall paints as children may eat the fallen paint chips. While this leads in the first place to lead poisoning, we need to keep in mind since this metal is divalent, it will competitively inhibit iron absorption and lead to worsening of iron deficiency which can exacerbate PICA and so on. While lead poisoning leads to basophilic stippling in red blood cells, the associated microcytotis is caused by concurrent iron deficiency.

Anemia of inflammation has been recognized for a long time but the explanation of this phenomenon lagged for decades after that. Inflammatory mediators can be a potent positive feedback regulator of hepcidin levels. This results in inhibition of cellular export of iron and a picture compatible with iron deficiency anemia. The main benefit of this is the body trying to cut off iron supplements needed for bacterial DNA replication thus in a form fighting infection. Since this mechanism is not specific to infection, this explains the anemia seen in non-infectious inflammatory disorders such as SLE.

We present folate and vitamin B12 (Cobalamin) deficiency together here as there are many similarities between them in presentation and pathophysiology. Since both are essential for DNA synthesis and regulation, they will present with very similar hematologic pictures including anemia, macrocytotis and hyper segmented neutrophils. Severe deficiency of either can lead to pancytopenia that can be frequently misdiagnosed as or confused with other entities. We call this picture megaloblastic anemia and is due to impaired DNA replication. This results in nuclear-cytoplasmic desynchrony

with abundant cytoplasm and impaired nuclear maturation. Bone marrow findings are similar to peripheral blood. Megaloblastic anemia is considered among the causes of ineffective erythrocytosis, a group of disorders with different pathophysiologies resulting in the premature death of red blood cells. This can be accompanied by hyperbilirubinemia. While these two entities present with similar hematologic pictures, we have to note that only cobalamin deficiency presents with neurologic abnormalities of these two. Both folate and cobalamin deficiency can result in the physical finding of a smooth tongue. There are other causes of megaloblastic anemia such as thiamine deficiency, inherited metabolic disorders (hereditary orotic aciduria), MDS, AML M6, drugs and toxins. We also need to differentiate megaloblastic anemia from other causes of macrocytotis such as hemolysis, liver disorders and hypothyroidism.

Folate is abundant in diet especially green leaves, fruits and vegetables making folate deficiency relatively rare except in cases of severe malnutrition and increased demand such as pregnancy and hemolytic anemias. Folate malabsorption can be seen in gluten sensitive enteropathy (celiac disease), severe Crohn's disease and jejunal resection. Other causes of folate deficiency include the use of anti-folate drugs such as methotrexate and alcohol consumption. It's absorbed to a small extent in the duodenum but mainly in the proximal jejunum and is stored in the liver. Non-hematologic effects of folate deficiency include neural tube defects in babies and elevated plasma homocysteine levels.

Cobalamin is absorbed in the terminal ileum by binding to the cubulin receptor after it forms a complex with the intrinsic factor produced by gastric parietal cells. It's also stored in the liver and typically enough is stored for a several-year supply. Cobalamin is found only in foods of animal origin so strict vegetarians (Vegans) are at a high risk for deficiency. Among the other causes of vitamin B12 deficiency are gastrectomy, autoimmune gastritis (pernicious anemia), ileal resection, Crohn's disease, imerslund- Grasbeck syndrome, severe pancreatic disease and trans cobalamin II deficiency. Once absorbed, cobalamin is transported in the plasma by trans cobalamin I and II and then will enter the cells with the help of trans cobalamin II.

There are 2 subtypes of pernicious anemia. The adult type, or autoimmune form, is relatively common and rarely acquired during childhood. It's characterized by reduced or absent production of intrinsic factor by gastric parietal cells and is associated with other autoimmune disorders. Autoantibodies are directed against parietal cells, intrinsic factor, or both. Antibodies against the intrinsic factor are specific but not sensitive and it's the other way around for anti-parietal cell auto antibodies. Testing today is based mainly on evaluating for the presence of these antibodies as compared to the historically used Schilling test that utilizes the oral administration of radiolabeled B12, with or without intrinsic factor and measures B12 absorption indirectly by measuring radioactivity in the urinary excreted vitamin B12. This way, we can distinguish vitamin B12 malabsorption due to internist factor deficiency from other causes. The other form is the rare, autosomal recessive juvenile pernicious anemia caused by mutations in the intrinsic factor gene. The mainstay of treatment of both forms is parenteral B12 supplements.

Other rare congenital causes of vitamin B12 deficiency include imerslund- Grasbeck syndrome where an autosomal recessive mutation in the cubulin gene results in early infancy presentation of megaloblastic anemia associated with proteinuria. This results in an abnormal Schilling test not corrected by the addition of intrinsic factor. Trans cobalamin II deficiency is one rarer autosomal recessive disorder that presents during early infancy with pancytopenia, diarrhea and failure to thrive. It's characterized by normal plasma vitamin B12 due to the presence of trans cobalamin I but it's not able to enter the cells to exert its biological activity due to the absence of trans cobalamin II. Since vitamin B12 is absorbed here, Schilling test will be normal. Treatment is with high doses of IM vitamin B12.

Once treatment is initiated, the first notable response would be reticulocytosis within a few days up to a week. Anemia is usually corrected within a month and this is followed by normalization of the MCV within 1-2 months. It's extremely important to understand that the hematologic picture of vitamin B12 deficiency can be corrected using high doses of folic acid. This, however, doesn't reverse the neurologic abnormalities which may continue to worsen in the face of improving anemia. Thus, correct diagnosis is of paramount importance.

There are other less common forms of nutritional anemia. Notable examples are copper deficiency resulting in microcytic anemia (since copper is needed for iron transport, hephastin) and in severe cases, neutropenia. Further causes include severe protein malnutrition (Kwashiorkor) or anorexia nervosa.

Hemolytic anemia results from abnormally increased destruction of red blood cells. This is usually associated with a compensatory increase in erythrocytic activity and jaundice. Other associated clinical findings with hemolysis include splenomegaly, dark urine and gallstones. Hemolytic disorders can be either congenital or acquired and can be driven by factors related to the red blood cell membrane, inside the red blood cells or outside the red blood cell.

The first group of hemolytic disorders has to do with red blood cell membrane abnormalities. These disorders are hereditary in nature. The most famous example is hereditary spherocytosis which is the most common cause of non-immune hemolytic anemia. Most cases are autosomal dominant with about a quarter due to sporadic mutations thus lacking a family history. The less common autosomal recessive cases tend to be more severe. The biconcave shape of normal red blood cells provides more membrane surface area compared to the cell volume thus making gas exchange across the membrane more efficient. This effect is lost in hereditary spherocytosis in addition to making the cells less deformable when passing through capillaries and eventually reducing the life span of erythrocytes and subjecting them to destruction when they pass through the spleen. The degree of anemia can be variable depending on the specific mutation. About a quarter of patients have mild compensated hemolysis with no clinical anemia. Often, the first presentation is neonatal jaundice shortly after birth or as exacerbation of physiologic hemoglobin nadir of infancy. Gallstones and splenomegaly are common findings and family history is usually positive except when it's a new onset mutation. These patients are at high risk for parvo virus B19 associated aplastic crisis. Hereditary spherocytosis is suspected based on the clinical picture and the presence of spherocytes in peripheral blood in addition to indirect hyperbilirubinemia, increased mean corpuscular hemoglobin concentration (MCHC) and reticulocytosis. It can be confirmed with the osmotic fragility test where red blood cells are incubated in increasing concentrations of saline. Normal cells can tolerate hypotonic solutions down to 0.5% saline while cells affected with hereditary spherocytosis tend to lyse at higher concentrations. For clinically severe cases, splenectomy can correct the anemia and jaundice. This, of course, won't correct the morphology of the erythrocytes and puts the patient at a high risk for post splenectomy sepsis.

A related condition is hereditary elliptocytosis, characterized by elongated, elliptical, cigar shaped red blood cells and is usually inherited in an autosomal dominant pattern. It tends to be much less symptomatic than hereditary spherocytosis. Splenectomy can be helpful in severe cases.

Moving to hemolysis secondary to abnormalities arising from within the erythrocytes. These are usually inherited and can be either hemoglobin related disorders or enzymopathies. We differ discussing hemoglobinopathies to another section. Enzymes inside the red blood cell carry diverse and vital functions including energy production, maintaining the ion gradient across the cell membrane and keeping the hemoglobin iron in the reduced ferric state in addition to many others. Abnormalities in these enzymes can lead to various clinical problems but here we will focus on those with hematologic effects.

Glucose-6-phosphate dehydrogenase, G6PD, deficiency is the most prevalent red blood cell enzyme disorder with over 100 million people affected worldwide and is inherited in an X-linked recessive manner. G6PD is required for the synthesis of NADPH which maintains reduced glutathione stores in the cells that protect hemoglobin from oxidation. Its deficiency results in hemolytic disease upon exposure to oxidative stress such as infection, fava beans and naphthalene. Denatured hemoglobin can be seen on the peripheral smear as Heinz bodies. The most common, less severe form, usually affects people of African descent and usually presents with mild hemolytic disease under stressful conditions such as infections. This is in contrast with the Mediterranean more severe form that can result in more significant hemolysis with minor oxidative stress. Since reticulocytes have a much higher G6PD content, measuring the enzyme

activity just following an acute hemolytic episode can result to false negative testing. Pyruvate kinase deficiency is an autosomal recessive disorder resulting in decreased ATP production inside the red blood cell causing membrane damage and eventual hemolysis.

The third category of hemolytic anemias include conditions that arise from outside the red blood cell. This is a very diverse group of unrelated conditions that are acquired for the most part. These are usually subcategorized into mechanical, immune mediated and complement mediated conditions. Mechanical causes include hypersplenism, some toxins or drugs (e.g., alpha methyl dopa), thermal burns and microangiopathic hemolytic anemia (DIC, thrombotic thrombocytopenia purpura, hemolytic uremic syndrome etc.) where hemolysis can be induced by injury to red blood cells from fibrin strands.

Immune mediated hemolytic anemia can be subclassified into alloimmune and autoimmune. Alloimmune hemolytic anemia is when the origin of antibodies causing hemolysis in the patient is from another human. The classic example of this is neonatal alloimmune hemolytic anemia or hemolytic disease of the newborn (or erythroblastosis fetalis). The process is initiated by transplacental passage of maternal alloantibodies of the IgG subtype specific to fetal red blood cell antigens to the fetal circulation leading to hemolysis of fetal or newborn red blood cells resulting in anemia and jaundice with the risk of developing hydrops fetalis and kernicterus. This used to be a leading cause of kernicterus and cerebral palsy in the past. The most well-known cause to this is Rh incompatibility (although many other blood groups may cause it) where feto-maternal hemorrhage, either spontaneous or following trauma or a procedure, leads to maternal (Rh negative mother) sensitization to the D antigen on fetal red blood cell. The first pregnancy is spared since antibody production requires several weeks but these antibodies will be available to inflect damage on subsequent fetuses with Rh + blood. These infants present with an anemia along with a positive direct globin test (direct coombs test) and a peripheral smear containing nucleated red blood cells with polychromasia. Maternal antibody screen (indirect coombs test) will also test positive. This situation can be prevented by administering anti D antibodies to Rh negative mothers before birth to neutralize Rh + fetal cells in maternal circulation before recognized by the maternal immune system.

There are several types to autoimmune hemolytic anemia. The most widely recognized is warm autoimmune hemolytic anemia that is IgG mediated most of the time. This is a form of extravascular hemolysis since IgG autoantibodies attaching to RBCs do not initiate a hemolytic reaction on their own but rather antibody tagged red blood cells are marked for clearance by splenic macrophages. While this form of hemolytic anemia is generally idiopathic in children, it can be secondary to conditions like lymphomas, SLE, immune deficiencies and post bone marrow transplant. Direct Coombs test (direct anti globin test or DAT) is generally positive in this situation for IgG and sometimes for C3. This form of hemolytic anemia is usually sensitive to corticosteroids. Other lines of treatment include splenectomy, IVIG and immunosuppressive therapy.

Cold antibody hemolytic disease on the other hand is generally IgM mediated. It's named so because the antibody-RBC immune complex is formed at lower temperatures in the peripheral blood vessels under the skin and gets activated when warmed centrally when the blood returns to the heart. Hemolysis occurs intravascularly. This form of hemolysis can be associated with mycoplasma and EBV infection. Direct antiglobin test is usually positive for C3. Treatment is usually conservative by keeping the patient warm in the colder weather and removing excess antibodies by plasma pheresis in severe cases. Its worth noting that plasma pheresis is not generally utilized in warm antibody disease since the volume of distribution of IgG is much larger than IgM making removal from the circulation by this method impractical.

Paroxysmal cold hemoglobinuria is considered to be a rare entity in childhood. It's usually associated with acute illness such as viral URI, measles, mumps, mycoplasma, varicella. It's mediated through a cold reactive IgG antibody (Donath Landsteiner antibody) that is specific to the P antigen on RBC's. This entity is typically self-limiting and may last several weeks.

Less common forms of hemolytic anemia include drug induced hemolytic anemia, thrombotic thrombocytopenic purpura PNH (Paroxysmal nocturnal hemoglobinuria) and others. Among the drugs that may precipitate hemolysis are: Penicillins, some cephalosporins such as Ceftriaxone, Tacrolimus and Fludarabine. Mechanisms are diverse and are not of interest here. Some toxins and infections might also precipitate hemolysis such as clostridium sepsis in addition to spider bites, snake venom etc. Finally, burns and trauma might also precipitate hemolysis.

Paroxysmal nocturnal hemoglobinuria (PNH) is now recognized as an acquired stem cell disorder. Where a clone of the red blood cells that is susceptible to complement mediated hemolysis (fragile) takes over erythropoiesis. This clone is characterized by the lack of GPI linked proteins (membrane proteins). Diagnosis in the past realized on Ham's test but now a days we can identify this clone via flowcytometry looking for CD55 and or CD59 deficient clones. Until recently, the only treatment for this was bone marrow transplant but now with the availability of eculizumab we can block the complement pathway and prevent hemolysis.

Finally, thrombotic thrombocytopenic purpura is characterized by the classical pentad of fever, microangiopathic hemolytic anemia, thrombocytopenia, renal dysfunction and neurological changes. Of note, there has been a shift in the recent decades to diagnose this entity without the need for documenting all five elements. This disorder is caused by abnormally elevated levels of high molecular weight Von Willebrand factor due to deficiency of the cleaving protease encoded by ADAMTS13 gene. The presence of very large molecular weight vWF multimers leads to microvascular fibrin deposition followed by platelet trapping leading to thrombocytopenia and microangiopathic hemolytic anemia characterized by the presence of peripheral schistocytes. This disorder could be congenital due to the absence of the enzymes encoded by the gene or acquired resulting from an autoantibody to the vWF cleaving protease (ADAMST13 protein). Treatment of choice is plasmapheresis for the acquired form while the congenital form responds to ADAMST13 protein supplement through fresh frozen plasma infusions.