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The Crux

BIMONTHLY FORUM FOR THE LABORATARIANS

CONTENTS

Editorial

We have had to more than double the production of copies of The Crux being printed in the first year itself. The ever-increasing demand for The Crux and the appreciation it has received at home and from far and wide (overseas) has served as a reward and motivation for us. Our efforts have borne fruit! Laboratarians have been able to ward off incoming problems by appropriately answering the queries put forward by the clinicians. Many laboratarians have further refined their working procedures after going through the TROUBLE SHOOTING sections of the previous volumes. As it is the job of the Laboratarian to provide normal reference ranges and usually the interpretation of the results obtained and also to suggest further investigations, we have learnt that the INTERPRETATION segment has cleared many a doubt pertaining to the aspects already considered. The chief topic of each issue namely DISEASE DIAGNOSIS has refreshed the clinico-pathologic knowledge of the readers. We have covered current international problems like Malaria, HIV/AIDS, Syphilis and seasonal problems like Dengue Fever etc. From this issue we are starting with the rarer but significant entities.

The DISEASE DIAGNOSIS portion in this issue discusses at length G6PD deficiency and the related clinco-diagnostic aspects. If overlooked, the anemia developed due to malaria may be further aggravated by treatment on account of G6PD deficiency in affected individuals. Immediately after the hemolytic episode, the MRT or the quantitative G6PD test may give false negative results (because of release of G6PD rich reticulocytes and younger RBCs). In chronic blood loss disorders or megaloblastic diseases G6PD values may consistently remain high. Drugs are not the only reason why G6PD deficient subjects suffer from hemolytic episodes/crises.

Prostate Specific Antigen is interpreted for you and the latest thoughts regarding utility of Total and Free PSA ratio are presented. Like FOBT provides a quick screening method for detecting Gastro-intestinal malignancies, PSA RDTs can also provide useful semi-quantitative tool to quickly differentiate between benign and malignant prostatomegalies. Quick bedside or rural environment (even at PHC level) diagnosis is not only conceivable but also possible these days.

TROUBLE SHOOTING segment defines for you the QUALITY ASSURANCE standards that must be followed in the blood banks or Diagnostic Laboratories that perform immuno-hematological investigations.

He who laughs at himself is usually liked by all, BOUQUET does exactly that. In lighter vein is directed towards the medical fraternity. Take it lightly!

TÜV MANAGEMENT SERVICE ISO 9001

Tulip Group

DISEASE DIAGNOSIS

GLUCOSE-6- PHOSPHATE DEHYDROGENASE DEFICIENCY

G6PD catalyses the initial step in the HMP shunt, and is now known to be critical in protecting RBC from oxidant injury. In most patients with G6PD deficiency, there is no anemia in the steady state, reticulocyte counts are normal, but RBC survival may be slightly diminished, however, episodic exacerbations of hemolysis accompanied by anemia occur in association with the administration of certain drugs and with some infections. In a few cases, G6PD deficiency is associated with a chronic hemolytic process. Till date, over 400 G6PD variants have been recognised.

Genetics

The G6PD gene is located on the X chromosome (band X q 28). The fact that normal males and females have the same enzyme activity in their red cells is explained by the Lyon hypothesis. This hypothesis maintains that one of the two X chromosomes in each cell of the female embryo is inactivated and remains inactive throughout subsequent cell division for the duration of life. Enzyme deficiency is expressed in males carrying a variant gene, whereas heterozygous females usually are clinically normal. However, depending on the degree of lyonisation and the degree to which the abnormal G6PD variant is expressed, the mean RBC enzyme activity in female may be normal, moderately reduced or grossly deficient. A female with 50 per cent normal G6PD activity has 50 per cent normal red cells and 50 per cent G6PD deficient red cells. However, the G6PD deficient cells in females are as valuable to hemolysis as are enzyme deficient red blood cells in males.

Prevalence and Geographic Distribution

Over 20 crore people are affected worldwide (mainly in the tropical and subtropical zones of the eastern hemisphere). About 2.6 per cent of Indian males are thought to be affected. Because of the high incidence among populations in which malaria was endemic, G6PD deficiency is thought to confer a selective advantage against infection by falciparum malaria. The parasitic growth is inhibited in the G6PD-deficient red cells. Why? One possibility is that the oxidant stress that causes GSH instability and destroys the host RBC also kills the parasite. An alternative explanation is provided by the observation that infected G6PD-deficient red cells are unable to generate the ribose derivatives needed by the parasite for nucleic acid synthesis. Thus, inhibition of parasite growth, rather than oxidative destruction of the parasite or host cells, may be an important mechanism for the balanced polymorphism of the G6PD gene.

G6PD Enzyme and its Variants

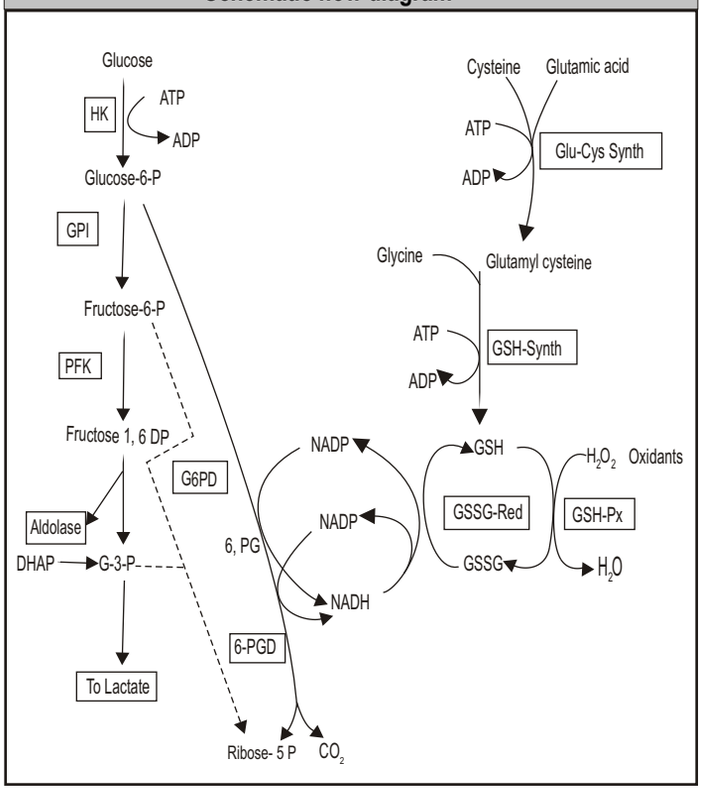
The monomeric form of G6PD contains 515 amino acids and has a molecular weight of over 59,000 Da. The active form of G6PD *in vivo* is a dimer that contains tightly bound NADP. The normal or wild type enzyme is G6PD B, although hundreds of variant enzymes have been identified. Currently about 442 distinct forms of G6PD are recognised (overlapping of distinct forms is a possibility).

The WHO has further classified the different G6PD variants on the magnitude of the enzyme deficiency and also the severity of hemolysis. Class I variants have very severe enzyme deficiency (<10 % of normal) and have chronic hemolytic anemia. Class II variants also have severe enzyme deficiency, but there is usually only intermittent hemolysis. Class III variants have moderate enzyme deficiency (10-60 % of normal), with intermittent hemolysis usually associated with infection or drugs. Class IV variants have no enzyme deficiency or hemolysis. Class V variants are those in which enzyme activity is increased.

Variants in the last two groups although of much interest to biologists, geneticists and anthropologists, are of no clinical significance.

The normal wild type enzyme, G6PD B, is found in most Caucasians, Asians and a majority of blacks. It has normal catalytic activity and is not associated with hemolysis (class IV). A commonly encountered variant is G6PD A^+, which is found in 20-30 per cent of blacks from Africa. It has normal catalytic properties and does not cause hemolysis (class IV). It differs from G6PD B in that it has a much faster electrophoretic mobility.

Schematic flow diagram



Hk=Hexokinase, GPI= Glucose phosphate isomerase, PFK= Phosphofructokinase, G-6-PD= glucose-6-P dehydrogenase, 6,PGD=6, phosphogluconate dehydrogenase, GSSG-Red= glutathione reductase, GSH-Px=glutathione peroxidase, GSH-synth= glutathione synthetase, ϒ-GluCys-Synth=ϒ- glutamyl-cysteine-synthetase G-3-P= glyceraldehyde-3-phosphate, DHAP= dihydroxy acetone phosphate, Ribose-5-P=Ribose5-Phosphate, 6,PG=6, phosphogluconate GSH= reduced glutathione, GSSG=oxidised glutathione

Another common variant is, G6PD A^- is the enzyme responsible for primaquine sensitivity in blacks, and it is the most common variant associated with mild to moderate hemolysis (Class III). This G6PD variant is found in 10-15 per cent of African Americans and with similar frequencies in western and central Africa. It has an electrophoretic mobility identical to that of G6PD A^+. However, this is an unstable enzyme and its catalytic activity although nearly normal in bone marrow cells and reticulocytes decreases markedly in older RBCs. Hence this variant is designated G6PD A^- (the + and - denote enzyme activity). G6PD Mediterranean is the most common abnormal variant found in Caucasians, particularly those whose origins are in the Mediterranean area. Its electrophoretic mobility is identical to that of G6PD B, but it is synthesised at a reduced rate, its catalytic activity is markedly reduced.

Because leucocyte and platelet G6PD is regulated by the same gene as that of red cells, documentation of decreased activity the WBCs and platelets of deficient subjects is not surprising. Because of normally short survival of WBCs and platelets, however, subjects with unstable variants rarely manifest functional impairment. Phagocytic and bactericidal activity of granulocytes from deficient subjects are characteristically normal.

Pathophysiology

As red cells age, the activity of G6PD declines exponentially. The normal enzyme (G6PD B) has an *in vivo* half-life of 62 days. Despite this loss of enzyme activity, normal old RBCs contain sufficient G6PD activity to generate NADPH and thereby sustain GSH levels in the face of oxidant stress. In contrast, G6PD variants with hemolysis have much shorter half-lives. The activity of $G6PDA^-$ in reticulocytes is normal, but it declines rapidly thereafter with a half-life of only 13 days. The instability of G6PD Mediterranean is even more pronounced, with a half-life measured in hours. The clinical correlation of this age related enzyme instability is that hemolysis in patient with $G6PDA^-$ is mild and is limited to the older deficient erythrocytes. In contrast, the enzymatic defect in G6PD Mediterranean is caused by a much greater enzyme instability, and RBCs of all ages are grossly deficient. Consequently, the entire RBC population of people with G6PD Mediterranean is susceptible to oxidant-induced injury, and this can lead to severe hemolytic anemia.

G6PD deficient erythrocytes exposed to oxidants (infection, drugs, fava beans) become depleted of GSH. This reaction is central to the cell injury in the disorders because once GSH is depleted there is further oxidation of other RBC sulfhydryl-containing proteins. Oxidation of the sulfhydryl groups on hemoglobin leads to formation of denatured globin or sulfhemoglobin. The latter form insoluble masses that attach to the red cell membrane, possibly by disulfide bridges, and these are known as Heinz bodies. In addition to hemoglobin oxidation, the direct oxidation of membrane sulfhydryl groups leads to the accumulation of membrane polypeptide aggregates, presumably because of disulfide bond formation between spectrin dimers and between spectrin and other membrane proteins. The result of these changes is the production of rigid, nondeformable erythrocytes that are susceptible to stagnation and destruction by reticuloendothelial macrophages in spleen and liver. Although hemolysis is mainly extravascular, intravascular hemolysis also occurs, giving rise to hemoglobinemia and hemoglobinuria. It should also be remembered that some patients with unstable hemoglobinopathies also may manifest oxidant injury because these abnormal hemoglobins are inordinately susceptible to mild oxidant stress. In the vast majority of cases, however, when hemolysis occurs with oxidant injury it is caused by G6PD deficiency.

Hematologic and Clinical Features

The clinical expression of G6PD variants encompasses a continuous spectrum of hemolytic syndromes. In most affected people the deficiency state goes unrecognised, but in some it causes episodes or chronic anemia. Four clinical states are recognized (1) acute hemolytic anemia (2) congenital nonspherocytic hemolytic anemia (3) neonatal hyperbilirubinemia, and (4) favism.

Acute Hemolytic Anemia

With the most prevalent G6PD variants (G6PD A and G6PD Mediterranean), severe hemolysis occurs only after exposure to certain offending agents. The steady state is associated with no anemia or alteration in blood morphology, although a modest shortening of red cells survival can be demonstrated by using isotopic methods. Sudden destruction of the older, more deficient erythrocytes is triggered by drugs having a high redox potential and by selected infections or metabolic perturbations. A classical example is set by primaquine induced hemolysis. After 2 to 4 days of primaquine ingestion all the signs, symptoms and laboratory results characteristic of an acute hemolysis and dark urine with or without abdominal and back pain, are sudden in onset. An abrupt decrement of 3-4 gm/dl in hemoglobin concentration occurs. Cell fragments, microspherocytes and eccentrocytes or bite cells are found in peripheral smears. In response to anemia, red cell production increases; an increase in reticulocytes is apparent within 5 days and is maximal by 7 to 10 days after onset of hemolysis. Despite continued drug exposure, the acute hemolytic process ends spontaneously after about 1 week, and hemoglobin concentration thereafter return to normal levels. The anemia is self-limited because the older, vulnerable population of erythrocytes is replaced by younger RBCs with sufficient G6PD activity to withstand an oxidative assault. Although red cell survival remains shortened as long as use of the drug continues compensation by the erythroid marrow effectively abolishes the anemia in $G6PDA^-$ subjects. In contrast, the hemolysis occurring with the G6PD Mediterranean variants is more severe because this variant has a very short intraerythrocytic half-life, and thus a larger population of circulating erythrocytes is vulnerable to injury. Sequestration of damaged RBCs occurs equally in liver and spleen. Hemolytic crises occur in heterozygous female subjects as well as homozygous male patient. In virtually all cases acute hemolytic episodes are caused by the administration of drugs, are associated with infection or rarely occur with diabetic acidosis

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Drug Induced Hemolysis

Primaquine is one of several drugs that can precipitate hemolysis. The common denominator of these drugs is their interaction with hemoglobin and oxygen, thus accelerating the intracellular formation of H_2O_2 and other oxidising radicals. Aspirin can be given to patients with class II and III G6PD variants.

Drugs and chemicals unsafe for class I, II and III G6PD variants

Acetanilid, Primaquine, Furazolidone (Furoxone), Sulfacetamide, Methylene Blue, Sulfamethoxazole, Nalidixic acid, Sulfanilamide, Naphthlene (Moth balls), Sulfapyridine, Nitrofurantion (Furadantin), Thiazolsulfone, Phenazopyridine (Pyridium), Toluidine Blue, Phenylhydrazine, Trinitrotoluene (TNT).

Drugs and chemical safe for class II and III G6PD variants

Acetaminophen, Ascorbic acid, Probenecid, Procainamide, Aspirin, Pyrimethamine, Chloramphenicol Quinidine, Chlorquine, Streptomycin, Colchicine, Sulfamethoxypyridazine, Diphenhydramine, Sulfisoxazole, Isoniazid, Trimethoprim, Menadione, sodium bisulfite, Tripelennamine, Phenacetin, Vitamin K, Phenylbutazone, Phenytoin.

Infection Induced Hemolysis

Infection is probably the most common factor inciting hemolysis. About 20 per cent of G6PD deficient subjects with pneumonia experience an abrupt drop in hemoglobin concentration. Various infectious agents implicated are: Salmonella, *Escherichia coli,* β-hemolytic Streptococci and

Rickettsiae. Hemolysis is particularly prominent in G6PD deficient subjects with viral hepatitis. The accelerated destruction of red cells imposes additional bilirubin load on an already damaged liver, resulting in an exaggerated increase in serum bilirubin level. Despite the magnitude of bilirubin retention, however, convalescence is generally complete and uneventful. Although hemolysis triggered by infection characteristically is mild, on rare occasion acute renal failure secondary to massive intravascular hemolysis has been observed. The exact mechanism for destruction of G6PD-deficient red cells during infection is not known. A possible explanation for this relationship between infection and hemolysis is that oxidant generated by phagocytosing macrophages diffuses into extracellular medium where they pose an oxidative threat to G6PD deficient erythrocytes.

Hemolysis Induced by Diabetic Acidosis

Diabetic ketoacidosis can trigger destruction of G6PD deficient red cells. Correction of acidosis and restoration of glucose homeostasis reverses the hemolytic process. Changes in pH, glucose and pyruvate have been proposed as possible mechanisms for hemolysis. Also, occult infection may be a common trigger for inducing both acute hemolysis and diabetic acidosis.

Congenital Nonspherocytic Hemolytic Anemia

With some G6PD variants, the deficiency of enzyme activity is sufficiently great that life-long hemolysis occurs in the absence of infection or drug exposure. These class I variant enzymes are extremely heterogenous with respect to biochemical kinetics, but have in common low *in vitro* activity and marked instability. Most of these variants have DNA mutations at the glucose-6-phosphate or NADP binding sites. The hemolytic anemia associated with class I variants is indistinguishable from the congenital nonspherocytic hemolytic syndromes related to glycolytic enzyme deficiencies.

Anemia and jaundice often are noted first in the newborn period. Hyperbilirubinemia often necessitates exchange transfusion. Typically hemolysis occurs in the absence of a recognised triggering factor, although exposure to drugs or chemicals with oxidant potential exaggerates an already established hemolytic process. Beyond infancy, signs and symptoms of the hemolytic disorder are subtle and inconstant. Pallor is observed infrequently, scleral icterus is noted intermittently, and rarely the spleen is enlarged. The course may be complicated by parvovirus-induced aplastic crises. A temporary arrest of erythropoiesis, usually associated with a febrile illness, is attended by an abrupt drop in hemoglobin concentration. Often such a crises is the event that first leads to examination of blood. Exaggeration of anemia occurs after exposure to drugs with oxidant properties, even those that are safe for patients with class II and III G6PD variants. Hemolysis also can be accelerated with exposure to fava beans.

No hematologic alterations of the class I variants are distinctive. The hemolytic process may be fully compensated, although mild to moderate anemia is the rule (Hb. 8-10 gm/dl). Under basal conditions the usual reticulocyte count is 10 to 15 per cent. Splenectomy generally is of little use.

In a few rare instances, leukocyte dysfunction caused by G6PD deficiency patients has been described in severely deficient patients. The abnormality is characterized functionally by defective bactericidal activity (but normal chemotaxis and phagocytosis), and clinically by recurrent infections with catalase-positive organisms. Overall, however, clinical infections are not a major problem in G6PD.

Neonatal Hyperbilirubinemia

Few G6PD variants that cause acute hemolysis also are associated with hyperbilirubinemia in the newborn period. At particular risk are neonates with the rare class I G6PD variants. From a practical perspective, however, neonatal hyperbilirubinemia is seen with the more common G6PD Mediterranean (class II). G6PD Canton may also be responsible. Untreated hyperbilirubinemia often leads to kernicterus with severe neurologic injury or death. The cause of hyperbilirubinemia in G6PD deficient infants is not clear. The most common consideration is that it reflects increased bilirubin production caused by accelerated red cell breakdown. However, often no obvious external oxidant is responsible for red cell injury. The herbs used in China or clothing impregnated by naphthalene balls or drugs or chemicals taken by the mother in late gestation have been implicated as the inciting stimulus. However, because neonatal hyperbilirubinemia most commonly occurs in the absence of obvious exogenous oxidants, it has been suggested that hyperbilirubinemia may have another etiology, possibly related to impaired hepatic clearance of bilirubin. This hypothesis is supported by the observation that carboxyhemoglobin production, a marker of hemolysis or RBC breakdown, is the same in G6PD Mediterranean deficient neonates with and without hyperbilirubinemia. Also neonates with G6PD Mediterranean have been demonstrated to have a partial defect in bilirubin glucuronide conjugation, similar to that seen in Gilbert's disease. Taken together, these observations suggest that hyperbilirubinemia in these infants may be related to impaired liver clearance of bilirubin, although as yet no known role of G6PD is established in bilirubin conjugation.

Favism

The fava bean (Vicia fava) is toxic for G6PD deficient individuals (mostly associated with G6PD Mediterranean variants and hence usually encountered in Italy and Greece but few cases are also observed in Asia too). Most reported cases of favism result from ingestion of fresh beans with peak seasonal incidence of this disorders coinciding with harvesting of the bean (April and May). Hemolysis of comparable severity can follow consumption of dried beans. The syndrome has been observed in nursing infants of mothers who have eaten the beans, as well as in a newborn infant whose mother had eaten fava beans 5 days before delivery. Moreover, inhalation of pollen from the fava plant has also been incriminated.

Favism occurs most commonly in children between ages of 1 and 5 years. Like other G6PD deficiency syndromes, it occurs primarily in males. Symptoms of acute intravascular hemolysis occur within 5 to 24 hours of ingestion of the bean. Headache, nausea, back pain, chills and fever are followed by hemoglobinuria, anemia and jaundice. The drop in hemoglobin concentration is precipitous and often severe. In the absence of transfusion anemia can be fatal.

Favism does not occur in all susceptible, G6PD people and it is thought that an additional genetic factor is involved, presumably related to how fava beans oxidants are metabolized. A factor other than enzyme deficiency is operative. A decrease in the urinary excretion of D-glucaric acid and impaired formation of salicylamide glucuronide in subjects with favism have been interpreted to reflect an abnormality in the hepatic conjugation of glucuronide and, by inference, are related to the toxicity of some factor derived from fava beans. Two pyrimidine aglycons, divicine and isouramil, have been implicated as the toxic components of fava beans.

Both compounds rapidly overwhelm the GSH-generating capacity of G6PD deficient cells and reproduce many of the metabolic derangements noted during hemolytic episodes. Till date, however, there are no convincing data to explain the erratic hemolytic episodes in favism.

Diagnosis

Most commonly, anemia is first recognised during or after an infectious illness or after exposure to one of several suspect drugs or chemicals. Clinical and hematologic findings reflect the severity of hemolysis but are not themselves results of G6PD deficiency. Irregularly contracted erythrocytes (eccentrocytes with hemoglobin puddled to one side of RBC) and bite cells are seen in the Wright-stained peripheral blood smear. Earlier, these bite cells were considered a consequence of splenic removal of Heinz bodies. Now, however, it is understood that these RBCs contain a coagulum of hemoglobin that has separated from the membrane, often leaving an unstained non-hemoglobin containing cell membrane (creating the appearance of a bite removed from the cell). These morphologic alterations are a consequence of the oxidative assaults on hemoglobin. Brilliant cresyl blue supravital stains of the peripheral blood may reveal Heinz bodies during hemolytic episodes.

The specific diagnosis of G6PD deficiency is made by adding a known amount of hemolysate to an assay mixture containing substrate (glucose-6-phosphate) and cofactor (NADP) and then spectrophotometrically measuring the rate of NADPH generation. Alternatively a variety of screening tests that use hemolysate as a source of enzyme can also be used. The fluorescent spot test is the simplest, most reliable, and most sensitive screening method. This test is based on the fluorescence of NADPH indirectly by measuring the transfer of hydrogen ions from NADH to an acceptor. In the methemoglobin reduction test (MRT), methylene blue is the acceptor used for the transfer of hydrogen from NADPH to methemoglobin, thereby facilitating its reduction. It is important to mention this test because, when combined with a technique for the elution of methemoglobin from intact cells, it can be used to detect relative G6PD sufficiency of individual RBC, thereby detecting the carrier state with approximately 75 per cent reliability. Regardless of the specific test used to detect G6PD deficiency, however, false-negative reactions occur if the most severely enzyme-deficient RBCs have been removed by hemolysis. In such cases the family members should be studied. Another approach is to wait until the hemolytic crisis is over and re-evaluate the patient after the RBC mass is repopulated with cells of all ages (approximately 2-3 months).

G6PD values may be increased in : Pernicious anemia, Myocardial infarction, Werlhof's disease, Hepatic coma, Hyperthyroidism, Chronic blood loss, Other megaloblastic disorders.

The normal value is 8.6 to 18.6 U/Gm of Hb.

Therapeutics

G6PD deficiency management is determined by the syndrome with which it is associated. Acute significant fall of hemoglobin requires RBC transfusion. This is the case more commonly in G6PD Mediterranean (class II) than in G6PDA$^-$ (Class III). All affected individuals should avoid exposure to drugs known to trigger hemolysis. Pregnant and nursing women known to be heterozygous for the deficiency should avoid ingestion of drugs with oxidant potential because some gain access to the fetal circulation and breast milk. If indication for its use is sound, however, an offending drug may be given despite modest shortening of red cell survival. For example, primaquine is safely given to patients with the G6PDA$^-$ variants, provided it is started cautiously (15 mg/day or 45 mg once or twice weekly) and the blood count is monitored closely. The mild anemia caused by its administration is rapidly corrected by a compensatory erythropoietic effect and does not recur unless the dose of drug is enhanced. Transfusion therapy is unnecessary unless hemolytic episodes are compounded by a concurrent arrest of erythropoiesis.

Chronic nonspherocytic hemolytic anemia is caused by class I G6PD variants may need more active intervention. Exchange transfusion during the first week of life often is needed to prevent bilirubin encephalopathy. Beyond the newborn period, anemia persists, but it rarely is severe enough to require blood transfusion. During aplastic crisis, however, transfusion may be lifesaving. As with other syndromes resulting from G6PD deficiency, drugs capable of exaggerating hemolysis should be avoided. Splenectomy occasionally brings about a modest improvement in hemoglobin concentration, but it is generally without benefit. Because of the antioxidant properties, vitamin E has been proposed as a therapeutic agent, but subsequent evaluation of large doses of the vitamin failed to demonstrate an ameliorative effect on anemia.

Screening

Blood bank screening of donors should be conducted in areas where G6PD deficiency (presumably class II variants) is common. The recommendation currently is not a standard blood bank procedure. Screening for public at large is unwarranted.

BOUQUET

In Lighter Vein

When a panel of doctors were asked to vote on building a new hospital,
The Allergists voted to scratch it.
The Dermatologists recommended no rash Moves.
The Gastro-enterologists had a Gut feeling about it.
Neurologists thought the administration had a lot of nerve.
Obstetricians stated they were laboring under a mis-conception.
The Ophthalmologists considered the idea short-sighted.
The Pathologists yelled, "Over my dead body!"
The Pediatricians said, "Grow up!"
The Psychiatrists thought it was madness.
The Surgeons decided to wash their hands of the whole thing.
The Radiologists could see right through it!
The Internists thought it was a bitter pill to swallow.
The Plastic Surgeon said, "This puts a whole new face on the matter."
The Podiatrists! thought it was a step forward.
The Urologists felt the scheme wouldn't hold water.
The Anesthesiologists thought the whole idea was a Gas.
The Cardiologists didn't have the heart to say no.
During the argument the Proctologists stayed in the rear.

Wisdom Whispers

"If Columbus had an advisory committee he would probably still be at the dock."
"People don't notice whether it's winter or summer when they're happy."
You can live to be a hundred if you give up all the things that make you want to live to be a hundred."
"Turn your wounds into wisdom."
"The best way to cheer yourself up is to try to cheer somebody else up."
"A great deal of intelligence can be invested in ignorance."
"There are no shortcuts to any place worth going."
Youth is when you're allowed to stay up late on New Year's Eve. Middle age is when you're forced to."
"Happiness is nothing more than good health and a bad memory."

Brain Teasers

1. In an oncocytoma the oncocytes
   (A) Have sac-like mitochondria (B) Have large number of phagosomes
   (C)Have disrupted Golgi apparatus (D) Have aggregates of immunoglobulins
2. Enzymic fat necrosis may be associated with
   (A) Acute pancreatic necrosis (B) Acute appendicitis (C)Ulcerative colitis
   (D) Sarcoidosis
3. Midzonal necrosis in liver may occur in
   (A)Yellow fever (B) Enteric fever (C) Scarlet fever (D) Rheumatic fever
4. The most comfortable site for taking a biopsy for diagnosing secondary amyloidosis is
   (A) Kidney (B) Rectum (C) Spleen (D) Tongue

ANSWERS : 1. A, 2. A, 3. A, 4. B

INTERPRETATION

PROSTATE SPECIFIC ANTIGEN (PSA)

- It is a 33 KDa single chain glycopeptide produced only in the prostatic secretory epithelium.
- It is a major protein in the seminal plasma.
- Because it is a serine protease, it forms complex in the serum with various protease inhibitors.
- The major PSA complex detected in the serum is the PSA 1 antichymotrypsin (PSA ACT) complex. It is also bound with 2-chymotrypsin (PSA A2M)
- The tissue specificity makes it the most useful marker for the diagnosis and treatment of prostate cancer
- A small portion of PSA remains free in the blood unbound to any carrier protein. This portion is "FREE PSA".

Usage

Assists in the identification, differentiation, classification, staging, and localization of tumor; monitoring preoperatively, postoperatively, and for recurrent tumor; assists in the selection of therapeutic interventions or cytotoxic drug therapy; and assists in assessment of tumor response to treatment protocols.

Issues of PSA Diagnosis

Determination of PSA value in the serum is a good indicator for prostate cancer. But like any laboratory test, there is a significant overlap between PSA levels found in cancer and benign prostatic hyperplasia. Thus, it is important to obtain sequential levels in low or borderline elevated values. A rise in the level as compared to an earlier measurement is an ominous sign.

What are the limitations of PSA testing?

Detection does not always mean saving lives.

False positive tests:

False positive test results (also called false positives) occur when the PSA level is elevated, but no cancer is actually present. False positives may lead to additional medical procedures, with significant financial costs and anxiety for the patient and his family. Most men with an elevated PSA test turn out ***not*** to have cancer. False positives occur primarily in men age 50 or older. In this age group, 15 of every 100 men will have elevated PSA levels (higher than 4 ng/ml). Of these 15 men, 12 will be false positives and only three will turn out to have cancer.

False negative tests:

False negative test results (also called false negatives) occur when the PSA level is in the normal range even though prostate cancer is actually present. Most prostate cancers are slow-growing and may exist for decades before they are large enough to cause symptoms. Subsequent PSA tests may indicate a problem before the disease progresses significantly.

Elevation in different conditions:

PSA can be raised in Benign Prostatic Hyperplasia, Prostatic cancer or infarct, Proststatic needle biopsy, Prostatitis, Transurethral resection (TUR), Urethral instrumentation, and Urinary retention.

Role of FREE PSA

The introduction of free PSA (f-PSA) testing has introduced a greater level of specificity in identifying early prostate cancer. In 1998, the FDA approved the use of Total and free PSA estimation as a diagnostic tool to aid in the stratification for Total PSA values that lie between 4.0-10.0. This has often been the diagnostic gray zone.

New Concepts in Prostate Cancer Diagnosis

The value of total PSA, even though an indicator for prostate cancer, does not differentiate from BPH (Benign Prostatic Hyperplasia).
In this circumstance it is advisable to perform free PSA and PSA RATIO.

What is PSA RATIO?

It is the ratio of free and bound PSA in the body. It is also known as FREE PSA%.

$$\text{FREE PSA \%} = \frac{\text{FREE PSA in sample}}{\text{Total PSA in sample}} \times 100$$

Advantages of PSA RATIO TESTING

- It enhances the specificity of PSA testing in prostate cancer.
- Combined with total PSA, DRE (digital rectal examination) and biopsy findings, help to predict the post-operative pathological stage and grade and may assist the patient and physician in making more informed treatment decisions.
- Can help differentiate CaP (Carcinoma of Prostate) from BPH and reduce unnecessary biopsies.

PSA VELOCITY:

It is defined as the change in PSA concentration over time and should be estimated over minimum of 3 samples with an interval of 12 to 18 months between individual PSA values. This approach places considerable demand on assay stability and consistency over time, and the careful timing of sample collection to avoid other extraneous elevations of PSA.

PSA DENSITY:

PSA density considers the relationship of the PSA level to the size and weight of the prostate. In other words, an elevated PSA might not arouse suspicion in a man with a very enlarged prostate. The use of PSA density to interpret PSA results is controversial because cancer might be overlooked in a man with an enlarged prostate.

PSA HALF LIFE

The half-life for total PSA is 3 days and for free-PSA is 16 hours. Stability of PSA at -20°C is 3 months, at 4-8°C is 2 days and at ambient room temperature (20-25°C) it is just a single day. Therefore inappropriately stored or transported and samples processed late, really have no meaning. Such reports should not be considered at all.

IMMUNOCHROMATOGRAPHIC DEVICES SERVE AS RAPID SCREENING TOOLS AND THE PATIENT CAN GET REPORT WHILE HE WAITS. IT DOES GIVE A BROAD IDEA AS TO WHETHER THE PATIENT'S PSA VALUE IS NORMAL, WITHIN THE GREY ZONE OR BEYOND IT. ON DILUTING THE SAMPLE, FURTHER SEMI-QUANTITATIVE BUT USEFUL INTEREPRETATIONS CAN BE OBTAINED.

TROUBLE SHOOTING

Quality Assurance In Routine Immuno-hematology

Every laboratory that performs immuno-hematological examinations must regularly conduct internal quality controls and participate in external quality control trials.

Reasons

- Clinical validation of the results is not feasible
- Immuno-hematological methods are not quantitative
- Biological reagents with great variability and storage instability are used
- Reagents are subject to only limited controls by official overseeing institutions or government regulatory agencies
- Reagents and methods need to be compatible with each other
- Numerous sources for potential methodical errors as well as many patient-specific interfering factors exist

Goals

- Confirmation of the adequate sensitivity and specificity of the methods used by external quality control, e.g. inter laboratory surveys
- Regular internal control of the quality of the reagents used
- Regular internal control of the equipment used
- Constant monitoring of the accuracy of test performance, test sensitivity, and test specificity by internal quality controls
- Detection and differentiation of patient-specific interfering factors by regular internal quality control

Internal Quality Control

Internal quality control includes controls of the tests, the reagents, and the equipment.

Test controls

Test controls as performed in the individual test methods. They include positive and negative controls as well as auto-controls. These controls are indispensable even in emergency cases; they should not be postponed until after the other tests have become positive.

Reagent controls

All reagents used on a daily basis should be examined under identical conditions (quality control location) at least once a week according to a standard protocol, if possible in the test methods used; the results of these examinations should be compared with a target-value protocol. If abnormalities are found, the test needs to be repeated. If the abnormal result is confirmed, the test is repeated again using a new sample from the same batch.

Reagents	Examination (frequency)
Test cells	Hemolysis (t), reactivity (w)
Test sera	Contamination (t), reactivity (w)
Albumin/LISS	Contamination (t), reactivity (w), pH (b), albumin contents (b), molarity (b)
Enzymes	Contamination (t), reactivity (w)
Wash solution	Contamination (t), pH (daily), electrolytes (b)
AB serum	Contamination (t), pH (t'), reactivity (w)

'If used for hemolysis tests

In Table given above controls are listed that should be performed either each time the reagents are used (t), weekly as part of the reagent control (w) or only in the case of a new batch (b).

The presence of hemolysis or contamination is checked by visual inspection before the reagents are stirred and pipetted.

Control of the reactivity of reagents

The weekly reagent controls serve to check sensitivity and specificity

Target value protocol in conjunction with reagent controls

Sera/media	Test media						
	A1	A2	B	O	Coomb's controls	O D ^{weak}	O D ^{neg}
AB serum	-	-	-	-	-	-	-
Anti-A	~4+	~2+	-	-			
Anti-B	-	-	~4+	-			
Anti-D				~4+		-	-
Anti-D						*~2+	-
Anti human globulin serum				-	~2+		
Anti Le				~2+			

* In the Indirect Antiglobulin Test

Equipment controls

The demands placed on equipment controls, as far as immuno-hematology is concerned, do not differ from other, customarily performed equipment-specific controls in laboratories; some of these controls are also performed according to the manufacturers' specifications. Therefore, no further details are presented here.

External Quality Controls

External quality control is primarily based on regular participation in inter laboratory surveys. In addition, all unresolved immuno-hematological problems should be mailed to reference laboratories for further examination.

As part of inter laboratory surveys, all parameters should always be checked which are investigated in that particular laboratory. In the immuno-hematological laboratory, this applies especially to the ABO and Rh blood groups including the subgroups and weak variants, antibody screening, identification and quantification as well as the characterization of auto antibodies. If different methods are used for antibody screening and cross-matches, these tests must be checked independently from each other.

Analyses for inter laboratory surveys must be performed with coded material under routine conditions.

TULIP NEWS

Tulip Group launches two novel HCV detection immunoassays,

Qualisa HCV & FLAVICHECK-HCV WB

Qualpro, a Tulip group company, achieved a breakthrough in the field of HCV detection by developing two major products **Qualisa HCV & FLAVICHECK-HCV WB** after extensive R & D.

Qualisa HCV, an ELISA based test using unique **DHS**^{3} Technology ensures detection of all the 6 major genotypes of HCV with 100% Sensitivity, Specificity and Stability. The other advantages of the system being unmatched flexibility, high detectability and reliability and breakable wells pouched as 3x8 wells strips, to eliminate wastage. The kits are available in pack sizes of 96, 192, 480 Tests.

FLAVICHECK-HCV WB is an advanced 4th generation rapid assay for the detection of total HCV antibodies (viz. IgG, IgM & IgA) in serum, plasma and whole blood samples with the **"365 advantage"** having 100% Sensitivity and Specificity >99%. This rapid test is available in pack sizes of singles, 10, 25, 50 Tests.

HEPATITIS C

BRIEF NOTE:

Hepatitis C is a highly asymptomatic liver disease. This disease has become a major health concern these days as it afflicts as many as 170 million people world wide and about 10.9 million people in India.

Hepatitis C has the tendency to develop into long term liver disease, causing cirrhosis or even hepatocellular carcinoma.

Apart from the health implications, a great threat Hepatitis C poses is that it can pass undetected for a long time. Thus, a person with a chronic infection stays healthy for a long time without even knowing that he is infected.

HCV transmission occurs primarily through exposure to infected blood. This exposure exists in the context of injection drug use (IDU), blood transfusion, solid organ transplantation from infected donors, unsafe medical practices, occupational exposure to infected blood, birth to an infected mother, multiple heterosexual partners, and high-risk sexual practices.

HCV is an RNA virus of the Flaviviridae family. There are 6 HCV genotypes and more than 50 subtypes. These genotypes differ by as much as 30 to 50 percent in their nucleotide sequences. The virus also has a high propensity to mutate creating a slightly different version of itself every time it replicates, the virus is able to evade the body's immune system.
The lack of a vigorous T-lymphocyte response appears to promote a high rate of chronic infection.

The extensive genetic heterogeneity of HCV has important diagnostic and clinical implications, perhaps explaining difficulties in vaccine development and the lack of response to therapy.

FLAVICHECK-HCV WB and **Qualisa HCV**, with advanced technology ensures detection of all the 6 major genotypes of HCV and is set to establish a foray into HCV detection field as the front line test all 365 days in a year.

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