Saint Luke's Regional Laboratories
Clinical Laboratory Letter

April 2005

Which Testosterone should be measured for Older Men?

Testosterone circulates in the blood 98% bound to protein. In men, approximately 40% is bound with high affinity to sex hormone binding globulin (SHBG) and approximately 60% is bound weakly to albumin. The testosterone fraction that is bound to albumin dissociates freely in the capillary bed, becoming available for tissue uptake. Only 2 to 3% of testosterone exists in the free state. All non-SHBG bound testosterone is considered to be bioavailable.

After the age of 40 years, men's total testosterone levels begin to decline about 0.4% per year. Men with chronic illnesses have testosterone levels that are 10–15% below that of healthy age-matched men. SHBG increases with age, causing free and bioavailable testosterone to decrease to a greater extent than total testosterone. Gonadotropins usually do not increase above the normal range with aging.

Young men have a circadian rhythm of testosterone, with the zenith occurring in the morning between 0600 and 0800 h and the nadir in the late afternoon between 1700 and 1800 h. This circadian rhythm disappears in elderly men. The difference in testosterone levels between young and elderly men is most pronounced when measurements are made in the morning.

Total testosterone is the most appropriate test to determine whether an older man is hypogonadal or not. If the total testosterone level is below 2 ng/mL, the individual should be considered hypogonadal, regardless of age. The question that cannot be answered today is whether men with total testosterone levels between 2 and 3 ng/mL are hypogonadal and whether they would benefit from androgen replacement.

A symposium on "Issues in Testosterone Replacement in Older Men", concluded that no well designed clinical trials have indicated that one testosterone assay is better than any other at determining whether an older man is androgen deficient (J CE & M 1998;83:3436-38). Total testosterone is as good a measurement, and less expensive, than the more complex and labor intensive measurement of free or bioavailable testosterone. Measurements of free or bioavailable testosterone should be considered experimental, until they are clearly shown to be a better marker of hypogonadism in elderly men than total testosterone levels.

According to Mayo Medical Laboratories, total testosterone should be measured to monitor testosterone replacement therapy. During treatment with depot-testosterone preparations, trough levels of serum testosterone should remain within the normal range, while peak levels should not be significantly above the normal young adult range.

New Protein S Activity Assay

Protein S, a vitamin K-dependent protein, is one of the major naturally-occurring anticoagulants, functioning as a cofactor for protein C. Hereditary protein S deficiency is relatively rare, with a prevalence of approximately 1-2% of heterozygous deficiency in patients with venous thrombosis, and associated with an approximately 10-fold increased risk of venous thromboembolism. Acquired protein S deficiency is much more common than the hereditary form, and is seen in the following conditions:

- inflammatory disorders (since the binding protein for protein S is an acute phase reactant)
- pregnancy
- estrogen therapy
- thrombosis
- DIC
- liver disease
- warfarin therapy (since protein S is vitamin K-dependent)

Protein S is partially (60%) bound to the C4b binding protein; the remaining free portion is the functionally active component. There are three ways to measure protein S in the plasma: immunological assays for both total and free protein

S antigen, and a functional assay for protein S activity. Until fairly recently, protein S activity assays have not been considered reliable, consequently Saint Luke's Regional Laboratories has previously used the free protein S antigen assay as the initial screening test for protein S status. An acceptable protein S activity assay is now available, and will be adopted as our screening assay for protein S levels in April 2005.

There are three varieties of hereditary protein S deficiency, as shown in the table. Types I and III are quantitative deficiencies, differing only in the level of total protein S antigen, and Type II is a qualitative deficiency with normal levels of protein S antigen and a decrease only in protein S activity. Acquired protein S deficiency resembles either Type I or Type III deficiency. Using the protein S activity assay as a screening test will ensure that all subtypes are detected.

Sub-types of Hereditary Protein S Deficiency

Subtype	Total Protein S Antigen	Free Protein S Antigen	Protein S Activity
Type I	D	D	D
Type II	N	N	D
Type III	N	D	D

Protein S activity assay will be available as an individual test, and as a component of the venous thrombosis panels. The reference range for protein S activity is 57-172%. If the protein S activity level is decreased, assays for both free and total protein S antigen will be performed. A reliable protein S assay cannot be performed in a patient on warfarin anticoagulation. If anticoagulation cannot be discontinued, consideration should be given to stopping warfarin for 10 days prior to performing the assay, while the patient is temporarily anticoagulated with standard or low molecular weight heparin. In view of the high incidence of acquired protein S deficiency in hospitalized patients, it is preferable to perform the assay when a patient is clinically stable, ideally several weeks after an acute event. The assay is performed Monday, Wednesday and Friday, and one 5mL pale blue top tube is required.

Herpes Simplex Type-Specific Serology

Genital herpes simplex virus (HSV) infections are reportedly the most common sexually transmitted disease among women. The seroprevalence of HSV-2 has increased substantially over the last decade, and genital infections due to HSV-1 are becoming more frequent. Differentiation of HSV-1 from HSV-2 is important prognostically, since genital HSV-2 infection is twice as likely to reactivate and recurs 8-10 times more frequently than genital HSV-1 infections. Recurrence of genital HSV-1 is rare after the first year of infection. Acquisition of new HSV-1 infection in an individual with HSV-2 antibodies is unusual, however women with genital HSV-1 infection are still at risk for HSV-2 acquisition.

The American College of Obstetrics and Gynecology recently released Clinical Management Guidelines for Herpes Simplex Infections (ACOG Practice Bulletin #57, 11/04). In women with new or recurrent genital ulcers, PCR testing is 1.5 to 4 times more sensitive than culture for diagnosis. In the absence of lesions, or when PCR is negative despite high clinical suspicion, type-specific antibody testing is recommended. IgG antibody to HSV is detectable 2-12 weeks after infection and persists indefinitely. Only tests based on detection of antibody to HSV glycoprotein G-2 are type-specific due to cross-reactivity between viruses. Compared to Western blot, the sensitivity of type-specific HSV antibodies is 96-100%, with a specificity of 97-98%. It is also recommended that women who have partners with genital herpes should be tested with type-specific serology to assess risk of infection.

Saint Luke's Regional Laboratories replaced conventional virus culture with herpes simplex PCR for genital specimens in June 2003. Type-specific herpes antibody testing is also available through Mayo Medical Laboratories. The test should be requested as 'HSV Type 1 & 2 Specific Antibodies' and the specimen requirement is one red top tube of blood.