Anemia: classification challenge and clinical questions

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December 2014—Anemia is in the eye of the classifier. While that's not as elegant as the "beauty-beholder" saying, it's much more important. To be able to effectively treat and diagnose anemia, "You have to know what is causing the decrease in red cells," said Sherrie Perkins, MD, PhD, speaking at an AACC workshop this year.

There are plenty of definitions to choose from, said Dr. Perkins, of the University of Utah/ARUP Laboratories, Salt Lake City. At the most basic level, she noted, anemia is a pathologic condition marked by a reduced capacity of blood to transport and deliver adequate oxygen to tissues. In short, anemia is a manifestation of disease, not a disease itself.

Dr. Perkins

The most common CBC parameters used to establish anemia include hemoglobin concentration, hematocrit, red blood cell concentration, and mean corpuscular hemoglobin. "That will give us some very good information," but it's far from perfect, she said, since hemoglobin concentration and hematocrit utility may be affected by altered plasma volumes, chronic oxygenation status, and hemoglobin variants/hemoglobinopathies.

The NHANES definition uses the lower limit of normal adult hemoglobin (from 10 to 44 years): 13.2 g/dL in men, 11.7 g/dL in women. The WHO, relying on a technique common among checkbook balancers, "tends to round to make things a little bit easier" and bases its definition on 13.0 g/dL in men and 12.0 g/dL in women. Beyond this, Dr. Perkins said, it's well understood that acceptable levels are lower in children and in women during pregnancy; the African-American population also tends to have slightly lower (0.5 to 0.6 g/dL) values. Men tend to have slightly higher hematocrit levels than women—also considered to be "completely normal," she said.

Anemia is dependent on the RBC lifespan. To understand it, "We have to know where the red cells come from." Under normal conditions, bone marrow erythroid production is constant. The normal 70-kg adult RBC mass is about 2,000 mL, or 300 × 109 RBCs/kg. The normal RBC lifespan is 100 to 120 days, so any alterations that affect RBC production or lifespan may cause anemia. Replenishment requires adequate bone marrow hematopoietic pools, nutrients, and stimulation of proliferation by factors such as erythropoietin.

On a practical level, the various definitions and symptoms create an interesting classification challenge. Historically, noted Dr. Perkins, people used CBC data and red cell morphology, primarily the mean corpuscular volume (i.e. RBC size). Under this scheme, anemias were determined to be microcytic (95 fL). This remains a common diagnostic approach because it provides useful pathophysiologic insights.

Another useful way to think about anemia classification is pathogenetically. Anemias can occur because of impaired proliferation or maturation. This could be related to the bone marrow, such as in diseases like aplastic anemia or myelodysplastic syndromes. Tumors can also cause bone marrow infiltration, leading to less bone marrow capacity for red cell production. Other possible culprits include vitamin deficiencies; marrow suppression from drugs, radiation, or infections; and chronic disease/inflammation.

On the other hand, anemia can be caused by increased destruction (i.e. hemolysis) of red cells. Shortened RBC lifespan often results from inherent abnormalities of the red cells, such as membrane or enzyme defects. It can also be caused by hemoglobinopathies, immune-based hemolytic anemia, infections of red cells (e.g. malaria), or splenic removal. Finally, there's plain old blood loss.

Dr. Perkins divided pathogenetic classification into three basic categories:

- hypoproliferative: bone marrow damage, deficiencies (e.g. iron), and decreased stimulation by EPO (renal disease, inflammation, metabolic disease);
- maturation disorders: myelodysplasias, and other conditions, such as vitamin B12 and folate deficiencies;
- inability to sufficiently replace red cells with a decreased lifespan due to rapid turnover from hemorrhage or hemolysis.

What parameters are useful for classifying anemia?

The reticulocyte count is the best way to identify whether there's been a bone marrow response, said Dr. Perkins, and is the best test to distinguish between hypoproliferative anemias and those related to problems with RBC lifespan or blood loss.

Reticulocyte counts haven't always been easy, however. When done manually, there was a high degree—20 to 25 percent—of imprecision. "And you had to count a huge number of red cells manually to be able to get a good reticulocyte count."

Fortunately, this parameter has moved from the counting chamber to automated CBC analyzers. With some of the more recent analyzers, it's even possible to look at subpopulations of reticulocytes, including immature reticulocyte fraction, nucleated RBCs, as well as reticulocyte hemoglobin concentration. To understand anemia in a useful way, said Dr. Perkins, it's necessary to correct the reticulocyte count for the degree of anemia. "That also helps us to know whether we're getting a good bone marrow response or whether there may be some problem at the bone marrow level."

Dr. Perkins called immature reticulocytes an interesting new parameter. The immature reticulocyte fraction or reticulocyte maturity index enables a comparison between immature reticulocytes (which contain the most RNA) and mature reticulocytes. "That is very useful to see if the bone marrow is beginning to respond," said Dr. Perkins, in cases involving, say, recovery from chemotherapy or engraftment post-transplant. The IRF will increase in such cases. The IRF can also be used to monitor response to therapy in iron deficiency, though Dr. Perkins cautioned this application "is not very well

developed. I think we'll be seeing this evolving over the next couple years" before it becomes readily available.

Nucleated RBCs are commonly seen in neonates. When seen in adults or older children, they represent an abnormal finding, one that's usually associated with extreme erythrocytic activity or marrow damage. It's important to identify NRBCs because they may spuriously elevate white cell counts.

When evaluating anemia in the laboratory initially, it's important to ask certain clinical questions as well, Dr. Perkins said. (See page 18.)

A good place to start: Is the anemia associated with other hematologic abnormalities? If thrombocytopenia or leukopenia is involved, for example, "then you may want to do a bone marrow examination" to assess for leukemia, aplastic anemia, myelodysplasia, etc. If not, then check to see if there is an appropriate reticulocyte response to anemia. "If there is, then that will point us toward hemolysis," said Dr. Perkins. "And if there is not, then we start wondering about the red cell indices so that we can classify it by the more classical means."

When Dr. Perkins began her career as a hematopathologist some 25 years ago, she recalled, "We used to do bone marrows all the time for anemia." That's no longer the case. Now, "It's most useful when you have reticulocytopenia or other abnormalities in your blood count or peripheral smear, or if you think there may be possible systemic disease such as a metastatic tumor causing your anemia."

Microcytic anemia—one of the more classic ways of thinking about anemia—can have several causes, including disorders of iron metabolism, globin synthesis defects, disorders of heme synthesis (either hereditary or acquired). "And occasionally we can see it with lead intoxication," she added.

Hypochromic microcytic anemias have a complex testing algorithm. "Typically we start with our CBC and that very important reticulocyte count," Dr. Perkins said. It's also common to include iron studies. If those don't yield a clear answer, looking at bone marrows would be next. "Oftentimes we want to do a smear review. If we see abnormal morphology, such as target cells, we would think about thalassemia or perhaps other hemoglobinopathies or liver disease." If there are no other diagnostic changes, consider looking at the red cell distribution width (high RDW could indicate iron deficiency and should be followed by iron staining/iron studies) and red cell count.

Reticulocyte counts can also help identify the underlying cause of microcytic anemia. Low or normal counts are more likely to be associated with iron deficiency, anemia of chronic disorders, some of the thalassemic traits, and sideroblastic anemia. If it's increased, that could point to hemoglobinopathies, red cell membrane disorders, or other hemolytic anemias.

Iron deficiency anemia is one of the most common nutritional deficiencies in the world, Dr. Perkins said—and thus it's a common visitor to laboratory workups. The key proteins to think about are transferrin, transferrin receptor, and ferritin. There's also hepcidin, an acute phase reactant produced in the liver; it's upregulated in inflammation and downregulated in iron deficiency. It's "emerging as one of the important pathophysiologic mediators of iron transport" and might play a role in future laboratory testing.

Elliptocytes can be "a very good morphologic clue" to identifying iron deficiency. Target cells are uncommon, but thrombocytosis may be present. "And oftentimes we'll see anisopoikilocytosis (high RDW on CBC)" as well as a low reticulocyte count, because the marrow lacks enough iron to make more

red cells.

Anemia of chronic disorders, or ACD, is the second most common cause of anemia and a common differential diagnostic consideration, especially in hospitals. Patients, after all, are there because they're ill. This often is mild or moderate anemia and characterized by low serum iron. Unlike iron deficiency, however, ferritin will be normal or increased. ACDs are seen with chronic infections (subacute bacterial endocarditis, osteomyelitis, TB), collagen vascular disease (rheumatoid arthritis), inflammatory states, and malignancy. "So you can see that we're covering a wide range of the patients [who] would be seen in a hospital setting."

The pathogenesis of ACD has become clearer in recent years. It's multifactorial but due primarily to cytokine effects on the bone marrow. "We see increased levels of IL-1, IL-3, and IL-6"—inflammatory cytokines that are seen in infection and collagen vascular diseases that dysregulate iron transport and erythropoiesis. These cytokines also divert iron into storage pools, making less of it available for RBC production, and they may inhibit erythroid proliferation, erythropoietin responses, and RBC lifespan. "If we do a bone marrow in a patient who does have anemia of chronic disease, we tend to see a lot of iron, which is trapped in the histiocytes as storage iron, but we do not see any going to the erythroid precursors."

Looking at the morphologic findings in hypochromic microcytic anemia, beware of significant overlap, Dr. Perkins cautioned. "Typically we're going to see decreased red cell numbers for chronic disorders as well as iron deficiency," Dr. Perkins said. The RDW is increased with iron deficiency; it's variable in chronic disorders and oftentimes not increased. Basophilic stippling is not seen in either type.

To differentiate between iron deficiency and anemia of chronic disorders, iron studies can be useful. Serum ferritin in particular is an excellent marker and is starting to replace bone marrow iron stain as the gold standard for bone marrow iron stores. Soluble transferrin receptor is also useful because it's not altered by inflammation. But again, Dr. Perkins raised the red flag of overlapping studies. Serum iron, for example, is often decreased in both iron deficiency and chronic disorders; there can also be significant overlap in transferrin saturation and in total iron-binding capacity. Ferritin, on the other hand, is oftentimes elevated in chronic disorders and decreased in iron deficiency. "Typically if you have a nondiagnostic ferritin, then you go ahead and look at your TIBC," Dr. Perkins said. If it's low, then it's likely anemia of chronic disorders. "You can also look at your percent transferrin saturation and your serum soluble transferrin receptor" to distinguish the two entities. "And hopefully, with all these tests, you will not need to do a bone marrow."

Dr. Perkins is intrigued by the unfolding story of hepcidin and its role in ACD. This iron regulatory hormone, synthesized in the liver, was discovered in 2001. Hepcidin expression is increased by lipopolysaccharide (from infections) and IL-6 (from infections and inflammation). It appears to block duodenal absorption of iron, Dr. Perkins said, and blocks release of iron from macrophages. "So this may be the hormone that is altered when we have anemia of chronic disorders." And it could explain the decreased iron but increased or normal ferritin often seen in these disorders.

Hepcidin can be measured in the blood by mass spectrometry and in urine by cation exchange chromatography. Antibody testing is in development, "but because hepcidin is bound to serum protein complexes, it's not always accurate." Nevertheless, she predicted that measurement of hepcidin levels could eventually help diagnose and classify iron storage disorders, including ACD and hereditary hemochromatosis.

CBC detection of so-called functional iron deficiency also looks promising, she said. Typical iron studies do not reflect the entire lifespan of red blood cells, and thus are not sensitive to early deficiencies or treatment response. It can take 10 days to two weeks for hematocrit levels to rise after treatment with iron, and use of reticulocyte hemoglobin level will provide information on early response to iron therapy. Not only would this measurement be useful in treatment monitoring for resistant iron deficiency, said Dr. Perkins, but it could also be helpful when erythropoietin stimulation of erythropoiesis is used in other disorders to evaluate response. “I think this is going to make it into general utilization in the next two to five years.”

Reticulocyte hemoglobin content is assessed with flow-type systems that measure red cell volume and hemoglobin content of reticulocytes based on light and/or forward scatter properties, she said. The reference value is typically 30.8 pg/reticulocyte, but the lower limit is 28 pg. “It really does provide quite nice data about early responses for red cells that have been produced over the last three to four days,” she said, making it particularly helpful for noting early response to IV iron therapy or erythropoietin responses.
Shifting gears, Dr. Perkins turned to normocytic and hemolytic anemia—those more likely to be medical emergencies.

Pathogenetically, normocytic anemias can be classified as anemia associated with appropriately increased erythrocyte production (usually post-hemorrhagic or hemolytic anemia); anemia with impaired marrow response (aplasias, hypoplasias, infiltration, myelodysplasia); and anemia associated with decreased erythropoietin secretion (renal and hepatic anemias “that are very commonly seen in our hospital-based populations,” Dr. Perkins said).

There’s nothing radical about evaluating these anemias. “We want to look at a smear and get the reticulocyte count,” said Dr. Perkins. But there’s also strong incentive to obtain good clinical information or screen for liver, endocrine, or renal disease, because these often underlie the etiology of normocytic anemia. Iron studies for early (premicrocytic) iron deficiency or anemia of chronic disease may also be warranted. In some cases of a normocytic hypoproliferative anemia, a bone marrow biopsy might also be useful.

If reticulocytes are increased, “you want to know a lot of historical information about the possibility of hemorrhagic or hemolytic anemia. If you have increased bilirubin and LDH, you need to think about hemolysis.”

Renal, hepatic, or endocrine function should also be looked at if reticulocytes are normal or decreased, she continued. It might also be wise to evaluate erythropoietin levels and to consider the possibility of anemia of renal disease, liver disease, or endocrine failure. A low serum iron gives rise to the possibility of anemia of chronic disorders or early iron deficiency.

Peripheral smear evaluations can be quite useful in evaluation of normocytic anemias, Dr. Perkins said. Seeing nucleated RBCs or leukoerythroblastosis would suggest myelophthisic processes, such as infiltration of the marrow by a hematopoietic or metastatic tumor or marrow fibrosis. This would prompt a bone marrow, as would the presence of abnormal blood cells, such as blasts or lymphoma cells.

Hemolytic anemias are typically normocytic anemias, but they can be slightly macrocytic, especially if there’s a very high reticulocyte count. They’re also characterized by biochemical evidence of RBC destruction, such as increased LDH, increased bilirubin, decreased haptoglobin, and the evidence of

hemosiderin, which is an insoluble iron oxide that oftentimes is deposited in tissues and may be seen in urine.
"Pathophysiologically," said Dr. Perkins, "we want to think about whether the defects causing the hemolysis are intrinsic, which are due to defects in the red cells themselves." This includes membrane and enzymatic defects and hemoglobinopathies. Extrinsic causes are usually caused by immune-mediated hemolysis or physical damage to the red cells—exposure to toxins, drugs, or microangiopathic processes.

Labs need to identify microangiopathic hemolytic anemias (MAHA) "because they can be medical emergencies." Microangiopathic hemolytic anemia is marked by inappropriate intravascular clotting, which in turn leads to consumption of platelets and hemolytic anemia due to red cell destruction. Dr. Perkins identified the three major types of MAHA: thrombotic thrombocytopenia purpura (TTP), hemolytic uremic syndrome (HUS), and disseminated intravascular coagulation (DIC).

The coagulation associated with TTP can cause severe central nervous system symptoms and often requires plasmapheresis for treatment. HUS is caused by the Shiga toxin and occurs with improper handling of cow fecal waste that contaminates meat or water used in agriculture—and often receives a lot of press, she said, recalling the Jack in the Box E. coli outbreak of 1993 and later outbreaks associated with spinach and other vegetables. Of the three, DIC is the one most commonly seen in hospitals, where infections, obstetric complications, traumas, tumors, and other causes can lead to massive induction of intravascular clotting/coagulation. "When we look to make a diagnosis of DIC, probably the most specific thing that we can use is a markedly elevated D-dimer." Decreased platelets, decreased hematocrit, and shistocytes are not specific. "But it's important to identify DIC, because you need to treat the underlying disorder to treat the inappropriate coagulation."

In short, if labs suspect a microangiopathic hemolytic anemia, based on anemia, thrombocytopenia, and presence of shistocytes on the smear, "then we need to do additional testing to identify the cause," Dr. Perkins said. "So we do tests for coagulation, Shiga toxin, and ADAMTS-13." An ADAMTS-13 deficiency identifies TTP. Elevated D-dimer, as noted, is linked to DIC. And if neither is the case, "we can start considering HUS."

Dr. Perkins concluded her talk by addressing macrocytic anemias, which are megaloblastic—or not. Those that aren't are associated with liver disease, hypothyroidism, and myelodysplasia whereas the megaloblastic macrocytic anemias are associated with vitamin B12 and folate deficiencies.

In patients with a macrocytic anemia, laboratories should start with a blood smear. "Do we have any hypersegmented neutrophils or macro-ovalocytes?" The former are an important morphologic clue to megaloblastic anemias and should lead to B12 and folate testing. A drop in B12 should point in the direction of a GI cause leading to poor absorption. A decreased folate may be due to poor diet, GI disease, or the demands of pregnancy, infancy, or chronic hemolysis.

A lack of hypersegmented neutrophils should turn labs in the direction of nonmegaloblastic anemia. Look at the reticulocyte count. If it's increased, there might be an unrecognized hemolysis or hemorrhage. If it's normal or decreased, consider etiologies such as alcohol toxicity, hypothyroidism, or liver disease.

And if none of these approaches yields answers, it's time, once again, to turn to the bone marrow, to rule out the bone-marrow–related causes of megaloblastic anemia, such as myelodysplasia and aplastic anemia. Myelodysplasia is probably the most common, particularly in older patients, and can be seen in

other dysplastic changes in the marrow, such as hypolobated and hypogranulated neutrophils and blasts.

Despite her expansive survey, Dr. Perkins didn't address hemoglobinopathies and other causes of anemia. It's a common problem, she noted, with "a huge number of different pathologic processes that can result in anemia."

Anemia, its causes, and related tests and data—including emerging hematologic analyzer parameters—can feel "overwhelming when trying to identify a cause for a specific patient," she said.

"We want to be efficient in utilization of laboratory testing to efficiently and cost-effectively identify the cause of the anemia so that appropriate therapy can be instituted. There's nothing more disturbing than [having] a patient coming in with a megaloblastic anemia, and they've got all these iron studies, and then they've got a bone marrow ordered, and multiple other tests that are not rationally driven. It's more of a shotgun approach."

Hematology analyzers, pages 21–38

What is Dr. Perkins' first step? "I want to stop," she said. "Really stop and think." Once she's identified an anemia based on CBC, she'll look at morphologic features on the blood smear; look at other CBC data; and listen carefully to glean clues from the clinical history. "Then, at that point, I talk to my clinicians so that we can order, cost-effectively, tests that will help define the [underlying] etiology and drive appropriate therapy."

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