Assays of Serum Testosterone

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KEYWORDS

• Adult • Humans • Testosterone/blood • Steroids/blood • Circadian rhythm
• Hypogonadism/metabolism • Testosterone/deficiency • Immunoassay

KEY POINTS

- Hypogonadism is increasing in prevalence as the population ages and obesity rates climb.
- The diagnosis of hypogonadism depends on an assessment of the clinical signs and symptoms of hypogonadism and the determination of serum testosterone levels.
- Significant variability can exist in serum total and free testosterone levels due to intraindividual variation and assay variability.
- Current serum testosterone assay methods include immunoassay and mass spectrometry. However, no current gold standard assay exists for the assessment of total testosterone levels.
- Standardization programs are in place to improve the accuracy and comparability of testosterone assays in clinical and research laboratories.

INTRODUCTION

Male hypogonadism is defined by the Endocrine Society as a clinical syndrome that results from the inability of the testes to produce physiologic levels of testosterone (T) and a "normal" number of spermatozoa secondary to a dysfunction in the hypothalamic-pituitary-gonadal axis (HPG).[1] The diagnosis of hypogonadism depends on the assessment of the clinical signs and symptoms of hypogonadism and serum T levels assayed on at least 2 different occasions.[2] The symptoms most suggestive are low libido followed by a reduced quality of erections; however, these markers are subjective and nonspecific.[3] The laboratory's assessment of serum T levels, in contrast, provides an objective measure of the gonadal status that can support or refute clinical signs and symptoms. Nevertheless, serum T levels can vary widely between samples drawn from the same patient and between the various laboratory assay platforms due to a multitude of factors, such as diurnal variation, systemic illnesses, and seasonal variation, as well as assay-specific factors, and must be interpreted with caution.

Current clinical laboratory assay platforms include immunoassays and mass spectrometry (MS). Despite significant advances to improve the accuracy and precision of currently available assays, limited comparability exists between assays at the lower and upper extremes of the T range. Moreover, there is no currently accepted gold standard method of assessment. In this review, the growing significance of T assays in the aging population is highlighted, the indications

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for the various assays used to assess free and total T are discussed, and the impact of various preanalytical and analytical factors that can influence the results of T assays are analyzed.

PREVALENCE

Late-onset hypogonadism is considered to be a disease of aging. As the population ages, the epidemiologic burden of male hypogonadism is expected to proportionally increase. The World Health Organization estimates that by 2020, the number of people over the age of 65 will for the first time surpass the number of people less than 5 years of age.[4] According to the same estimates, the number of people over the age of 65 will nearly double from a current population of 524 million people to roughly 1.5 billion by 2050. A population-based observational study by Araujo and colleagues[5] estimated the crude prevalence of biochemically confirmed, symptomatic male hypogonadism (total T <300 ng/dL) to be 5.6% in a cohort of 1475 US men aged 30 to 79 and 18.4% in subset of men aged 70 to 79. The investigators projected in this study that in 2025, as many as 6.5 million American men will exhibit symptomatic androgen deficiency. In addition, comorbidities that accumulate as part of aging can also increase the epidemiologic burden of hypogonadism. The Healthy Man Study showed a strong association between comorbidities, such as obesity and ex-smoker status, and T values over repeat measures drawn over a 3-month duration.[6] Based on these trends, a significant increase in utilization of T assays can be expected, and a thorough understanding of the nuances and limitations of the various T assay platforms will be invaluable in the care of the hypogonadal patient.

DIAGNOSING HYPOGONADISM

Male hypogonadism results from a reduction of androgen levels due to a dysfunction in the HPG axis. The presence and magnitude of symptoms associated with male hypogonadism depend on the concentration of T available to the target organ. Although the total testosterone (TT) concentration is often used as a surrogate for the amount of T available for the target organ, the bioavailable fraction of the TT is a more accurate measure of T concentrations at the tissue level and correlates better with clinical symptoms than TT.[3,7,8] T circulates in either a protein-bound or non-protein-bound (free) state. In a healthy adult man, most T circulates in a protein-bound state attached to either albumin ~50%, sex hormone-binding globulin (SHBG) ~44%, or cortisol-binding globulin ~3.5%. The remaining 2% to 3% of T circulates as unbound, free testosterone (FT).[9,10] The bioavailable fraction of the TT consists of albumin-bound and FT. Unlike the TT level, the bioavailable fraction is not susceptible to SHBG concentration, which can fluctuate with age, thyroid function, drugs and alcohol consumption, and disorders of the pituitary and the liver.[11]

Diagnosing hypogonadism is challenging in the setting of a patient with the signs and symptoms consistent with hypogonadism, borderline normal TT value, and possible alteration of SHBG level. Under these circumstances, the Endocrine Society recommends measuring free or bioavailable testosterone (BioT) levels with an accurate and reliable assay.[1] Several methods have been used to assess FT and BioT levels, such as equilibrium dialysis (FT_D),[12] direct estimation of serum free T by an analogue ligand immunoassay (FT_A),[13] ammonium sulfate precipitation of SHBG-bound T,[14] calculation of the free androgen index (FAI) using the formula TT/(SHBG $\times$ 100),[15] and multiple algorithms to calculate the free testosterone index (FTI) based on the concentration of albumin and SHBG. Although the FT_D is considered the gold standard, this method is time-consuming and expensive.[16] In contrast, calculation of the FTI using published algorithms, such as the Vermeulen equation,[17] is inexpensive and has been shown to have good correlation with gold standard methods of comparison.[17–22]

The Vermeulen equation, which is widely used, determines the FT level using the concentration of T bound to albumin and the albumin concentration.[17] The equation operates under the assumption that the albumin concentration is in the physiologic range of 40 to 50 g/L (5.8–7.2 $\times$ 10^{-4} mol/L).[17] There are several shortcomings to this equation. If the albumin concentration is expected to deviate from the physiologic range, Vermeulen and colleagues[17] recommend calculating the albumin concentration in order to account for the reduced amount of albumin-bound T. In addition, the Vermeulen equation assumes that T does not face significant competition from other steroid hormones, such as estradiol (E2) and dihydrotestosterone (DHT), for its binding site on albumin.[16] Supraphysiologic levels of E2 and DHT will confound the calculation, leading to underestimation of the FT.

In a cross-sectional study of 50 men aged 28 to 90, Morley and colleagues[22] compared the results of different tissue-available T assays, including the ammonium sulfate precipitation method, FT_D, FT by ultracentrifugation (FT_U), FT_A, FTI, and TT, to determine the utility of each assay in the assessment of gonadal status. The investigators