In-Common Laboratories

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Lab Testing: Testosterone

The Debate

Aging is accompanied by various physical and mental changes, reduced sexual function and libido and declining vitality. In males, aging is also accompanied by a reduction in circulating testosterone (T), the major androgen hormone. A causal connection between aging and reduced T has not been established but is widely debated and occurrence of the Testosterone Deficiency Syndrome is often accepted as fact. Thus the possibility of reversing some of the effects of aging in males by testosterone supplementation is an on-going debate. A very vigorous debate considering that supplementation can cost a patient thousands per year. The controversy extends to diagnostic criteria as well as treatment.

Guidelines

Over the years multiple diagnosis and management guidelines have been issued, by Endocrine Society in 2010 (), European Association of Urology in 2014 (2), International Society for Sexual Medicine in 2015 (3) and The International Society for the Study of the Aging Male (ISSAM) Hypogonadism panel in 2015 (4).

Canadian Guidelines

The Canadian guideline issued in 2015 by the Canadian Men's Health Foundation Multidisciplinary Guidelines Task Force on Testosterone Deficiency (5) is aimed at Canadian physicians. Of particular interest to laboratorians are the following recommendations:

- The initial biochemical test should be total testosterone level measured in serum samples taken in the morning; determinations of bioavailable testosterone or free testosterone should be restricted to patients with equivocally low total testosterone levels (strong recommendation; high-quality evidence).
- We recommend that sample collection for testosterone measurement occur between 7 am and 11 am, or within 3 hours after waking in the case

of shift workers (strong recommendation; moderate-quality evidence).

- Testosterone levels should be measured with the use of testosterone assays traceable to internationally recognized standardized reference material; commercial assays should be certified by the testosterone standardization program of the US Centers for Disease Control and Prevention (strong recommendation; high-quality evidence).

- Measurement of sex hormone–binding globulin with calculated free or bioavailable testosterone should be restricted to men with symptoms of testosterone deficiency and equivocally low testosterone levels (strong recommendation; moderate-quality evidence).

The Measurements: Total Testosterone

Testosterone circulates free or bound to proteins. The major portion is tightly bound to sex hormone binding globulin (SHBG). A portion is bound less tightly to albumin and the small remainder fraction is free. The free and albumin-bound fractions are considered biologically active or bioavailable.

The most commonly available clinical assays for testosterone are chemiluminescent immunoassays or chromatographic-mass spectrometry assay (e.g. LC-MS/MS or GC-MS). Isotope-dilution mass spectrometry is the reference method and not generally used for clinical specimens. The MS methods are generally more sensitive, more specific (less interference from other steroids) and more precise, especially in the lower range commonly found in women, children and older men. For this population, immunoassays are less desirable, given their dependence on the ability to free the bound forms of testosterone prior to measurement, and on issues of specificity and sensitivity (6, 7). LC-MS/MS has been recommended as the clinical method of choice for all sex steroids (8). In an Editorial in The Journal of Applied Laboratory Medicine, William E. Winter states “I am hopeful that continued efforts by the CDC will provide firm guidance that testosterone in women and children (and possibly in hypogonadal men) should always be measured by mass spectrometry”. (9)

The Measurements: Free Testosterone

Free testosterone can be assessed by physical separation methods that are technically challenging and generally not used in clinical service. Equilibrium dialysis is the reference method. Methods using testosterone analogues were once popular but are no longer considered valid for clinical use.

The bioavailable fraction can be assessed by differential-ammonium sulphate precipitation or calculated on the basis of testosterone, SHBG and

albumin and their dissociation constants.

Specimen Collection

Given the diurnal variation of testosterone, indeed of most steroids except progesterone (10), it is recommended that specimens be collected in the morning. However the diurnal effect is blunted in older men. (11)

Total Testosterone: Assay Standardization

All assays should be traceable to international reference preparations. Given the technical variability between methods and labs, each lab should validate their methods and develop clinically relevant reference values. Variation between labs is most pronounced when multiple imprecisions must be accounted for, as in bioavailable testosterone assays or calculated free testosterone.

Testosterone Supplementation and Aging Males – The Testosterone Trials

In 2004, the Institute of Medicine "was struck by the paucity of randomized controlled clinical trials, particularly in middle-aged or older men". A recommendation was made to initiate a coordinated set of clinical trials to evaluate the efficacy of testosterone supplementation in aging men while assessing the benefits and risks. The National Institutes of Health sponsored the trials, issuing the plan in 2014 as "The Testosterone Trials: The Design of Seven Coordinated Trials to Determine if Testosterone Treatment Benefits Elderly Men" (13). The Trials included the Physical Function Trial, Sexual Function Trial, Vitality Trial, Cognitive Function Trial, Anemia Trial, Bone Trial, and Cardiovascular Trial.

The inaugural report covering the Physical, Sexual and Vitality Trials was published in *The New England Journal of Medicine* (NEJM) in February 2016 (14). Testosterone was measured using a LC-MS/MS assay certified by the Centers for Disease Control. While the results show that there was some benefit, they do not yet unequivocally support clinical benefit of testosterone supplementation and the safety issues could not be adequately assessed. An NEJM Editorial considered this "a landmark study in the field of men's health and no doubt a bell-wether for additional important contributions from the Testosterone Trials."

[The Testosterone Trials are reminiscent of the fifteen-year, multi-million dollar Women's Health Initiative (WHI), also sponsored by the National Institutes of Health (USA) and initiated in 1991. The WHI consisted of three Clinical Trials (Hormone Therapy, Dietary Modification and Calcium/Vitamin

D Intervention) and an Observational Study

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“Inside ICL”: Dr. Shash Tilak

Dr. Shashank (‘Shash’) Tilak was educated at the University of Toronto where he pursued studies in Biochemistry, Cell Biology and Clinical Chemistry. He obtained a B.Sc. and Ph.D followed by the post-doctoral training program in Clinical Chemistry and is a Certified member of the Canadian Society of Clinical Chemists. His professional career was launched with ICL in 1985. Over the years Shash has enjoyed varied tasks and responsibilities in the Laboratory, Specimen Referral and Community Services (Specimen Collection) Divisions. He is currently Privacy Officer, Safety Officer, and the Laboratory and Scientific Director.