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The Crux

BIMONTHLY FORUM FOR THE LABORATARIANS

CONTENTS

Editorial

Our last issue introduced you to two of our latest diagnostic ventures that we have whole-heartedly stepped into. Subsequent to exhaustive in-house R&D lasting for several years and following multiple external and internal quality control trials we proudly presented the whole range to you. Now you can confidently grow microbes and also safely eliminate them from your working environment as and when you desire.

BioShields and **Accumix** are two more feathers in our cap, a rather heavy and colourful cap but we do it happily and with immense pleasure so that your cap can grow bigger and become as multihued too. It will happen. This is not a doubtful prediction but a confident promise! The previous issue was as unique in colours and presentation as were the products it carried. Happy Diagnosing!! The Crux

The usual format of is back.

This issue confronts you with a not so uncommon a problem faced routinely- HAEMOLYTIC DISEASE OF THE NEWBORN or HDN in short. All aspects of presentation, causes, cardinal features, epidemiology, demographics, diagnosis and its workup along with therapeutic options and preventive modalities are covered in detail.

DISEASE DIAGNOSIS diagnoses HDN for you and another twin section under TROUBLE SHOOTING presents THE GUIDELINES FOR BLOOD GROUPING AND ANTIBODY TESTING IN PREGNANCY Both articles complement each other. Hopefully you will never ever in future be faced with unresolved issues pertaining to HDN- its diagnosis and prevention.

INTERPRETATION discusses CHOLINESTERASE, a rare but important enzyme that most often becomes very important in cases of insecticide poisoning cases. Related diagnostic protocols are provided and interpreted.

The section we would like to call as sweet chilly - BOUQUET - is quietly waiting for your kind attention too.

TÜV ISO 9001

Tulip Group

DISEASE DIAGNOSIS

HEMOLYTIC DISEASE OF THE NEWBORN (HDN)

Description
- Low hemoglobin in the absence of hemorrhage, increased reticulocyte count
- Jaundice with hyperbilirubinemia
- Hydrops fetalis in severe cases

Synonyms
- Erythroblastosis fetalis
- Neonatal immune hemolytic anemia
- Rhesus (Rh) disease
- Rh isoimmunization
- Rh incompatibility
- ABO incompatibility

Urgent action
- Infants with severe hemolysis may develop hydrops fetalis secondary to congestive heart failure with generalized edema, pericardial and pleural effusions and ascites. They require intensive supportive care and exchange transfusions may be needed to correct anemia
- Even well-appearing infants with hemolysis must be closely monitored for increasing hyperbilirubinemia. Kernicterus, an irreversible neurotoxicity, may result from severe hyperbilirubinemia. Serum bilirubin levels can be controlled by the use of phototherapy and, if necessary, exchange transfusions

Background
Cardinal features
- Low hemoglobin in the absence of hemorrhage
- Increased reticulocyte count
- Jaundice with unconjugated hyperbilirubinemia
- Coombs' positive (DAT positive)
- Hydrops fetalis in severe cases
- Intrauterine death in severe cases
- Pallor
- Hepatosplenomegaly
- Nucleated red blood cells
- Rh disease: infant's blood type is Rh positive and mother's is Rh negative
- ABO incompatibility: infant's blood type is A, B, or AB and mother's is O

Causes
Common causes
- RhD incompatibility: occurs when an RhD negative mother is exposed to the RhD positive red blood cells of her fetus. This usually occurs as a result of a transplacental hemorrhage either during gestation or at delivery but sensitization can occur during a previous pregnancy, including a miscarriage. The mother develops anti-D antibodies that cross the placenta and cause the destruction of fetal red cells. Red cell destruction continues in the infant to varying degrees
- ABO incompatibility: after exposure to fetal blood, maternal anti-A or -B antibodies enter the fetal circulation and react to the fetal red blood cells' A or B antigens

Rare causes
- Maternal autoimmune disease (autoimmune hemolytic anemia, systemic lupus erythematosus): maternal antibodies enter fetal circulation and result in fetal or infant red cell destruction
- Minor blood group antigen incompatibility (Kell, Duffy, M, S)
- Drug-induced hemolysis (e.g. penicillin, acyclovir)
- Infection: cytomegalovirus, toxoplasmosis, syphilis, sepsis
- Disseminated intravascular coagulation (DIC)
- Hereditary red blood cell disorders (hereditary spherocytosis/elliptocytosis, thalassemias, glucose-6-phosphate dehydrogenase deficiency, pyruvate kinase deficiency)
- Metabolic abnormalities (acidosis, galactosemia)
- Angiopathic hemolysis (cavernous hemangioma, large vessel thrombi, renal artery stenosis, severe coarctation of the aorta)

Serious causes
- Rh incompatibility is the most likely cause of severe hemolysis in the newborn period. The resulting severe anemia may lead to hydrops fetalis. Further, severe hemolysis may lead to acute hyperbilirubinemia that, if untreated, may lead to kernicterus
- Regardless of the cause of hemolysis, hyperbilirubinemia may result. Significant hyperbilirubinemia in a newborn is always serious and requires treatment and close observation

Contributory or predisposing factors
- Father Rh positive and mother Rh negative; previous infant with Rh incompatibility; previous pregnancy even if pregnancy did not go to term
- Family history for rare hereditary red blood cell disorders

Epidemiology
Incidence and prevalence
Frequency
- Rh incompatibility: 15% of Caucasians, 7% of African, and 2% of IndoEurasians do not express the D antigen and are, thus, Rh negative. If the father's blood type is not available, the chance that the Rh-negative woman is carrying an Rh-positive fetus is about 60%. For pregnancies complicated by Rh incompatibility, the risk of maternal isoimmunization to the D antigen is about 8% if no prophylaxis is given. Fortunately, with the common use of prophylaxis, the incidence is less
- ABO incompatibilty: about 12% of pregnancies. Evidence of fetal red blood cell sensitization (i.e. Coombs' or DAT positive) in 3% of births. <1% of live births are associated with significant hemolysis secondary to ABO incompatibility . The incidence of late HDN is about 4.4-7.2/100,000 live births

Demographics
Age
Newborn period.

Race
Most common in Caucasians.

Genetics
Genes determine parent and, thus, infant blood types. For hereditary blood disorders, genetic testing and prenatal screening may be warranted.

Socioeconomic status
Plays a role only in as much as it influences the mother receiving prenatal care, screening, and RhD prophylaxis if indicated.

Diagnosis
Clinical presentation
Signs
- Pallor
- Jaundice
- Tachycardia
- If severe anemia: respiratory distress, hepatosplenomegaly, hypotension, signs of congestive heart failure, hydrops fetalis

Differential diagnosis
Hemorrhage in the newborn
Features
- Prenatal: fetomaternal, intraplacental, retroplacental, twin-to-twin transfusion
- Perinatal: umbilical cord abnormalities, placental abnormalities, e.g.

abruptio placentae and placenta previa
- Postnatal internal bleeding: cephalohematoma, subdural or subarachnoid hemorrhage, intracerebral/intraventricular hemorrhage, subcapsular hematoma or rupture of the liver, splenic rupture
- Postnatal external bleeding: umbilical bleeding, gastrointestinal bleed, iatrogenic blood loss

Failure of red blood cell production in the newborn

Features
- Acquired: viral infection, anemia of prematurity
- Congenital: Diamond-Blackfan anemia (pure red cell aplasia), Fanconi's anemia, Aase syndrome

Conjugated hyperbilirubinemia

Conjugated hyperbilirubinemia is rare in neonates.

Features
- Newborn sepsis
- Congenital biliary atresia
- Newborn hepatitis

Workup

Diagnostic decision

Diagnosis is based on the blood tests.

Don't miss!

As a consequence of hemolysis, newborn infants are prone to develop hyperbilirubinemia. Hyperbilirubinemia in infants must be monitored closely as kernicterus (irreversible brain damage) can occur.

Summary of tests

It is helpful to remember that it is often very difficult to obtain blood from newborn infants. Parents and the patient greatly appreciate minimizing needle sticks; so, whenever possible, group the blood tests. Also, keep in mind that normal blood test values for infants are different from those for children and adults. In cases of anemia in the newborn, all of the following tests are indicated:
- **Complete blood count (CBC)**: if anemia in the newborn is suspected, you may start by checking the hemoglobin alone, but evaluation of the white blood cell and platelet count can help narrow the differential diagnosis. Very high or low white blood count may suggest infection. Low platelet count may suggest disseminated intravascular coagulation (DIC)
- **Reticulocyte count**: a low reticulocyte count in the context of anemia suggests an inability to produce red cells (e.g. congenital hypoplastic anemia or drug-induced red cell suppression). In hemolytic anemia, the reticulocyte count should be high or normal
- **Direct Coombs' test (DAT)**: a positive DAT indicates sensitization to red blood cell antigen. DAT is positive in Rh disease but frequently negative in ABO incompatibility
- **Bilirubin:** Rh disease is characterized by marked hyperbilirubinemia as compared to ABO compatibility
- **Blood type**: for Rh disease, the mother must be Rh negative and the infant Rh positive; for ABO incompatibility, the mother is typically O and the infant A or B
- **Haptoglobin**: binds free hemoglobin and is decreased with hemolysis
- **Lactate dehydrogenase and aspartate transaminase**: the intracellular enzymes are increased with hemolysis
- **Head ultrasound:** this noninvasive imaging can detect intracranial bleeding that could be the cause of anemia in a sick newborn
- **Peripheral blood smear:** can be used to evaluate the number and morphology of the different cell lines

Tests

Complete blood count

Advantages/disadvantages

Advantage: inexpensive and widely available.

Normal

Newborn (first week):
- Hemoglobin: 16-20g/dL (160-200g/L)
- Hematocrit: 54-68%
- Reticulocytes: 1.8-4.6%
- White blood cells: range 10,000-34,000/mm^3 (10-34x10^9/L)
- Platelets: 90-450x10^3/mm^3 (90-450x10^9/L)

Abnormal
- It is important to remember that reticulocyte count must be considered in the context of the hemoglobin, e.g. in the newborn without anemia, a reticulocyte count of 3 is appropriate. However, in the context of anemia, the reticulocyte count should be higher, as a reflection of red cell production
- Results must be interpreted in conjunction with clinical condition

Cause of abnormal result

RhD and ABO incompatibility are most likely causes.

Direct Coombs' test (DAT)

Description

Patient's red cells are washed and tested with Coombs' serum.

Advantages/disadvantages

Advantage: DAT is positive in Rh disease and frequently positive in ABO. Thus, while it may not be specific for the precise etiology of hemolysis, DAT is a relatively sensitive test to determine immune-mediated hemolysis in the newborn.

Normal

Coombs negative.

Abnormal

Coombs positive.

Cause of abnormal result

Sensitization to red blood cell antigen.

Medications, disorders and other factors that may alter results

DAT may be positive in certain drug reactions and in individuals with autoimmune conditions.

Bilirubin level

Description

Venous blood sample.

Advantages/disadvantages
- Advantage: high bilirubin levels may not determine precise etiology of anemia, but it is vital to follow bilirubin levels if kernicterus is to be prevented. In infants with hemolytic disease, the bilirubin is typically unconjugated
- Disadvantage: hyperbilirubinemia is common in the newborn without hemolytic anemia. Common causes include breast-feeding jaundice, breast milk jaundice, and physiologic hyperbilirubinemia of the newborn

Normal

A full-term newborn infant (gestational age of at least 37 completed weeks) with physiologic jaundice generally does not have a total bilirubin level peak >12mg/dL (>120mg/L) and the majority of the fractionated bilirubin is unconjugated.

Abnormal
- Clinical jaundice within the first 24h of life in a term infant is considered pathologic and requires a full evaluation. Otherwise, the level of bilirubin at which one considers intervention with phototherapy and/or exchange transfusion depends upon the age of the infant.
- Premature infants are at greater risk of kernicterus-related hyperbilirubinemia and thus proportionately lower levels of bilirubin will trigger an aggressive response. Typically,

premature infants weighing <1000g will all require some phototherapy to speed the metabolism of the bilirubin. Infants who weigh between 1000 and 1500g will receive phototherapy at levels of 7-9mg/dL (70-90mg/L) whereas infants between 1500 and 2000g can tolerate levels of 10-12mg/dL (100-120mg/L) prior to the initiation of phototherapy. Babies with a birth weight of 2000-2500g will receive phototherapy at levels similar to their full-term counterparts: 13-15mg/dL (130-150mg/L)

Blood type

Description

Important to determine blood types of the infant and mother.

Advantages/disadvantages

Advantage: determination of blood types may indicate a 'set-up' for immune hemolysis.

Abnormal

- Rh disease: mother Rh negative and infant Rh positive
- ABO incompatibility: mother typically O and infant A or B

Ultrasound

Advantages/disadvantages

Advantages:

- Fast and relatively noninvasive
- In the ill newborn with significant anemia, it is important to consider an ultrasound of the head and brain to look for a bleed that may explain the anemia
- Allows serial follow-up

Disadvantage:

- May be more difficult to rely on results of an examination performed by someone who does not frequently perform ultrasounds on infants

Abnormal

Significant central nervous system bleeds should be detectable by ultrasound performed by an experienced pediatric radiologist.

Cause of abnormal result

- Intraventricular hemorrhage
- Subdural hematoma
- Cephalohematoma

Peripheral blood smear

Description

Slide is made of a sample of the patient's blood for microscopic review.

Advantages/disadvantages

- Advantage: may help to illustrate morphologic abnormalities in blood, but these will not necessarily explain etiology
- Disadvantage: needs to be interpreted by an individual with experience reviewing blood smears

Clinical Hallmarks

- Jaundice in an infant <24h old should always prompt a thorough investigation
- Severe or prolonged jaundice or jaundice that is not responsive to phototherapy should elicit suspicion for hemolytic disease and other disorders

  Hemolytic anemia in the newborn secondary to ABO incompatibility is typically mild, and while hyperbilirubinemia may develop, referral to a neonatologist or hematologist is usually not necessary. However, referral is necessary in some situations:
- Hemolysis from unclear etiology
- Persistent hemolysis, even if etiology seems clear
- Infants who have poor feeding, lethargy, respiratory difficulty, temperature instability, other signs of illness, or are preterm may necessitate early referral for evaluation and monitoring of hemolysis and hyperbilirubinemia

Treatment

Goals

- To provide supportive care and maintain hemodynamic stability
- To prevent severe hyperbilirubinemia and subsequent kernicterus
- To educate parents regarding potential for similar issues in subsequent children and to communicate with mother's physician to ensure proper prophylaxis and monitoring in subsequent pregnancies

Immediate action

- Most critical issue is to ensure patient is hemodynamically stable and, if not, he or she must be transferred to a neonatal intensive care unit (NICU) as soon as possible
- If urgent blood transfusion is needed before blood types and diagnosis are known, O negative blood that is cytomegalovirus negative, filtered, and irradiated is preferable
- If patient appears clinically stable, it is important to send blood tests in order to confirm diagnosis

Therapeutic options

Summary of therapies

Best treatment of Rh incompatibility is prevention. However, even if the mother did receive optimal prenatal care, the newborn may still be affected.

- If severe hemolytic disease has been diagnosed prenatally by ultrasound or amniocentesis, intrauterine transfusion is recommended
- In severe cases when hydrops fetalis has developed in the newborn, adequate ventilation must be established and baby should be transferred to a NICU for management of metabolic imbalances and circulatory support as needed
- If delivery of a baby with Rh disease is expected, type O blood crossmatched with the mother's serum should be available at delivery for use if needed for the newborn
- Extremely close monitoring of hemoglobin (every 4-6h), bilirubin (every 8-12h), and reticulocyte count (daily) are indicated
- Hyperbilirubinemia must be monitored and treated in order to prevent kernicterus. Most newborns with Rh disease and ABO incompatibilty can be managed with phototherapy
- Indications for exchange transfusion depend on patient's age, bilirubin level, and degree of ongoing hemolysis. It should only be performed in the setting of an intensive care unit. Because preterm infants are at greater risk of developing kernicterus than full-term infants, exchange transfusion should be employed at relatively lower levels of bilirubin
- Some studies suggest that high-dose intravenous immunoglobulin in the newborn with Rh disease decreases the need for exchange transfusion. This is not widely accepted and is best considered in consultation with a pediatric hematologist or neonatologist
- Infants with hemolytic anemia that do not require exchange transfusion are at high risk of developing significant anemia within the first 8 weeks of life because of persistent maternal antibody causing hemolysis. Some hematologists advocate the use of erythropoietin (red blood cell growth factor) in an effort to mitigate the impending anemia. This is not widely accepted and is best considered in consultation with a pediatric hematologist or neonatologist
- Many physicians would prescribe iron in anticipation of future anemia. However, if the patient has received transfusions, it is likely that the patient's iron stores are replete, and supplemental iron may not be necessary

Efficacy of therapies

- Considering the variety of causes of hemolytic anemia in the newborn, and the highly variable severity of hemolysis amongst the causes, it is difficult to make a blanket statement about the efficacy of combined therapies and outcomes. Whatever the cause, effort should be made to establish diagnosis and institute appropriate treatment early
- For the most common causes of hemolysis in the newborn, Rh and ABO incompatibility, outcome is generally very good and hemolysis is usually mild to moderate. While many infants require phototherapy, the majority do not require exchange transfusion. Hemolysis may continue for several weeks (as long as maternal antibodies

INTERPRETATION

CHOLINESTERASE

Cholinesterases hydrolyze acetylcholine. Two related enzymes are known:
- Acetylcholine acetylhydrolase, formerly also called specific cholinesterase, true cholinesterase or cholinesterase I. It is found in the erythrocytes, the gray matter of the central nervous system, in the sympathetic ganglia of the motor end plate of the myocyte, in the lungs and the spleen but not in the plasma. The enzyme hydrolyzes only choline esters and not aryl or alkyl esters. Acetylcholine acetylhydrolase cleaves the acetytcholine released at the nerve endings. It thus modulates the transmission of the nerve impulse across the synapse to the end organ.
- Acylcholine acylhydrolase, formerly also called non-specific cholinesterase, pseudocholinesterase, benzoyl cholinesterase, cholinesterase II or S-type cholinesterase. It is found in the plasma, liver, intestinal mucosa, pancreas, spleen and in the white matter of the central nervous system. Acylcholine acylhydrolase cleaves not only choline esters but also benzoylcholine and butyrylthiocholine and aryl and alkyl esters. The function of the enzyme occurring in the serum is not known.

Acetylcholine acetylhydrolase and acylcholine acylhydrolase are competitively inhibited by the alkaloids prostigmine and physostigmine.
This article deals only with acylcholine acylhydrolase i.e. the enzyme activity measurable in the plasma. This is the enzyme referred to as cholinesterase (ChE).

Indication
- Suspected liver parenchymal damage with impaired organ function
- Prior to administration of muscle relaxants of succhinylcholin type in patients with
  a history suggesting the presence of a cholinesterase variant
- Prolonged apnea after surgery
- Pesticide poisoning
- Monitoring of workers exposed to pesticides

Method of determination
Photometric rate assay using acylthiocholine esters as substrate
Principle: substrates such as butyryl-, acetyl- or propionylthiocholine are hydrolyzed in a comparable manner to acetylcholine.
Butyrylthiocholine+H_2O (in the presence of ChE) Thiocholine+Butyric acid
The resulting thiocholine is detected in a chromogenic reaction. Measurement at 405 nm
Benzoylcholine method
Principle: Benzoylcholine is hydrolyzed by ChE yielding the reaction products benzoic acid and choline. The decrease in the absorbance of benzoylcholine is measured at 340 nm.
Methods for detection of plasma cholinesterase genetic variants:
The synthesis of ChE in the plasma is controlled by a gene locus on the long arm of the third chromosome. The alleles known today are U, A, S, F H, J, K, which are potentially responsible for the synthesis of 28 different phenotypes. In some individuals more than one mutation occurs in the same gene. Hence, the phenotype is not always identical with the genotype. For example, the phenotype UA can correspond to the genotype UA or UAK. Altogether up to 45 different diploid genotypes, but only 11 distinctive phenotypes, are possible.
Some of the cholinesterase variants lead to reduced ChE activities in the plasma, do not hydrolyze succinylcholine and are responsible for the prolonged apnea after surgery using muscle relaxants of the succinylcholine type.

The classical method for biochemical genotyping of ChE in plasma is by inhibition tests with dibucaine and fluoride.

Clinical significance
ChE in liver disease
ChE is an excretory enzyme of the liver, normal activities in serum are therefore dependent on adequate functioning of all liver parenchymal cells. In the healthy population there is a broad reference interval with coefficients of between 10 and 40% reported for interindividual variation, and of about 10% for intraindividual variation. It is therefore difficult to obtain sufficient information on a patient's liver function from an isolated measurement of ChE activity.
More information can, however, be derived from monitoring the course of ChE activity in an individual patient. A continuous decline suggests deterioration of liver function, an increase is a sign of improvement or successful treatment.
In general, determination of ChE does not contribute greatly to the diagnosis of liver disease. In a patient population in which 8% of the patients have independent hepatobiliary diseases
ChE decrease can be associated with acute hepatitis, chronic hepatitis, liver cirrhosis, acute liver failure, chronic liver congestion, hepatic tumours and liver transplantation.

ChE can also be diminished in severe illness, shock, critically ill patients, septic shock, chronic inflammatory bowel disease, progressive muscular dystrophy, Thomsen's disease, myocardial infarction, pernicious anaemia and trichinosis

Decrease in ChE by drugs
The alkaloids prostigmine and physostigmine cause reversible inhibition of ChE. Both alkaloids compete with the choline residue of acetylcholine for its binding site on the enzyme. Other drugs and substances cause irreversible inhibition of ChE.
Inhibition of cholinesterase by drugs and pesticides
Inhibition less than 15%
- non-depolorizing muscle relaxants, e.g. pancuronium, verocuranium
- antibiotics: penicillins, streptomycin

Inhibition of 20-100%
- carbamate esters which are used as parasympathomimetics, e.g.neostigmine, edrophonium, pyridostigmine, physostigmine
- organophosphate esters used as pesticides in the form of alkylphosphates
- cardiovascular drugs, e.g. quinidine, esmolol
- cytotoxic drugs, e.g. cyclophosphamide; reduction on the first day of treatment already, normalization within one week
- hormones, e.g. corticosteroids
- hormonal contraceptives
- psychotropic drugs, e.g. lithium, phenelzine
- bronchodilators, e.g. Bambuterol
- anti-glaucoma drugs, e.g. ecothiopate
- muscle relaxants, e.g. succinylcholine

ChE reduction due to organophosphate and carbamate esters
Both groups of substances are used as pesticides.
Organophosphate esters: alkylphosphates, e.g. ethylparathion, methidathion, carbophenothion, mevinphos, chlorpyrifos, dimethoate, naled, EPBP, phosalone.
Carbamate esters: formethanate-HCl, methomyl, carbaryl.
Organophosphate esters and carbamate esters inhibit both acetylcholine acetylhydrolase and acylcholine acylhydrolase.
Exposure to pesticides leads to inhibition of the acetylcholinesterase

modulation of neuronal impulses. The decrease in plasma ChE activity is a measure of the degree of pesticide exposure.

Acute intoxication: clinical symptoms are miosis, hypersalivation, nausea, vomiting, increased muscle tremor and sweating. Symptoms do not occur until the ChE decreases to 60% of the lower reference limit as a result of incorporation of the insecticides.

Organophosphate poisoning is classified as follows on the basis of the ChE activity:

- mild (ChE 60-40% of the lower reference limit), with mainly the clinical symptoms described above
- moderately severe (ChE 40-20% of the lower reference limit), with tightness in the chest and muscle pain in addition to the above symptoms
- severe (ChE < 20% of the lower reference limit) with respiratory distress as the predominant clinical feature.

After complete inhibition ChE returns to levels within the reference interval after 30-40 days.

Chronic pesticide exposure: can be asymptomatic or there can be nonspecific symptoms such as diarrhea, weight loss, muscle weakness and psychiatric symptoms.

Monitoring exposed persons:

Agricultural workers who spray pesticides can be monitored for possible influence by measurement of ChE in serum or erythrocytes according to the following recommendation: if spraying is to be carried out on more than 6 days per month, before beginning the spraying work two baseline values are obtained at an interval of 3 (not more than 14) days, followed by 3 measurements at monthly intervals. A relative risk for pesticide poisoning exists if the ChE falls to 60-80% of the individual baseline value measured before the beginning of the spraying season. Individuals whose levels fall to < 60 % must stop spraying.

Increase in ChE activity levels has no clinical significance, however, it can occur in Diabetes mellitus, Coronary heart disease, Fatty liver, Nephrotic syndrome, exudative enteropathy, Hyperthyroidism, severe obesity, Gilbert's syndrome, Cynthiana variant.

Reference interval

Typically, normal pseudocholinesterase values range between 8- 18 U/ml or (2.17- 5.17 IU/ml). In the neonatal period and the subsequent weeks the ChE level in serum is only about 50% of the level measured in adults. It then increases gradually, reaching adult values by the age

BOUQUET

IN LIGHTER VEIN

- Jones applied to a finance agency for a job, but he had no experience. He was so intense that the manager gave him a tough account with the promise that if he collected it, he'd get the job.
Two hours later, Jones came back with the entire amount.
"Amazing!" the manager said. "How did you do it?"
"Easy," Jones replied. "I told him if he didn't pay up, I'd tell all his other creditors he paid us."

- How bankers do it...
Bankers do it risk-free.
Bankers do it just for money.
Bankers charge a fee each time they do it.
Bankers do it with varying rates of interest.
Bankers do it with a penalty for early withdrawal.

- A man walks into a New York City bank and says he wants to borrow $2,000 for three weeks. The loan officer asks him what kind of collateral he has. The man says "I've got a Rolls Royce -- keep it until the loan is paid off -- here are the keys." The loan officer promptly has the car driven into the bank's underground parking for safe keeping, and gives the man $2,000.

Three weeks later the man comes into the bank, pays back the $2,000 loan, plus $10 interest, and regains possession of the Rolls Royce. The loan officer asks him, "Sir, if I may ask, why would a man who drives a Rolls Royce need to borrow two thousand dollars?"

WISDOM WHISPERS

- Though no one can go back and make a brand new start, anyone can start from now and make a brand new ending.
- Let him who would enjoy a good future waste none of his present.
- Look at life through the windshield, not the rear-view mirror.
- Destiny is not a matter of chance; but a matter of choice. It is not a thing to be waited for, It is a thing to be achieved.
- To expect defeat is nine-tenths of defeat itself.
- Defeat never comes to any man until he admits it.
- I have not failed. I've just found 10,000 ways that won't work.

BRAIN TEASERS

1. "Owl Eye" appearance is seen in
   A.Hodgkins's disease B. Infectious mononucleosis C. CML
   D. Hairy Cell Leukemia
2. Cabot's rings are observed in
   A. Iron deficiency anemia B. Megaloblastic anemia C. CLL D. ALL
3. "Starry Sky" appearance is typical of
   A. Burkitt's lymphoma B. Follicular lymphoma C. Multiple myeloma
   D.Myelosclerosis
4. Which of the following is not a germ cell tumour?
   A.Teratocarcinoma B. Embryonal cell carcinoma C. Seminoma
   D. Leydig cell tumour
5. "Drop Met" is referred to spread of which tumours?
   A. CNS tumours B. Lung tumours C. Ovarian tumours D. Testicular tumours

Answers: 1. A, 2. B, 3. A, 4. D, 5. A, 6. D.

TROUBLE SHOOTING

GUIDELINES FOR BLOOD GROUPING AND ANTIBODY TESTING IN PREGNANCY

Introduction

Purpose of the Guideline

The purpose of the guideline is to define the red cell immunohaematology tests which should be applied in pregnancy. The aim of the testing programme is the prevention of haemolytic disease of the fetus and newborn. Since the majority of publications use the term 'haemolytic disease of the newborn', HDN, to refer to both fetus and newborn, it is used here too.

The Significant Developments

The use of monoclonal reagents and automation has considerably improved the related diagnostic scenario. The Serious Hazards of Transfusion [SHOT] haemovigilance scheme has focused attention on blood grouping and red cell serology practice and revised guidelines for compatibility testing in blood transfusion laboratories have been brought to the fore.

The introduction of non-invasive techniques to monitor fetal anaemia has influenced the management of allo-immunised pregnancies and the concentration of care of these cases in fetal medicine units has resulted in improved outcomes of intra-uterine transfusion.

It is endorsed that routine antenatal anti-D prophylaxis (RAADP) should be offered to all D negative women who have no detectable immune anti-D. Injections at 28 weeks and again at 34 weeks gestation are recommended. As the relative incidence of immune anti-D has declined the incidence of positive antibody screens due to prophylactic anti- D has increased and the two types of antibody cannot be distinguished by laboratory tests.

The risks associated with the misinterpretation of passive and immune anti-D are clear: if passive anti-D is misinterpreted as immune, anti-D prophylaxis may be omitted leaving the women unprotected from sensitization. If immune anti-D is misinterpreted as passive, appropriate follow-up of the antibody level during pregnancy may be curtailed putting the fetus at risk. The testing protocols recommended here are designed to provide clarity for practice in order to protect pregnant women including those who are D negative, and their infants

Informed consent

Providing information about any blood test and obtaining consent is a clinical responsibility and ideally informed consent should be obtained and documented prior to samples being taken

Purposes of laboratory tests

ABO and D typing to identify D negative women who require anti-D prophylaxis.

Screening and identification of red cell alloantibodies

To detect clinically significant antibodies which might affect the fetus and/or newborn to highlight possible transfusion problems

Follow-up tests when clinically significant red cell antibodies are present:-

To monitor the strength of antibodies to identify those pregnancies which are at risk of HDN and to predict fetuses/infants who are likely to require treatment for HDN.

To identify additional maternal alloantibodies. Women who have developed one or more antibodies may go on to form further antibodies of different specificities.

Recommendations for samples and request forms

Identification of samples

It is essential that samples from pregnant women are correctly identified and that request forms are accurately completed. SHOT reports provide evidence that errors in patient identification and sample labelling may lead to ABO incompatible transfusions. The record of ABO/D type derived from an antenatal sample may be used as the basis for the provision of suitable blood for transfusion, and the sample could be used for a crossmatch.

Misidentification can also lead to failure in, or inappropriate, administration of prophylactic anti-D.

Therefore, the same minimum patient identification on antenatal samples and request forms is required as for pre-transfusion samples] i.e.

i] Surname/family name [correctly spelt]

ii] First name[s] in full

iii] Date of birth [not age or year of birth]

iv] Unique identifier number e.g. Hospital number or IPD/ OPD number. When these are not readily 'address' is a suitable alternative identifier if it is given on both the sample and the request form.

Recommendation 1: Samples for antenatal screening are identified to the same standard as pre-transfusion samples (Good Practice Point [GPP])

Recommendation 2: Samples should be dated, labelled and signed by the person taking them, in the presence of the patient who should be asked to confirm demographic details. Any labels pre-printed away from the phlebotomy procedure, should not be accepted on the specimen

It is essential that any previous administration of prophylactic anti-D in the current pregnancy, including date and dose, is recorded on the laboratory request form.

Clinical history, particularly of HDN and previous transfusions, is also essential information on the request form.

Recommendations for laboratory testing

All test procedures must be well established and validated in compliance with published guidelines. Ideally testing should be performed on automated equipment which ensures positive sample identification and with electronic transfer of results to the woman's computer record.

Recommendation 3: ABO and D grouping must be performed in accordance with the guidelines for compatibility procedures in blood transfusion laboratories.

D grouping

Monoclonal IgM anti-D grouping reagents which do not detect DVI should be

TULIP NEWS

Tulip Group a Class Apart

Tulip Group is LEADING THE WAY IN IMMUNOHEMATOLOGY since its inception in 1988. Manufacturing products conforming to stringent international standards has enabled Tulip get **ISO** accreditation and **CE** (conforming to European quality standard) certification.

Apart from manufacturing and marketing excellent quality products, Tulip stands out for its excellent customer service, be it product complaint handling, sample related queries or technical support, Tulip's R & D goes an extra mile ahead in its quest for answers to sample-related issues and has assisted customers in identification of rare

We would like to illustrate a recent interesting case of rare blood group sample received at Tulip R & D for evaluation. The sample presented as A1 group, but was reacting with all ABO cells in reverse grouping.

Patient Information: 62 yrs, Male
Patient ID: (UHID: WHBG.0000008346)
History: No previous history of transfusion.
Other information:
(a) Patient posted for coronary artery bypass graft after a recent diagnosis of inferior wall myocardial infarction. (b) Preoperative lab workup was unremarkable except for blood bank workup, results of forward and reverse grouping were not matching. (c) On forward grouping patient's sample showed 'A1' positive. Reaction with Anti-H lectin was weak, but confirmed microscopically. (d) Reverse grouping the patient's serum showed weak agglutination with all cells 'A' 'B 'O' pooled cells as well as 'O' negative cells. Reactions were confirmed at 37°C. (e) DAT negative. IAT showed a titre of 16. (f) Random crossmatch of A positive, A negative and O negative blood also showed incompatibility (4/6 units)
Nature of the query: Identification of specific antibody for the patient sample

Further to this at Tulip we carried the following tests

1. Forward grouping (Slide test): With aforesaid patient's whole blood

Reagent	Lot. No	Avidity	Strength at 2 mins.
Eryscreen Anti-A	124621	2 secs	3+
Eryscreen Anti-B	124621	-	-
Eryscreen Anti-D	124621	6 secs	2+
Erybank Anti-A, lectin	107504	2 secs	3+
Erybank Anti-H lectin	108508	1 min	±

● Forward grouping was done on the patient's red cells and **the sample was found to be A1 Rh +ve group**.

2. Test for Autoantibody

Cells used	Serum used	Result
Patient's own cells	Patient's serum	-ve

No autoagglutination was observed, **therefore autoantibodies were absent in the patient's serum**.

3. Reverse grouping of the aforesaid patient's serum sample with known cells.

Known cells used	Result
A Rh +ve	+ve
B Rh +ve	+ve
O Rh +ve	+ve
A Rh -ve	+ve
B Rh -ve	+ve
O Rh -ve	+ve

The aforesaid serum reacted with all the cells indicating that the patient's serum contained an antibody that reacts with all these ABO cells. The common antigen in all ABO groups is the precursor H antigen.

The suspected antibody in the patient's serum could be **anti-H!**

- The patient's serum presumably containing (anti-H) had to be reacted with red cells in which the H antigen was absent i.e, Oh (Bombay Phenotype).

4. Tube test of the aforesaid patient's serum sample with Bombay phenotype cells

Known cells used	Result
Oh phenotype	-ve

The test gave negative result thus confirming the presence of **Anti-H antibody** in the patient's serum.

Secondly the tests also indicated that the patient's blood sample is compatible only with **Bombay Phenotype. (A good breakthrough that the patient could be transfused with Bombay phenotype blood).**

Confirmatory test for presence of Anti-H antibody in the patient' serum : is to check the reactivity of Anti-H with different blood groups. The order of reactivity of Anti-H with red cells of various ABO groups is **O A2 A2B B A1 A1B.** (O group has highest reactivity and A1B the least).

5.Tube test of the patient's serum with known cells (for order of reactivity)

Known cells used	Strength
O	3+
A2	2+
B	2
A2B	1+
A1B	

The order of reactivity observed with patient's serum sample was **O A2 B A2B A1B** The results were consistent with the graded reactivity pattern, except for the change in order of reactivity of **B** and **A2B** cells.
B cells showed unusually stronger reactivity than A2B.

This strong reactivity pattern with B cells could be due to the fact that the patient's blood group is A1(as observed from forward grouping results) therefore Anti-B could be present in the patient's serum in addition to anti-H.

To prove the presence of Anti-B, Anti-H antibody had to be totally removed from the patient's serum sample by adsorption with O group cells (O cells contains only H antigen) and perform the test No.5 once again

- The patient's serum sample was adsorbed of anti-H (anti-H was removed from the patient's serum sample by reacting the serum with O group cells). The supernatant serum devoid of anti-H was then tested again with the cells of (O, A1, A2, A1B, A2B).

6.Repeat of test No. 5 after adsorption of Anti-H antibody from patient serum

Known cells used	Strength
O	-
A2	-
B	2
A2B	1w
A1B	

All the tested cells gave negative results except for B, A2B and A1B.Thereby indicating that - Anti-B antibody in addition to Anti-H could be present in the patient serum.

Inference:
(a) The patient's sample could be Bombay phenotype A1 group with Anti-H & Anti-B antibodies in his serum. (b) The patient's serum sample did not react with Oh phenotype red blood cells (Bombay phenotype).

Vox Sang. 2006 Apr;90(3):195-7. Links
Detection of a new weak A blood-group allele (Aw11). Pruss A, Heymann GA, Braun J, Kiesewetter HH, Salama A.
BACKGROUND AND OBJECTIVES: Weak ABO variants may escape tests using unlicensed sera.
CONCLUSION: The use of CE-marked and licensed

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Fax: (0832) 2458544, E-mail: sales@tulipgroup.com. Website: www.tulipgroup.com

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