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The CRUX

BIMONTHLY FORUM FOR THE LABORATORIANS

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Editorial

We are completing two and half decades of the existence of our group and almost a decade of writing to you this so... loved communiqué aptly entitled "The CRUX". We are happy to inform you that reader participation has increased tremendously and we are doing articles on demand now. This issue for instance, goes deep into clinico-diagnostic aspects of Megaloblastic anemia. A hematologic disorder that is rampant in developing countries. Megaloblastic anemia is an anemia (of macrocytic classification) that results from inhibition of DNA synthesis in red blood cell production. When DNA synthesis is impaired, the cell cycle cannot progress from the G2 growth stage to the mitosis (M) stage. This leads to continuing cell growth without division, which presents as macrocytosis. The defect in red cell DNA synthesis is most often due to hypovitaminosis, specifically a deficiency of vitamin B₁₂ and/or folic acid. Vitamin B₁₂ deficiency alone will not cause the syndrome in the presence of sufficient folate, for the mechanism is loss of B₁₂ dependent folate recycling, followed by folate-deficiency loss of nucleic acid synthesis (specifically thymine), leading to defects in DNA synthesis. Megaloblastic anemia not due to hypovitaminosis may be caused by antimetabolites that poison DNA production directly, such as some chemotherapeutic or antimicrobial agents (for example azathioprine or trimethoprim). Just flip this page over to read all about Megaloblastic anemia under the heading of "DISEASE DIAGNOSIS".

The "INTERPRETATION" segment is also related to a diagnostic marker for an anemia where the red cells instead of becoming over-sized become smaller. Yes, you have guessed it right, iron deficiency or hypochromic anemia, the concerned space is occupied by "FERRITIN". Ferritin serves to store iron in a non-toxic form, to deposit it in a safe form, and to transport it to areas where it is required. When is it high? When is it low. You already know it for sure, just brush up and peep within the covers of what you are holding now.

The third portion of our communiques that we call as "TROUBLE SHOOTING" is also related to hematology and talks about Quality control in the new environment: "automated hematology". The hematology laboratory has undergone a technological revolution over the last 20 years-- from tedious manual methods to relatively simple instrumentation to complex multiparameter instruments. Any discussion of cost savings in quality control must begin with the question of whether QC methods have kept pace with the technological changes. Such important issues are considered here in this issue and will also be continued in the next one too.

"BOUQUET" can not be left out ever! "In Lighter Vein" jokingly pokes fun at Blondes. Not to offend anyone but to have a few laughs. Wisdom is whispering still – take it or not!. Brain Teasers are pictorial. Judge your photographic memory.....

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F O R P R I V A T E C I R C U L A T I O N O N L Y

DISEASE DIAGNOSIS

MEGALOBLASTIC ANAEMIA

INTRODUCTION: **Background:** Megaloblastic anemias are a heterogeneous group of disorders that share common morphologic characteristics. Erythrocytes are larger and have higher nuclear-to-cytoplasmic ratios compared to normoblastic cells. Neutrophils can be hypersegmented, and megakaryocytes are abnormal. On the molecular level in megaloblastic cells, the maturation of nuclei is delayed, while cytoplasmic development is normal. **Megaloblastosis** is a generalized disorder because nonhematopoietic cells, such as gastrointestinal and uterine cervical mucosal cells, can also have megaloblastic features. The etiology of megaloblastic anemias is diverse, but a common basis is impaired DNA synthesis. The most common causes of megaloblastosis are cobalamin (vitamin B₁₂) and folate deficiencies. The usual causes of cobalamin deficiency are pernicious anemia (PA, see Pernicious Anemia), failure of absorption of cobalamin in the terminal ileum, and the effects of medications. Folate deficiency is usually due to folate-poor diets but may also occur in patients with tropical sprue, in patients who are pregnant, and in patients on antifolate or other medications. Current routine folate replacement during pregnancy and folate-containing multivitamin supplementation for elderly persons has led to a decline in the frequency of folate deficiency. **Some patients** can be asymptomatic. The development of megaloblastic anemia is usually insidious; therefore, patients are often relatively asymptomatic because they have had time to adjust to the marked fall in hemoglobin (Hgb) levels. Patients with cobalamin deficiency may develop debilitating neurological impairment that may develop independently of anemia. **Recent trends** in medical care have emphasized early therapy. Folate supplementation is recommended to prevent the atherosclerosis and thromboembolic events by reducing homocysteine levels. Folate is given during pregnancy to prevent developmental defects in the fetus. Mild cobalamin deficiencies and incipient cobalamin-related neuropsychiatric abnormalities have recently been identified in some individuals, and prompt early treatment with cobalamin is recommended to avoid progression of mental deterioration and neurological complications. One review focuses on the relation between various outcomes of human reproduction (ie, pregnancy, lactation, male reproduction) and folate nutrition and metabolism, homocysteine metabolism, and polymorphisms of genes that encode folate-related enzymes or proteins. **Pathophysiology:** The molecular basis for megaloblastosis is a failure in the synthesis and assembly of DNA. The most common causes of megaloblastosis are cobalamin and folate deficiencies. Cobalamin metabolism and folate metabolism are intricately related, and abnormalities in these pathways are believed to lead to the attenuated production of DNA. **Methotrexate-induced** megaloblastosis has been ascribed to a deficiency in deoxythymidine triphosphate (dTTP) that is consumed by the methyl folate trap. Evidence exists that megaloblastosis is caused by interference of folate metabolism by the inhibition of methionine synthesis. However, because of dietary folate deficiency, the size of the dTTP pool is normal or increased in persons with megaloblastosis. **Impairment** in the deoxyuridine monophosphate (dUMP) – deoxythymidine monophosphate (dTMP) pathway may be responsible for nutritional megaloblastosis. Despite this information, the biochemical basis for megaloblastosis is not fully understood. This is especially true of the cobalamin-related neuropathy that can occur independently of megaloblastic changes in hematopoietic cells. One hypothesis for the cause of cobalamin neuropathy is that a defect exists in the conversion of adenosyl-cobalamin-dependent conversion of methylmalonyl coenzyme A to succinyl coenzyme A. **A hallmark** of megaloblastic anemia is ineffective erythropoiesis, as evidenced by erythroid hyperplasia in the bone marrow, a decreased peripheral reticulocyte count, and an elevation in lactate dehydrogenase (LDH) and indirect bilirubin levels. The pathogenesis of these findings is the intramedullary destruction of fragile and abnormal megaloblastic erythroid precursors. **An understanding** of the source of cobalamin and folate is important to understand the pathogenesis of the development of megaloblastosis. Dietary intake is the source of cobalamin and folate because humans cannot synthesize these substances. Cobalamin must be bound to intrinsic factor (IF), and this complex is taken up in the terminal ileum. Once absorbed, cobalamin is bound to another protein, transcobalamin II (TCII), and is transported to storage sites. Abnormalities in any of these steps in cobalamin transport can lead to deficiencies in this substance. Considerable amounts of cobalamin are accumulated in storage sites; this explains why years elapse before cobalamin deficiency develops in patients who cannot take up dietary cobalamin. **Although the processing** and transport of ingested folate is complex, folate-induced megaloblastosis is rarely caused by abnormalities in transport but instead is most often caused by dietary

insufficiency. Folate deficiency can be caused by malabsorption in patients with sprue. In contrast to cobalamin, very little folate is stored; this explains why folate deficiency can occur within months of cessation of folate ingestion. **Megaloblastosis** can also be caused by disorders in which cobalamin and folate uptake and metabolism are not affected. Myeloproliferative syndromes and viral infections (eg, HIV) can lead to megaloblastosis by disrupting DNA synthesis. Megaloblastosis can occur in patients who are on certain medications, including many cancer chemotherapy drugs. **Frequency:** Because the etiology is diverse, determining a numerical estimate of the frequency of megaloblastic anemias is difficult. **Dietary and pregnancy-related** folate deficiencies are probably the most common causes of megaloblastic anemias. However, current folate supplementation during pregnancy and vitamin supplementation for elderly persons has resulted in a low frequency of these forms of megaloblastosis. **Megaloblastosis** may be caused by a small number of drugs, for instance antifolates such as methotrexate, purine analogues such as azathioprine, pyrimidine analogs such as 5-fluorouracil, ribonucleotide reductase inhibitors such as hydroxyurea, anticonvulsants such as phenytoin, and oral contraceptives. **The frequency of pernicious anemia** in US is 0.25-0.5 cases per 1000 persons in their seventh decade of life. Other forms of megaloblastosis are rare. **The frequency of PA** is reported to be higher in Sweden, Denmark, and the United Kingdom (100-130 cases per 100,000 population). Note that the frequency of megaloblastosis is highest in countries in which malnutrition is rampant and routine vitamin supplementation for elderly individuals and pregnant women is not available. **Mortality/Morbidity:** The major morbidity of cobalamin deficiency is related to the severity of the anemia. In cobalamin deficiency, neurological impairment and anemia are major complications. Recent studies indicate that folate deficiency may also lead to neurological impairment. Megaloblastic anemia is more likely to be detected and treated in most industrial and Western nations. Therefore, the morbidity and mortality due to megaloblastosis have been reduced. **Neurological** impairment can occur in patients who are not anemic. The inadvertent treatment of patients with cobalamin deficiency with folate corrects the anemia but will not halt the progression of the neurological disorder. Therefore, neurological impairment continues to be a problem in some patients with cobalamin deficiency. **Evidence suggests** that folate deficiency during pregnancy can lead to neural tube defects and other development disorders in the fetus. However, folate supplements during pregnancy have reduced this morbidity. **Race:** Older literature indicated that pernicious anemia occurs primarily in white persons and is more likely to occur in persons of Scandinavian descent and others of northern European descent. Recent evidence suggests that pernicious anemia also occurs in Asian and African American persons, although with much lower frequency. **Age:** Pernicious anemia usually occurs in individuals older than 40 years, and the prevalence increases in older populations. Dietary folate deficiency also increases in older populations because of poor diets. Boiling foods in water dilutes folates, and excessive heating destroys folates.

CLINICAL: **History:** Anemia is a common feature of all megaloblastic anemias. However, most patients are relatively asymptomatic because anemia usually develops slowly. Therefore, the absence of symptoms of anemia does not exclude the diagnosis of megaloblastosis. When a marked decrease in Hgb occurs, patients can present with dyspnea, light-headedness, palpitations, and heart failure. Patients with cobalamin deficiency can present primarily with neurological impairment. Specific aspects of the etiology of cobalamin and folate deficiencies are described below. **When obtaining** a history with findings of possible cobalamin deficiency, obtaining evidence of anemia and neurological impairment first is important. **Some patients** can have gastrointestinal symptoms such as loss of appetite, weight loss, nausea, and constipation. **Patients** may have a sore tongue and canker sores. **Patients may** have symptoms of anemia. **Early neurological symptoms** include paresthesias in the feet and fingers, poor gait, and memory loss. At later stages, patients can have severe disturbances in gait, loss of position sense, blindness due to optic atrophy, and psychiatric disturbances. In some patients, neurological impairment can occur without anemia. Therefore, neurological symptoms may range from mild to severe, and cobalamin deficiency should be considered even with minimal neurological symptoms and the absence of anemia. **In the next phase** of eliciting relevant history, obtaining a history that can help distinguish between the causes, such as inadequate diets, malabsorption, medications, and congenital disorders, is important. **A history of folate** administration without vitamin B₁₂ therapy should be documented because folate may partially correct hematological abnormalities but will not stop the progression of neuropsychiatric complications. **Dietary insufficiency** of cobalamin is a rare cause of megaloblastosis. A history of a long-standing vegetarian diet without dairy products or eggs can suggest the possibility of this etiology. **Pernicious anemia** is associated with autoimmune disorders. A coexistent history of autoimmune disorders such as thyroid disorders,

type I diabetes, Addison disease, hypoparathyroidism, or autoimmune hemolytic anemia suggests the possibility of pernicious anemia. **A history of a gastrectomy** suggests the possibility of cobalamin deficiency. Approximately 3-5 years must elapse for cobalamin deficiency to occur after total gastrectomy and approximately 12 years must elapse after partial gastrectomy. **A history of ileal resection**, regional ileitis, and small intestinal lymphoma suggests intestinal malabsorption of cobalamin. **Previous gastric** or intestinal surgery may also suggest the possibility of blind loop syndrome. **Zollinger-Ellison** syndrome can cause megaloblastosis. **Handling or eating** raw fish tapeworm suggests that the entrenchment of the tapeworm in the small intestine may be responsible for cobalamin deficiency. **A history of taking medications** mentioned in Causes or exposure to nitrous oxide may suggest cobalamin deficiency. **A history of megaloblastosis** since childhood suggests a congenital cause of cobalamin deficiency. **Obtaining a history in support of folate deficiency** should focus on the patient's diet, evidence of increased folate turnover and consumption, indications of malabsorption and sprue, pregnancy, and medications. **Folate deficiency** develops rapidly because folate stores are minimal. **Folate deficiency manifests** primarily as anemia, but recent evidence indicates that folate deficiency may also lead to neurological syndromes. **Dietary insufficiency** is the most common cause of folate deficiency. A typical patient is an elderly person whose diet is inadequate or who cooks foods diluted in water with excessive heat. Dilution and heating can destroy folate. Alternative diets that are low in folate can produce folate deficiency. **Impaired absorption** may result in folate deficiency. Nontropical sprue should be considered in patients who have megaloblastosis and symptoms of malabsorption, such as weight loss, abdominal distention, diarrhea, and steatorrhea. These patients often have metabolic bone disease or bleeding due to deficiencies in vitamin K–dependent factors. They may describe a sensitivity to gluten. Megaloblastosis may not be evident because of the superimposed iron deficiency. **Tropical sprue** can cause folate deficiency. In addition to signs of malabsorption, these patients have a history of living or visiting tropical regions. Tropical sprue may develop years after the patients visited the tropics. **Other intestinal disorders** that may cause megaloblastosis as a result of folate malabsorption may include regional enteritis, intestinal lymphoma, surgical intestinal resection, amyloidosis, Whipple disease, and scleroderma. **Increased folate** requirements can occur during pregnancy because of transfer of folate to the fetus and during lactation. Dilantin therapy increases the requirement for folate. Patients with psoriasis and exfoliative dermatitis require additional folate because of the increased turnover of epidermal cells. **Miscellaneous causes** of folate deficiency can occur during hyperalimentation and hemodialysis because folate is lost in dialysis fluid. Megaloblastosis in persons with alcoholism is often due to coexistent folate deficiency. **A history of drugs** and antifolate agents mentioned in Causes should be elicited because these agents can cause folate deficiency. **Folate deficiency can occur** in infants who are fed goat milk (low folate content). Infants who are on synthetic diets for congenital disorders can develop folate deficiency. Premature infants can develop folate deficiency in the presence of infection or diarrhea. **A lifelong history** of megaloblastosis or folate deficiency suggests a congenital disorder. **A history of HIV infection** or a myelodysplastic syndrome may suggest that megaloblastosis is due to a direct effect of these disorders on bone marrow stem cells.

PHYSICAL: The physical examination may reveal findings indicative of the consequences of anemia in most persons with megaloblastic anemias. **Neuropsychiatric** signs are usually found only in patients with cobalamin deficiencies. **Findings may indicate** predisposing conditions or underlying disorders. For example, signs of autoimmune disorders that are associated with PA may be detected. **Physical examination** findings may range from barely detectable to markedly abnormal. **Evidence of malabsorption** indicates that the patient has sprue. **Patients** may have a lemon-yellow hue due to the combination of anemia and an increased level of indirect bilirubin. When the decrease in the Hgb level is severe, evidence of an uncompensated anemia is present, such as tachycardia and dyspnea. In many cases, the fall in the Hgb level is moderate and develops slowly; therefore, patients have compensated anemias characterized only by weakness. **Glossitis**, characterized by a smooth tongue due to loss of papillae, occurs in persons with cobalamin deficiency. **Dermatological signs** include hyperpigmentation of the skin and depigmentation of the hair because of increased melanin synthesis. **Neurological** signs occur primarily in persons with cobalamin deficiencies but may also occur in persons with folate deficiency. The signs can vary from minimal to severe. Peripheral neuropathy, abnormal gait, loss of balance, loss of proprioception and vibratory senses, blindness due to optic atrophy, depression, loss of memory, and psychiatric disorders may occur. **Neuropsychiatric complications** of folate deficiency are usually limited to irritability and minimal changes in personality. **Abdominal scars** may be evident from gastrectomies, ileal resections, or other

procedures that may lead to blind loop syndrome. **When cobalamin** deficiency is caused by lack of absorption in the terminal ileum due to regional ileitis, physical evidence of this disorder may be present. **Patients with pernicious** anemia may have signs of autoimmune disorders, including thyroid disorders, type I diabetes, and autoimmune hemolytic anemias. **Patients with nontropical** and tropical sprue may have signs of malabsorption such as weight loss, abdominal distention, diarrhea, and steatorrhea. These patients often have metabolic bone disease or bleeding due to deficiencies in vitamin K–dependent factors. **Patients** who have megaloblastosis due to HIV infection or myelodysplastic syndromes usually have signs of these disorders. **Children** with inborn errors that cause folate and cobalamin deficiencies or inborn errors that have a direct effect on stem cells may have signs of these congenital disorders.

CAUSES: Megaloblastic anemias can be caused by cobalamin deficiency or folate deficiency. **Disorders** such as myeloproliferative syndromes can disrupt DNA synthesis directly. Many pharmaceutical agents also interfere with DNA synthesis and cause megaloblastic changes in hematopoietic and other frequently dividing cells. The causes are diverse and are discussed below. **Cobalamin** (vitamin B₁₂) deficiency can be caused by impaired gastric or intestinal absorption, inadequate dietary intake, drugs, or congenital errors in metabolism. **Nutritional deficiency:** This is a rare etiology, but it can occur in individuals who are on vegetarian diets without milk, cheese, and eggs over a number of years because depletion of cobalamin reserves stored in the liver takes years. **Food-cobalamin malabsorption:** This is characterized by the inability to release cobalamin from food, possibly because of gastric acidity. As a result, cobalamin cannot bind to intrinsic factor (IF) and cannot be taken up. This entity has been recognized recently, and its prevalence as a cause of megaloblastosis requires further study. **Pernicious anemia:** This is the best-known cause of cobalamin deficiency. This disorder results from the absence of functional IF, which leads to impaired gastric absorption of cobalamin. In most cases, the loss of functional IF is caused by the autoimmune destruction of gastric parietal cells. However, some cases of pernicious anemia can be traced to a hereditary lack of production of IF. **Gastrectomy:** Patients develop pernicious anemia following gastrectomy because of the lack of a source of IF. Development of overt megaloblastosis requires approximately 3-5 years following total gastrectomy and approximately 12 years following partial gastrectomy. The lag is because of the time required to deplete cobalamin stores. **Zollinger-Ellison syndrome:** In this disorder, the secretion of large amounts of acid cannot be neutralized by pancreatic secretions. Therefore, the persistent acidity inactivates pancreatic proteases in the duodenum and prevents transfer of cobalamin from r-factor to IF. This factor (r-factor) is a cobalamin binder secreted by salivary glands. **Severe abnormalities** in the terminal ileum due to ileal resection, regional ileitis, or lymphoma: The terminal ileum is the site of uptake of cobalamin-IF complexes; therefore, these disorders can lead to cobalamin deficiencies. Several years are required for cobalamin deficiency to occur following the onset of these disorders because of the time required to deplete cobalamin reserves. **Diphyllobothrium latum (ie, fish tapeworm):** When the tapeworm is entrenched in the small intestine, it competes with the host for ingested cobalamin. The organism is most often found in Canada, Alaska, and the Baltic Sea. **Blind loop syndrome:** This syndrome involves bacterial colonization of intestines that are either deformed because of strictures, surgical blind loops, or anastomoses or abnormal because of scleroderma or amyloidosis. Bacteria compete with the host for cobalamin. **Nitrous oxide:** Methyl chloride is destroyed rapidly after prolonged exposure to nitrous oxide and can produce megaloblastosis. Folate deficiency can be due to dietary deficiency, lack of absorption, or increased folate consumption. In contrast to cobalamin deficiency, folate deficiency develops rapidly because folate stores are minimal. **Folate depletion:** This usually occurs because of dietary insufficiency, the destruction of folate by excessive heating of diluted foods, or consuming alternative diets that are low in folate. **Impaired absorption:** These patients often have metabolic bone disease or bleeding due to deficiencies in vitamin K–dependent factors. They may describe a sensitivity to gluten. Megaloblastosis may not be evident because of superimposed iron deficiency. **Tropical sprue:** Tropical sprue has a more severe effect on the distal ileum than nontropical sprue. Therefore, tropical sprue can lead to cobalamin deficiency and folate deficiencies. **Other intestinal disorders:** Megaloblastosis can occur because of folate malabsorption in patients with a history of regional enteritis, intestinal lymphoma, surgical intestinal resection, amyloidosis, Whipple disease, and scleroderma. **Increased turnover or requirements:** This can occur during pregnancy because of the transfer of folate to the fetus and during lactation. Dilantin therapy increases the requirement for folate. Patients with psoriasis and exfoliative dermatitis require additional folate because of the increased turnover of epidermal cells. **Infants:** Folate deficiency can occur in infants on a diet of goat milk (low folate content), premature infants with infection or diarrhea, and infants on synthetic diets

for congenital disorders. **Miscellaneous:** Folate deficiency can occur during hyperalimentation and hemodialysis because folate is lost in dialysis fluid. Megaloblastosis in persons with alcoholism is often due to folate deficiency.

DIFFERENTIAL DIAGNOSES: Macrocytosis: Other Problems to Be Considered: Occasionally, the morphological changes in megaloblasts and other cells may be extremely bizarre; these changes have been misinterpreted as neoplasia, acute leukemia, or myelodysplasia.

WORK UP: Laboratory Studies: A CBC count, RBC indices, platelet count, differential count, reticulocyte count, and microscopic examination of the peripheral blood smear should be performed. A typical patient with megaloblastic anemia presents with macrocytic anemia with thrombocytopenia and a decreased reticulocyte count. The mean cell volume can range from 100-150 fL or greater.

Hypersegmented neutrophils can be observed on the peripheral smear and represent an early phase of megaloblastosis in persons with nutritional megaloblastic anemias. Hypersegmented neutrophils contain 5 or more lobes, while normal neutrophils contain 3-4 lobes. **Macrocytes** are oval and have been called macroovalocytes. In persons with severe anemia, macrocytes with nuclear remnants and erythrocytes with megaloblastic nuclei can be present in the peripheral blood. Macrocytes can be found in the peripheral blood in patients with liver disease or hemolytic anemia (because of an increase in reticulocytes) and usually do not have oval features. However, macroovalocytes are characteristic of megaloblastic anemias. In general, the profoundness of megaloblastic changes is proportional to the severity of the anemia. In some cases of megaloblastosis, no anemia is present despite overt neuropsychiatric disease. One cause of this disparity is the administration of folic acid to patients with cobalamin deficiency. This therapy partially corrects the anemia, but the neuropathy is not affected and progresses. **Macrocytosis** due to cobalamin or folate deficiencies may be masked in patients with microcytic anemias because of thalassemia or iron deficiency. However, hypersegmentation of neutrophils may persist. Transfusion therapy or infections may modify the expression of megaloblastosis. **LDH and indirect bilirubin** assays should be ordered, and results are expected to be high because of intramedullary destruction of megaloblastic red cell precursors. LDH fraction 1 (LDH₁) and LDH fraction 2 (LDH₂) are elevated, with LDH₁ being greater than LDH₂. The LDH level is often extremely high, and, following therapy, the fall in the LDH level is an excellent indication of response to or failure of therapy. Increased LDH and indirect bilirubin levels along with a decreased reticulocyte count suggest ineffective hemopoiesis in which intramedullary hemolysis is occurring. **Serum iron and ferritin assays** should be ordered initially and during the treatment of megaloblastic anemias. These parameters may be high. Increased iron turnover occurs in persons with untreated megaloblastosis. However, serum iron and ferritin levels may also decrease because patients respond to therapy and consume iron stores for the production of new RBCs. If iron stores are depleted, patients have an incomplete response to cobalamin or folate therapy. **Tests for the diagnosis** of cobalamin deficiency are described as follows: **The most important test** is measuring the serum cobalamin level. In a typical clinical presentation of megaloblastic anemia, a low serum cobalamin level and a full response to cobalamin may be sufficient to establish a diagnosis. A Schilling test can be performed in patients who have been treated with cobalamin and folate. This test can be used to diagnose cobalamin deficiency and to distinguish between pernicious anemia and ileal malabsorption.

Serum for cobalamin levels should be drawn before transfusions or vitamin B₁₂ therapy. If the test cannot be performed within a reasonable time frame, serum should be frozen to preserve it for testing so that therapy can be started. Serum cobalamin levels are usually low in patients with anemia due to cobalamin deficiency. However, exceptions to this rule exist. **Cobalamin levels** may be falsely high in patients with megaloblastosis due to nitrous oxide, TCII deficiency, inborn errors in cobalamin metabolism, and myeloproliferative disorders. On the other hand, serum cobalamin levels can be falsely low with normal tissue levels in some patients with folate or iron deficiency, vegetarians, individuals on high doses of ascorbic acid, pregnant women, and persons with transcobalamin I (TCI) deficiency. **Serum samples for folate levels** should also be obtained and, if necessary, frozen prior to therapy in patients with possible cobalamin deficiency because patients with folate deficiency can have reduced cobalamin levels. **A Schilling test** is a radiometric test of cobalamin absorption. The test is given in 3 parts, as follows: **In the first part** of the test, radioactive cyanocobalamin is given orally. Unlabeled cyanocobalamin is given intramuscularly to inhibit the uptake of radioactive cobalamin by the liver. Next, the urinary secretion of radioactive cobalamin is measured to estimate whether the orally administered cobalamin has been taken up. Low secretion suggests either pernicious anemia or an abnormality in the terminal ileum that prevented the uptake of IF-cobalamin complexes. **The second part** of the test is performed in the same manner, except that IF is given orally along with radioactive cyanocobalamin. IF IF

restores the uptake of ingested radioactive cyanocobalamin, the patient most likely has pernicious anemia. However, if IF does not restore uptake, then an abnormality in the terminal ileum is most likely present. **A third phase** can be performed in which the patient is treated with antibiotics prior to administering radioactive cyanocobalamin. If antibiotics restore cobalamin absorption from the gastrointestinal tract, the patient most likely has a blind loop syndrome. **The main difficulty** with the Schilling test is inadequate collection of urine samples in patients who are either noncompliant or in renal failure. **The results of the Schilling test** may indicate cobalamin malabsorption in patients who have severe and long-standing folate deficiencies. This is because of the effect of severe folate deficiency on the ileal mucosa that leads to a decrease in cobalamin uptake in the terminal ileum. Treating patients with severe folate deficiency with both cobalamin and folate for a month may be advisable to restore the ileal mucosa before performing a Schilling test. **A Schilling test** has been valuable in distinguishing different causes for B₁₂ deficiency. Unfortunately, it is no longer available at most hospitals. **A protein-bound absorption test** (also known as food-cobalamin absorption test) should be performed if food-cobalamin malabsorption is suggested. In this disorder, IF is present, but cobalamin bound to r-binder is not released and thus cannot bind to IF. Results of a standard Schilling test are normal in persons with this disorder. However, if the Schilling test is modified by using in vivo cyanocobalamin-radiolabeled food or in vitro cyanocobalamin-radiolabeled chicken serum or eggs instead of free radiolabeled cyanocobalamin, the Schilling test result will be abnormal. The results of the modified Schilling test can help detect the failure of the release of cobalamin bound to foods. **Methylmalonic aciduria** is another test. Urinary excretion is a reliable index of cobalamin deficiency, provided the patient does not have renal failure. **Serum methylmalonic acid** and homocysteine test results are elevated in more than 90% of patients with cobalamin deficiencies. **Antiparietal cell antibodies** are rarely ordered in current practice. Of patients with pernicious anemia, 90% are positive for these antibodies. However, antiparietal cell antibodies are also present in patients with thyroid disease and other autoimmune disorders. **Anti-IF antibodies** (type I and II) are highly specific for pernicious anemia. However, tests for these are rarely ordered to diagnose or treat patients with megaloblastosis. **Tests for folate deficiency.** Serum folate is the earliest indicator of folate deficiency. Serum samples should be collected prior to therapy or transfusions. If necessary, serum can be frozen until the laboratory can perform the test. Folate levels respond rapidly to changes in dietary folate. A low folate level reflects dietary intake during the previous 2-3 days. Conversely, a single meal with normal folate content can restore serum folate levels to normal. **The RBC folate level** is usually low in patients with folate deficiency. Folate is incorporated into erythrocytes when they are formed, and folate levels do not fluctuate with changes in diet during the lifespan of the RBC. The RBC folate level may not be low in persons with rapidly developing acute folate deficiency. Another limitation of this test is that RBC folate levels are low in more than 50% of patients with cobalamin deficiency, and this test cannot be used to distinguish between these disorders.

IMAGING STUDIES: Abdominal x-ray films, upper and lower GI series, and CT scans may be useful for detecting and evaluating blind loop syndromes, strictures, and other gastrointestinal tract abnormalities that may cause a blind loop syndrome. **OTHER TESTS:** Cobalamin deficiency - Detection and evaluation of autoimmune disorders, regional ileitis, fish tapeworm infection, Zollinger-Ellison syndrome, pancreatitis, and myeloproliferative disorders. **Folate deficiency** - Detect and evaluate pregnancy, malnutrition, and other complications of sprue, chronic hemolysis, and exfoliative dermatitis. **Tests relevant** for the diagnosis and evaluation of inborn errors that cause or are associated with cobalamin or folate deficiency

PROCEDURES: Bone marrow aspiration and biopsy results are useful to confirm the diagnosis, to rule out myelodysplasia, and to assess the iron stores. Marrow is cellular with erythroid hyperplasia. Megaloblastic RBC precursors are abundant, and giant metamyelocytes are present. Iron stores may vary from high to low. The bone marrow begins to convert from megaloblastic to normoblastic within 12 hours, and normalization is complete within 2-3 days. Therefore, bone marrow aspiration should be performed as soon as possible and preferably before therapy if the procedure is considered useful for the patient's treatment.

HISTOLOGIC FINDINGS: Bone marrow is hypercellular. An increase in erythropoietic activity is reflected by a decreased or reversed myeloid-to-erythroid ratio. Erythroid precursors have megaloblastic features in that they are larger than normoblastic cells and they have immature nuclear development. Cytoplasmic maturation is normal, but nuclear remnants, Howell-Jolly bodies, may be present in the cytoplasm. Giant bands (neutrophils) may be present. Megakaryocytes may be large and hyperlobulated. Iron stores vary from being increased before therapy to decreased if iron is consumed during therapy for megaloblastosis. Bone marrow studies should be performed before therapy because therapy may restore normoblastic erythropoiesis rapidly.

TROUBLESHOOTING

QUALITY CONTROL IN THE NEW ENVIRONMENT: AUTOMATED HEMATOLOGY

The hematology laboratory has undergone a technological revolution over the last 20 years-- from tedious manual methods to relatively simple instrumentation to complex multiparameter instruments. Any discussion of cost savings in quality control must begin with the question of whether QC methods have kept pace with the technological changes. **In fact**, many hematology quality control procedures are really holdovers from that earlier era when instruments were fundamentally less reliable than they are today. Modern microprocessor-driven instruments are rather precise and stable and almost certainly do not need the degree of precision control common 15 to 20 years ago when procedures were semiautomated or manual. **Therefore**, many hematology laboratories could save money on QC while continuing to maintain quality standards. The way to do that, however, is not through a bottom-line approach that trims frequency of controls or cuts back on programs subscribed to in an attempt to reach some predetermined budget figure. **Instead**, we need to take a systems approach to the problem. This means analyzing every procedure in terms of the instruments and methods currently in use. The analysis should include not only the obvious costs of QC (reagents, computer time and programs, and labor) but also the hidden costs of too many repetitive runs. We need to discover how much extra cost is generated by quality control protocols that are too restrictive or rigid. **Within the systems analysis framework**, there are three specific programs we can institute that are highly cost-effective in hematology: the use of retained patient specimens, weighted moving averages of red cell indices, and clinical quality control. We will discuss these in some detail a little later in the article, along with quality control for some of the newer analyte additions to automated hematology. The systems approach requires us first to address the issues of selection, maintenance, and calibration of analytic systems.

Selecting and maintaining analytic systems. Selection of an analytic system is usually based on a number of factors, including costs of acquisition and operation (labor and consumables); throughput; ease of operation; training requirements; reliability in terms of downtime; accuracy, linearity, sensitivity, and precision data; and manufacturer support and reputation. Additional factors, harder to define, relate broadly to how the proposed instrumentation fits with the existing mix of space, personnel, and equipment. **We cannot ignore** these intangibles. Indeed, an analytic system cannot be viewed simply as an instrument. It is an integral unit composed of instrument, reagents, personnel, and performance documentation. For this unit to function effectively, appropriate training and continuing education of system operators are essential, as are readily available and user-friendly operating instructions, maintenance schedules, troubleshooting protocols, and convenient documentation. **To take this reasoning** a step further, selecting instrumentation on the basis of ease of process control and the ability to use available quality control material, techniques, and programs can itself be a prudent and cost-effective strategy.

Calibration. The International Committee for Standardization in Hematology has defined a calibrator as "a substance... used to calibrate, graduate, or adjust a measurement" that is "traceable to a national or international reference preparation or reference material." Once an instrument is calibrated, it does not ordinarily have to be recalibrated until there is a major change in an instrument component or a significant drift in control measurements. **Calibration** becomes an issue in hematology

because of the absence of stable standard substances and the relative instability of most materials that are available. Although there are exceptions, QC materials do not in general meet the ICSH guidelines and thus should not be used for calibration. **Indeed**, hematology calibration is mostly a patchwork affair. The cyanmethemoglobin method as described by the ICSH is the accepted reference standard for hemoglobin determination. Microhematocrit procedures, on the other hand, depend for precision on operational standardization with "selected" and "reference" methods. Calibration of hemacytometers can be done in two ways, neither very satisfactory. The choice is between tedious, poorly reproducible manual counting methods that identify cells and only cells and semiautomated counting instruments that may produce erroneous counts due to particles, bubbles, or other optical/electrical interference. Most labs opt for the latter, with appropriate precautions and corrections. **Automated instruments** present their own set of calibration problems. Some authorities still recommend repetitive analysis of fresh whole blood specimens, but the disadvantages of this approach are legion: the amount of blood needed; the time it takes to run 10 to 20 determinations on multiple specimens; and the cost of space, personnel, and instrument maintenance resources devoted solely to calibration. **Many laboratoris** find whole blood calibration impractical and have turned to commercial stabilized calibrators. The problem is that stabilized cells differ substantially from whole blood in the way they are processed and viewed by aperture impedance and optical light-scattering instruments and in their transferability from one instrument to another.

Process control with commercial reagents. A number of control materials and alternative strategies should be considered in establishing cost-effective and reliable quality control protocols. Manufactured quality control material offers convenience and stability, but it is expensive, and efforts to extend stability have often compromised the degree to which the material resembles fresh whole blood. Nevertheless, most laboratories buy stabilized commercial QC material. **It has been common practice** to document control determinations on Levey-Jennings charts and to look for shifts and drifts. Today, many laboratories are implementing alternative statistical options, including cusum analysis and multirule analyses of the Shewhart type.

Interlab comparison and proficiency testing. A valuable quality control tool--and one we recommend --is interlaboratory comparison. These programs, whereby laboratories share a common pool of extended-stability control material and a common database, may be sponsored by a vendor, a local pathology society, the CAP's QAS committee, or a multiregional group of labs. Whatever the source, the purpose of the program is to establish comparisons based on method/instrument/ reagent peer groups, as well as to provide intralaboratory summary statistics. **Limitations of interlaboratory** comparisons include their cost, the limited stability of control materials, and the difficulty in establishing meaningful databases because of small participant groups further splintered by numerous biases in methods, instruments, and reagents. In addition, problems are created by the characteristics of manufactured control products and their interaction with specific instruments. **The limitations aside**, participants can derive great benefit from interlab comparisons. The programs provide control materials at reduced cost as well as access to statistical resources and group data. Moreover, the spread of microcomputers and telecommunication increases the potential for on-line data acquisition and comparison. **Proficiency testing**, on the other hand, has limited value in real time. Its advantage lies in identifying trends and defining method bias, educating staff, and satisfying a need for peer group comparison and external validation of results.

Retained patient specimens. No other area of the laboratory is as adaptable as hematology is to the use of retained patient specimens.

With the exception of the white cell subpopulations, properly aliquoted, refrigerated blood specimens show no significant change in major hematologic parameters for 24 hours. This makes them ideal for run-to-run or shift-to-shift calibration control (at absolutely no cost). **The biggest advantage** of retained patient specimens is their transferability from major instruments to backup or satellite instruments, in contrast to commercial control materials, which are subject to significant instrument/method biases.

Weighted moving averages. The technique that offers a significant opportunity for cost savings in hematology also involves a conceptual leap from what has been standard in the past. Weighted moving averages is a patient-result-based system for process control of red-cell-related parameters. It is inexpensive and admirably suited to the control requirements of modern multiparameter automated hematology analyzers. **The technique** is based on the empirical observation that averaged red cell indices from patient populations in acute care general hospitals are approximately Gaussian, consistently stable, and similar in all institutions studied. These properties reflect the physiologic consistency of red cell size and hemoglobin content in health, disease, and even many hematologic disorders. The dimensions of the properties are expressed by the Wintrobe indices, ratios independent of certain procedural errors (dilution, inadequate mixing) that may seriously compromise hemoglobin and hematocrit measurements and red cell counts. **Weighted moving averages** anchors the validity of the indices by referencing one primary measurement, hemoglobin, to a defined calibration event. It then uses a complex, statistical algorithm to evaluate successive batches of patient sample indices and incorporate them into a continually updated mean. **Means are trimmed** (outliers eliminated) and

smoothed (data from previous batches incorporated into the new mean), thereby diluting the effect of random error and abnormal results. Deviations of the means from specific limits indicate loss of calibration, a shift in the characteristics of the population under study, or specific types of instrument malfunction.

Several caveats: The method should not be used by laboratories performing fewer than 100 CBCs daily. In addition, because of small sample sizes, random entry of raw data is mandatory, and each group of patients should be representative of the patient population as a whole. No more than one-third of a run should be made up of patients with mean corpuscular volume deviations in the same direction (chemotherapy, pediatric, iron-deficient). **The system takes** a little getting used to because it is so statistically intensive and because it completely abandons commercial controls. Many labs may opt for periodic use of manufactured controls or retained patient specimens as a kind of security blanket. **Controversy still colors** the subject of weighted moving averages. Some studies, concluding that stabilized whole blood controls are better at separating calibration change from patient variation, recommend that they be used in tandem with weighted moving averages. In addition, the system cannot be used for process control of leukocyte and platelet counts because of the very high physiologic variability of these analytes. **Nevertheless**, the hematology resource committee of the CAP has approved, with some reservations, the use of the weighted moving averages for longitudinal process control of seven- and eight-parameter analyzers. Recognition has also been accorded by the CAP's Commission on Laboratory Accreditation. Questions pertaining to the system are included in the current laboratory accreditation checklist.

To be continued...

BOUQUET

In Lighter Vein

Bare facts about Blondes

She was Sooooooo Blonde

- * She thought a quarterback was a refund.
- * She thought General Motors was in the army.
- * She thought Meow Mix was a CD for cats.
- * She thought Boyz II Men was a day care center.
- * At the bottom of an application where it says, "Sign here:" she wrote "Sagittarius."

She Was Soooooooooooooo Blonde...

- * She took the ruler to bed to see how long she slept.
- * She sent a fax with a stamp on it.
- * Under "education" on her job application, she put "Hooked On Phonics"

She was Soooooooooooooo Blonde...

- * She tripped over a cordless phone.
- * She spent 20 minutes looking at the orange juice can because it said "Concentrate."
- * She told me to meet her at the corner of "WALK" and "DON'T WALK."
- * She tried to put M&M's in alphabetical order.

She Was Soooooooooooooo Blonde ...

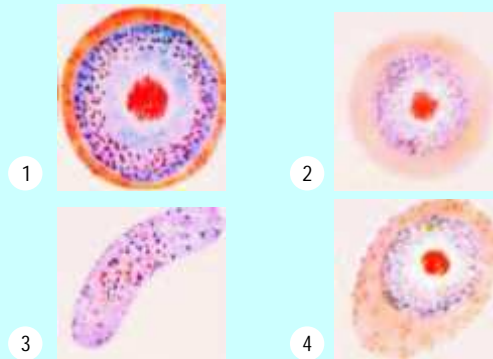
- * When she heard that 90% of all crimes occur around the home, she moved.
- * She thought if she spoke her mind, she'd be speechless.
- * She thought that she could not use her AM radio in the evening.
- * She had a shirt that said "TGIF," which she thought stood for "This Goes In Front."

Wisdom Whispers

- "The dogs may bark but the caravan moves on."
- "He is a fool who boasts of four things: that he has good wine, a good horse, a handsome wife, and plenty of money."
- "Industry is the parent of fortune."
- "Ill vessels seldom miscarry."
- "Patience is a virtue that causes no shame."
- "You cannot get a quart into a pint pot."
- "He travels fastest who travels alone."
- "Beauty without grace is like a hook without bait."
- "Empty sacks will never stand upright."
- "He who endures with patience is a conqueror."

Brain Teasers

Identify the species of the malarial parasites by looking at the images of their micro-gametocytes

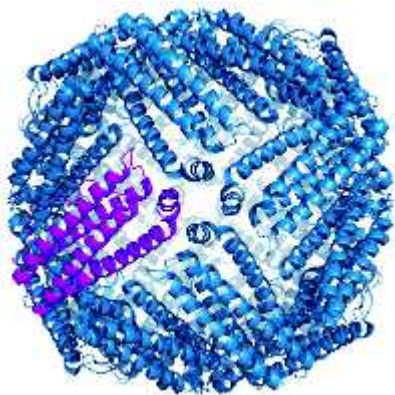


Answers: 1. *P. vivax* 2. *P. malariae* 3. *P. falciparum* 4. *P. ovale*

INTERPRETATION

FERRITIN

Ferritin is a ubiquitous intracellular protein that stores iron and releases it in a controlled fashion. The protein is produced by almost all living organisms, including bacteria, algae and higher plants, and animals. In humans, it acts as a buffer against iron deficiency and iron overload. Ferritin is a globular protein complex consisting of 24 protein subunits and is the primary intracellular iron-storage protein in both prokaryotes and eukaryotes, keeping iron in a soluble and non-toxic form. Ferritin that is not combined with iron is called apoferritin.



Description

Ferritin is a 450 kDa protein consisting of 24 subunits that is present in every cell type. In vertebrates, these subunits are both the light (L) and the heavy (H) type with an apparent molecular weight of 19 kDa or 21 kDa respectively; their sequences are about 50% homologous. Some ferritin complexes in vertebrates are hetero-oligomers of two highly-related gene products with slightly different physiological properties. The ratio of the two homologous proteins in the complex depends on the relative expression levels of the two genes. Mitochondrial ferritin was recently identified as a protein precursor. It is classified as a metal-binding protein which is located within the mitochondria. After the protein is taken up by the mitochondria it can be processed into a mature protein and assemble functional ferritin shells. Its structure was determined at 1.70 angstroms through the use of X-ray diffraction and contains 182 residues. It is 67% helical. The Ramachandran plot shows that the structure of mitochondrial ferritin is mainly alpha helical with a low prevalence of beta sheets.

Genetic structure

In human ferritin, introns are present between the 34/5th, 82/3rd, and 14/5th amino acid residues; in addition, one to two hundred untranslated bases grace either end of the combined exons. The Tyrosine residue at amino acid position 27 is thought to be associated with biomineralization.

Function

Iron storage

Ferritin serves to store iron in a non-toxic form, to deposit it in a safe form, and to transport it to areas that it is required. The function and structure of the expressed ferritin protein varies in different cell types. This is controlled primarily by how much mRNA is translated, and how stable the mRNA is. mRNA concentration is further tweaked by changes to how it is stored and how efficiently it is transcribed. The presence of iron itself is a major trigger for the production of ferritin, with some exceptions. Free iron is toxic to cells as it acts as a catalyst in the formation of free radicals from reactive oxygen species via the Fenton Reaction. Hence

vertebrates use an elaborate set of protective mechanisms to bind iron in various tissue compartments. Within cells, iron is stored in a protein complex as ferritin or hemosiderin. Apoferritin binds to free ferrous iron and stores it in the ferric state. As ferritin accumulates within cells of the reticuloendothelial system, protein aggregates are formed as hemosiderin. Iron in ferritin or hemosiderin can be extracted for release by the RE cells although hemosiderin is less readily available. Under steady state conditions, the serum ferritin level correlates with total body iron stores; thus, the serum ferritin is the most convenient laboratory test to estimate iron stores.

Immune response

Ferritin concentrations increase drastically in the presence of an infection or cancer; this is necessary to counter the infective agent's attempt to bind iron from the host's tissue. Infective agents may cause ferritin to migrate from the plasma to within cells, in order to deny iron to the infective agent.

Stress response

The concentration of ferritin has been shown to increase in response to stresses such as anoxia; this implies that it is an acute phase protein.

Mitochondria

Mitochondrial ferritin has many roles pertaining to molecular function. It participates in ferroxidase activity, binding, iron ion binding, oxidoreductase activity, ferric iron binding, metal ion binding as well as transition metal binding. Within the realm of biological processes it participates in oxidation-reduction, iron ion transport across membranes and cellular iron ion homeostasis.

Expression

In vertebrates, ferritin is usually found within cells, although it is also present in smaller quantities in the plasma.

Diagnostic uses

Serum ferritin levels are measured in patients as part of the iron studies workup for anemia and for restless legs syndrome. The ferritin levels measured have a direct correlation with the total amount of iron stored in the body including cases of anemia of chronic disease. A normal ferritin blood level, referred to as the "reference interval," is now determined by many testing laboratories. The reference ranges for ferritin are 30–400 ng/mL for males, and 13–150 ng/mL for females. Other tests/technologies may have different reference ranges.

Low

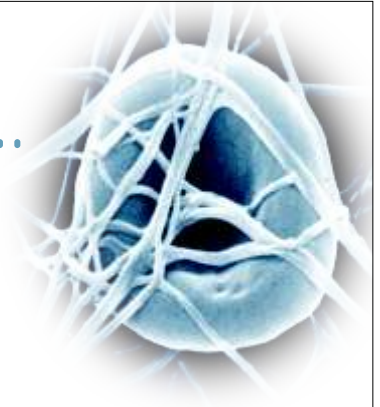
If the ferritin level is low, there is a risk for lack of iron, which could lead to anemia. Low ferritin levels (<50 ng/mL) have however been associated with the symptoms of restless legs syndrome, even in the absence of anemia and sickness. In the setting of anemia, serum ferritin is the most sensitive lab test for iron deficiency anemia. Low ferritin may also indicate hypothyroidism or vitamin C deficiency. In a certain study in Paris, France, the level of iron in the blood (measured by ordering a ferritin serum test) has been connected to Attention Deficit Hyperactivity Disorders - ADHD in children. Specifically, the lower the iron level, the more severe the ADHD symptoms.

Elevated

If ferritin is high there is iron in excess. Ferritin is also used as a marker for iron overload disorders, such as hemochromatosis, hemosiderosis and porphyria in which the ferritin level may be abnormally raised. As ferritin is also an acute-phase reactant, it is often elevated in the course of disease. A normal C-reactive protein can be used to exclude elevated ferritin caused by acute phase reactions. Ferritin can be elevated during periods of acute malnourishment.

TULIP NEWS

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