New Recommendations for Chronic Hepatitis B Testing

Recently, the Centers for Disease Control (CDC) released new guidelines which expand the testing recommendations for chronic hepatitis B (HBV) infections. Although the incidence of new HBV infections has declined due to vaccine availability, there are an estimated 800,000 to 1.4 million people with chronic HBV infections in the U.S. Because the disease can be asymptomatic for years, those with chronic HBV may be unaware of their infection, and are at high risk for late complications of the disease as well as potentially transmitting the virus. There are an estimated 2,000 to 4,000 deaths in the U.S. annually attributed to hepatitis B infection, mostly due to cirrhosis and liver cancer.

Previously, the CDC recommended hepatitis B screening for pregnant women & infants of HBV-infected mothers, household contacts and sex partners of HBV-infected individuals, HIV-infected people, persons born in countries with HBV prevalence >8%, and post-occupational exposure. The new guidelines expand the testing recommendation to include essentially three new groups:

- Patients receiving cytotoxic or immunosuppressive therapy, including chemotherapy, organ transplant recipients, and those treated for rheumatologic or gastroenterologic disease.
- People born in geographic regions with HBV prevalence >2%. This includes Eastern Europe, Asia, the Middle East, and Pacific Islands.
- People with behavioral exposures to HBV, including past or current injection drug users, and men who have sex with men.

Hepatitis B surface antigen (HBsAg) is the serologic marker primarily used to identify and define chronic hepatitis B. Chronic infection is defined as the absence of concurrent hepatitis B core IgM antibody (IgM anti-HBc) and by persistence of HBsAg or HBV DNA for at least 6 months. All HBsAg-positive persons are considered infectious. In addition to HBsAg, serologic testing for hepatitis B surface antibody (anti-HBs) and total core antibody (anti-HBc) is advised for immuno-suppressed patients.

The new guidelines also include recommendations for medical management of chronic HBV and are available through www.cdc.gov, MMWR Recommendations and Reports, September 19,2008; Vol. 57 (RR-8).

Working up a Prolonged Activated Partial Thromboplastin Time

The activated partial thromboplastin time (APTT) is a measure of the integrity of the intrinsic and common pathways of the coagulation cascade. The APTT is the time, in seconds, for patient plasma to clot after the addition of an intrinsic pathway activator, phospholipid and calcium. The APTT reagent is called a partial thromboplastin because tissue factor is not included with the phospholipid as it is with the protime (PT) reagent. The activator initiates the contact system. Then, the remaining steps of the intrinsic pathway take place in the presence of phospholipid.

The concept of separate intrinsic and extrinsic pathways of coagulation is useful for understanding and diagnosing blood coagulation abnormalities in vitro, however it should be appreciated that in vivo there are multiple interactions between the two pathways. The APTT will generally be prolonged when a clotting factor level is less than 30-40%. Since the normal range for most clotting factors is 50-150%, a normal APTT does not rule out the possibility of a mild factor deficiency.

There are 6 causes of a prolonged APTT in the presence of a normal or slightly prolonged PT:

- Pre-analytical errors
- Heparin
- Lupus anticoagulant
- Coagulation factor deficiency associated with risk of hemorrhage
- Coagulation factor deficiency with no risk of hemorrhage
• Specific coagulation factor inhibitor

In the investigation of a prolonged APTT, pre-analytical errors should be ruled out first. The most common pre-analytical cause of a prolonged APTT is contamination with heparin in a sample drawn from an arterial or central line. The APTT is also affected by an altered plasma to citrate ratio in blue top collection tubes, which may be seen with a high hematocrit (>55%), or a sample with a short or long draw. Other pre-analytical problems include dilution of a sample drawn above an IV, clot formation due to inadequate mixing, and transport or processing delays >4 hours.

Therapeutic IV administration of unfractionated heparin is the most common cause of a prolonged APTT in inpatients. Subcutaneous administration of low molecular weight heparin seldom prolongs the APTT more than 40 seconds.

Lupus anticoagulants are acquired inhibitors directed against phospholipid-binding proteins and are a common cause of APTT prolongation. In vivo, lupus anticoagulants do not interfere with coagulation factor complex formation on the platelet surface, and are not usually associated with a bleeding tendency.

Hereditary coagulation factor deficiencies which selectively prolong the APTT and are associated with a bleeding tendency include factors VIII, IX and XI. Hereditary coagulation factor deficiencies which selectively prolong the APTT but are not associated with a risk of hemorrhage include factor XII, prekallikrein and high molecular weight kininogen (HMWK).

Approximately 15% of patients with severe factor VIII or IX deficiency develop alloantibodies (inhibitors) to transfused factor concentrate. Autoantibodies against clotting factors may also arise spontaneously, or associated with various diseases and drugs, most commonly directed against factor VIII. The inhibitor-factor complexes are rapidly cleared, resulting in factor deficiency and a severe bleeding tendency.

The common acquired coagulopathies such as liver disease, moderate to severe vitamin K deficiency, DIC and massive transfusion may cause prolongation of the APTT; however the PT will also be prolonged in these disorders, due to multiple clotting factor deficiencies.

A mixing study is used to differentiate between a coagulation factor deficiency and the presence of an inhibitor. It is usually performed by mixing equal volumes of patient plasma and normal pooled plasma and then repeating the APTT. The basic principle is that the normal plasma contributes a sufficient amount of clotting factor to correct for a factor deficiency. A mixing study that corrects the APTT is characteristic of a coagulation factor, whereas one that does not indicates a factor inhibitor. Mixing studies on mildly prolonged APTTs (<5 seconds above upper limit of normal) are confusing and difficult to interpret.

Inhibitors are classified into 3 general categories:

- Medications such as heparin and direct thrombin inhibitors (lepirudin, argatroban)
- Nonspecific inhibitors such as lupus anticoagulants
- Specific coagulation factor inhibitors such as a factor VIII inhibitor

Heparin and direct thrombin inhibitors can be ruled out by reviewing the clinical history and medication list. However, this review cannot detect a sample that is contaminated by heparin at the time of collection. Heparin can be confirmed by performing a heparin anti-Factor Xa assay.

In practice, two types of mixing studies are actually performed: immediate and incubated. In the immediate mix, the APTT is performed immediately after mixing patient and normal plasma. In the incubated mix, the APTT is performed after incubating the mixture for one hour. The incubated mix is not necessary if the immediate mix shows evidence of a fast acting inhibitor such as a lupus anticoagulant. However, the incubated mixing study is necessary to distinguish between a factor deficiency and a time dependent inhibitor such as a factor VIII inhibitor.

Two assays are commonly used to confirm the presence of a lupus anticoagulant. The hexagonal phase phospholipid test consists of adding phospholipid to the APTT. The second assay is based on adding phospholipid to the dilute Russell Viper venom test. Shortening of the clotting time in either or both of these assays confirms that inhibition of the APTT is phospholipid dependent.

Clinical Pathologists are always available to assist with interpretation of these coagulation workups.