The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine

eJIFCC

Testosterone deficiency – establishing a biochemical diagnosis

Yonah Krakowsky, Ethan D. Grober

Department of Surgery, Division of Urology, Mount Sinai & Women's College Hospital, Toronto, Ontario, Canada

ARTICLE INFO

Corresponding author:
Ethan D. Grober
Murray Koffler Urologic Wellness Centre
Mount Sinai Hospital
60 Murray Street, 6th Floor, Box 19
Toronto, ON M5T 3L9
Canada
Phone: 416-586-4800 ext. 3985
E-mail: egrober@mtsinai.on.ca

ABSTRACT

Testosterone deficiency is a common and often unrecognized disorder impacting the lives of many men. Symptoms related to low testosterone are relatively non-specific and clinicians must therefore ensure that a patients' symptomatology is supported by a biochemical profile suggestive of testosterone deficiency. There are many options available to determine a patient's testosterone level and laboratories will vary in the type of biochemical assessment they provide. In assessing patients with suspected low testosterone, the presence of symptoms and a low total testosterone is usually sufficient to initiate therapy. In equivocal cases, measurement of free or bioavailable testosterone with a reliable assay can further clarify the clinical picture. By understanding the differences between total, free and bioavailable testosterone, and the accuracy and reliability of their measurement, clinicians can better interpret their patients' biochemical testosterone profile.

INTRODUCTION

Testosterone Deficiency (TD), also known as Androgen Deficiency in the Aging Male (ADAM), hypogonadism and andropause is estimated to affect 10% of men older than 30 years of age and up to 40% of men older than 70 years of age[1]. Despite its prevalence, it is estimated that only 5-10% of men with low testosterone are being treated[2]. It is now well established that TD negatively impacts sex drive, erectile function, energy levels, mood, cognition, muscle mass, bone density and fat accumulation[3]. Further, it has been demonstrated that correcting testosterone to normal physiologic levels can lead to improvement of many of these conditions. Despite such a prevalent, reversible problem, most men are not evaluated or treated. Moreover, even when the diagnosis is suspected, primary care physicians, endocrinologists and urologists face the challenge of making an accurate biochemical diagnosis due to controversies regarding testosterone measurement. In this review paper, we aim to summarize the different options with regards to testosterone measurement and their associated strengths and limitations. Informed clinicians should be able to request and interpret biochemical results within this context to best assess and treat patients with TD.

WHAT IS TESTOSTERONE?

Understanding the concepts and controversies surrounding the biochemical evaluation of testosterone deficiency requires a fundamental understanding of the physiology of testosterone production, homeostasis and action. Testosterone is critical in the male for its contribution to libido, mass muscle, fat distribution, mood, energy and sexual function. Testosterone is largely (90%) produced by Leydig cells within the testes. Gonadotropin-releasing hormone (GnRH), released by the arcuate nucleus of the hypothalamus regulates the pituitary production of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH stimulates the production of testosterone while FSH binds to Sertoli cells and promotes spermatogenesis. Approximately 10% of male androgens are produced by the adrenal glands.

Testosterone is metabolized to dihydrotestosterone (DHT) and estradiol (E2) - active metabolites that provide negative feedback at the level of the pituitary. Testosterone exerts its effects through binding androgen receptors in target cells. In some instances testosterone is a prohormone and is converted by 5-alpha reductase enzymes to DHT. Two distinct 5-alpha reductase enzymes exist. Type 1 is androgen independent and is located in the skin, liver and brain. Type 2 is controlled by androgens and is distributed in the prostate, seminal vesicles and testicles. DHT has a 10-fold greater affinity for androgen receptors than testosterone.

Testosterone exists in the bloodstream in two forms, bound and unbound. The vast majority of testosterone is bound to plasma proteins with approximately 2-3% of total testosterone being unbound. The unbound component is referred to as free testosterone (FT) and is thought to be the component that has access to cells and possesses androgenic action. Testosterone that is complexed to albumin is weakly bound and has androgenic potential. Testosterone bound to sex hormone binding globulin (SHBG) is tightly bound and is essentially inactive. Bioavailable testosterone (BT) refers to both free testosterone and that which is bound to albumin.

TESTOSTERONE MEASUREMENT

Measuring serum testosterone has limitations both in theory and in practice. Circadian rhythms influence testosterone with levels peaking in the morning[4]. Reflecting a conservative approach to diagnosis, testosterone blood measurement is usually requested in the morning

hours between 8-10 am. However, more recently, there is evidence that as men age, such diurnal variations are blunted to some degree[5]. Interindividual variability in testosterone measurements has been demonstrated within the same week[6]. Acute illness will depress testosterone levels and misrepresent one's true hormonal status[7]

Many expert advisory panels and clinical guidelines have avoided strictly defined cut-off values for "normal" testosterone due to difficulties in measurement techniques and variability between institutions and patients over time. What form of testosterone should be measured and what method of measurement should be employed remain controversial but vitally important issues in the diagnosis and treatment of testosterone deficiency syndrome.

VARIATION OF TESTOSTERONE LEVELS BASED ON PATIENT FACTORS

Testosterone levels have been shown to vary with many pathological and physiological processes. From age 40, mean serum total testosterone levels have been shown to gradually decline at a rate of about 1% per year. Moreover, with age, SHBG increases thus lowering both bioavailable and free testosterone by removing more testosterone from the bioavailable pool, reducing the total amount capable of exerting androgenic activity. Various factors can alter physiological levels of SHBG and, with them, levels of bioavailable testosterone (see table 1). SHBG is found to be decreased in obesity, acromegaly and hypothyroidism. Aging, liver disease, hyperthyroidism, and anticonvulsant use all increase SHBG level and therefore decrease the bioavailable levels of testosterone[8].

Lifestyle choices and comorbid conditions may also lead to lowered levels of testosterone. Tobacco, alcohol use, caffeine, obesity and stress have all been demonstrated to be associated with lower testosterone levels[9]. Type 2 diabetes, hypertension and sleep apnea are independent risk factors for declining testosterone levels[10]. The Hypogonadism In Men (HIM) study found the odds ratio for having low total testosterone to be 1.84 for hypertension, 2.09 for diabetes, and 2.38 for obesity[11]. A significant association between Vitamin D and hypogonadism has been described in well-designed studies[12].

TESTOSTERONE DEFICIENCY - SYMPTOM INVENTORIES

A number of diagnostic inventories or questionnaires exist for recognizing symptoms of testosterone deficiency. As a group, these inventories have been shown to have high sensitivity

Table 1 Conditions impacting sex hormone binding globulin

Conditions that increase SHBG	Conditions that decrease SHBG
Aging	Obesity
Liver Disease	Acromegaly
Anorexia	Anabolic Steroids
Anticonvulsant use	Diabetes
Hyperthyroidism	Hypothyroidism

in diagnosing a testosterone deficient state, but lack specificity for the condition[13]. The three most commonly used inventories currently are the Androgen Deficiency in Aging Males (ADAM)[14], the Aging Male Scales (AMS)[15], and the questionnaire of the Massachusetts Male Aging Study (MMAS)[16]. These inventories address the main symptomatology associated with testosterone deficiency (see table 2).

OPTIONS IN MEASURING TESTOSTERONE

Total testosterone

Total testosterone measures all forms of testosterone in the serum, both bound and unbound. Due to cost and availability, clinical evaluation of a patient suspected to be testosterone deficient generally begins with total testosterone measurement. Assays for total testosterone in plasma pose a number of challenges. Concentration of total testosterone can vary throughout the day. Steroids of similar structure to testosterone have been shown to cause assay interference with some assays. Further, validated age and/or gender corrected normal ranges do not currently exist using a standardized assay and there is no universally recognized testosterone calibrating standard thus complicating the use of thresholds for normal values. Lastly, conditions that affect levels of SHBG may ultimately skew the interpretation and significance of the total testosterone measurements. For example, as SHBG increases with age, total testosterone may be determined to be "normal", yet bioavailable levels of testosterone may be low.

Total testosterone can be measured through immunoassays or mass spectrometry. Immunoassays can either be performed directly by radioimmunoassay (RIA), enzyme-linked immunosorbent

Table 2 ADAM questionnaire

Androgen Deficiency in Aging Males (ADAM)	Patient Response
Do you have a decrease in libido?	Yes or No
Do you have lack of energy?	Yes or No
Do you have a decrease in strength or endurance?	Yes or No
Have you lost height?	Yes or No
Have you noticed a decrease in your enjoyment of life?	Yes or No
Are you sad and/or grumpy	Yes or No
Have you noticed a deterioration in your ability to play sports	Yes or No
Are you falling asleep after dinner?	Yes or No
Has there been a deterioration in your work performance?	Yes or No
Are your erections less strong?	Yes or No

assay (ELISA) or chemiluminescence immunoassay (CLIA) or after extraction and chromatography. The extraction and/or chromatography procedures allow for the removal of interfering proteins and the use of a large serum samples to increase measurement sensitivity. It is widely agreed that adding extraction and chromatography steps increases the accuracy of the direct assay[17]. Using extraction/chromatography or mass spectrometry, however, requires technical expertise, special facilities with waste disposal capabilities, and adds significantly more cost. Direct assays are technically simple, automated and inexpensive compared to those combined with extraction and/or chromatography. They are also significantly quicker, catering to busy clinics and laboratories. Direct assays, however, may overestimate actual testosterone levels and show limited accuracy at lower testosterone levels[18].

While there are no universally recognized lower and upper limits to total testosterone values, it is generally agreed that serum total testosterone values greater than 15 mmol/L are unlikely to be associated with significant clinical testosterone deficiency. A symptomatic male with total testosterone between 8 mmol/L and 15 mmol/L can reasonably be offered a trial of therapy. Measuring SHBG, BT or FT may be of benefit in symptomatic individuals with normal total testosterone and conditions that may affect SHBG levels.

Free testosterone

Free testosterone (FT) refers to the testosterone that is unbound. In most men, this represents 2-3% of total testosterone. The general consensus is that FT best correlates to the true androgenic state of the patient. There are three ways of measuring FT- indirectly by equilibrium dialysis, directly using an analog-based RIA (analog FT), or through standardized calculations. Equilibrium dialysis is costly and manual but remains the gold standard in measuring FT. Equilibrium dialysis involves adding radiolabeled testosterone (3 H-T) to the sample being assayed. The free and bound 3H-T is then separated after equilibrium is attained. The percentage of free 3 H-T is multiplied by the total testosterone to calculate the FT of the sample. This methodology is not used clinically due to cost and convenience. It is currently limited to mainly reference laboratories but serves as the standard in all investigational studies of testosterone measurement.

The direct analog-based RIA method has been in widespread use in Canada for over a decade[19]. This assay consists of adding a radiolabeled testosterone analog to an unextracted serum sample. The direct analog-based assay has received criticism from experts due to the fact that its results are consistently lower than those obtained by equilibrium dialysis[20]. However, some experts feel that as long as analog FT results are interpreted with that correction in mind, they are clinically valuable. Studies comparing the analog FT immunoassay showed a strong and clinically meaningful correlation to the equilibrium dialysis when compared to the calculated FT[21]. Due to numerical differences each test would require unique reference values. A recent investigation has demonstrated high correlation between calculated FT and direct analog-based RIA measured FT when compared to equilibrium dialysis, the gold standard in FT measurement[22].

Due to the cost and effort associated with empiric measurement of free testosterone, calculated free testosterone is a reasonable and far more commonly performed method. By measuring total testosterone, SHBG and either assuming an albumin value or measuring it, a free testosterone calculation can be performed. Studies have shown that application of standardized albumin levels leads to very little variation in calculated free testosterone and is clinically acceptable[23]. There are many online testosterone calculators

available for clinical use[24]. Laboratories are now independently reporting calculated free testosterone which obviates the need for clinicians to look up and implement tedious equations. The Vermeulen and Sodergard algorithms are the most commonly used in both clinical practice and research protocols[25].

In summary, direct assays for free testosterone are simple, rapid, and can be automated. Equilibrium dialysis is relatively expensive and technically demanding but remains the gold standard for free testosterone measurement. Calculated measurements, while useful, are limited by the accuracy of measurement of total testosterone and SHBG but have also demonstrated good correlation to equilibrium dialysis (see table 3).

Bioavailable testosterone

Bioavailable testosterone (BT), which consists of FT plus albumin-bound testosterone, can be measured directly by adding 3H-T to the serum sample and precipitating out the SHBG-bound testosterone with ammonium sulfate. The fraction of 3H-T not precipitated out is used to calculated bioavailable testosterone by multiplying it by the total testosterone value obtained in a separate sample. This assay is costly and requires significant effort. As a result, even though the direct measurement of bioavailable testosterone is currently the predominant method for measuring bioavailable testosterone, most clinicians and laboratories rely on FT or calculated FT measurement in place of bioavailable testosterone.

TREATMENT

The primary goal in treating patients with TD is symptom improvement by achieving physiological levels of testosterone. Currently in Canada, injectable, transdermal and oral formulations are options for testosterone replacement (see table 4).

Intramuscular injections (testosterone cypionate and testosterone enanthate) are cost effective and long acting. Due to the pharmacokinetics of injectables, serum testosterone may be supra-physiological immediately at the beginning of the injection cycle and may be subtherapeutic towards the end of the cycle, leading to recurrence of TD symptoms. Some patients find

Table 3 Options in measuring free testosterone

Method	Description	Strength	Weakness
Direct Assay/ RIA (Analog T)	Radiolabeled testosterone analog is added to unextracted sample	Cheap, widely available, quick	Results consistently lower than equilibrium dialysis results
Equilibrium Dialysis	3H-T added to sample, free and bound separated and percentage calculated	Gold standard	Expensive, labour intensive, dependent on the TT assay,
Calculated Free T	Automated established equations using TT, albumin and SHBG	Correspond well with equilibrium dialysis, less expensive	Depended on accuracy of input variables, multiple equations available and not standardized

Table 4 Treatment options

Treatment	Advantages	Disadvantages
Injectables (Testosterone cypionate, Testosterone enanthate)	• Inexpensive • Effective • Long acting (24 weeks)	• Can produce supraphysiologic levels at cycle beginning • Waning effect at cycle end • Require injection
Oral (Testosterone Undecanoate)	• No injection required	• Absorption issues • May induce high levels of DHT • Twice daily dosing
Transdermal (Androderm (patch), Androgel, Testim, Axiron)	• Easy to apply • Consistent testosterone levels	• Minor skin reactions

the idea of weekly or monthly injections daunting and there is a higher risk of erythrocytosis.

In Canada, oral testosterone undecanoate is approved but may lead to supraphysiologic levels of DHT[26]. By design, it bypasses the liver through lymphatic absorption to enable delivery to the systemic circulation. The oral formulation should be taken with a high fat meal (at least 20 mg of fat) to promote absorption and clinical response. Due to a short half-life, the oral formulation requires multiple doses per day.

Transdermal products have demonstrated both effective therapeutic control of testosterone levels and high patient satisfaction. AndroGel and Testim are both gel-based products approved in Canada and provide steady-state, physiologic levels of testosterone. They are very well tolerated with minor skin reaction in a minority of patients. Application is recommended on the shoulder, upper arms or abdomen. The transdermal patch (Androderm) has a similar profile to the gel-based products but has been shown to cause significantly more local skin reaction. Lastly, Axiron is a transdermal preparation made specifically for application to the underarms. By applying to the underarms there is theoretically less risk of secondary exposure by skin-to-skin contact with another individual.

MONITORING

A diagnosis of male breast cancer or prostate cancer represents absolute contraindications to testosterone replacement therapy. Prior to initiating testosterone therapy, physicians are recommended to measure patient's prostate-specific antigen (PSA) and perform a digital rectal exam (DRE) (see table 5). Testosterone therapy may cause erythrocytosis[27] and patients should have their complete blood count monitored during therapy. Within the first year of therapy monitoring should occur about every 3-6 months and include evaluation of serum testosterone, hemoglobin and hematocrit, PSA and prostate health (by DRE). Liver function tests are not required with modern testosterone formulations. Testosterone may worsen sleep apnea and congestive heart failure and patients should have those conditions addressed prior to initiating therapy. Further, exogenous testosterone is not recommended for men seeking fertility as sperm production may be reduced.

Table 5 Testosterone monitoring

Testosterone monitoring	
Baseline	Symptoms, Testosterone, DRE, PSA
Follow-up (initially q3 months and then q6-12 months)	Symptom response, CBC, DRE, PSA

CONCLUSION

Testosterone deficiency is common yet most men are not offered evaluation or treatment. The symptoms associated with TD are non-specific and can be easily overlooked or misattributed to other medical or psychosocial causes. Aside from symptomatic relief, treating low testosterone can offer other significant health benefits.

There are many options to determine the biochemical level of testosterone. Measurement of total testosterone represents a reasonable initial screening assay for most men with convincing symptoms of testosterone deficiency. In cases where the total testosterone is equivocal despite consistent symptomatology, assessment of free or bioavailable testosterone may be informative. Clinicians need to be familiar with the various options to measure a patient's testosterone status and an appreciation for the strengths and limitations of the assays used to determine such measurements.

REFERENCES

1. Liu PY, Beilin, J., Meier, C. et al. Age-related changes in serum testosterone and sex hormone binding globulin in Australian men: longitudinal analyses of two geographically separate regional chohort. J Clin Endocrinol Metab 2007; 92:3599-603.

2. Carruthers, M. Time for international action on treating testosterone deficiency syndrome. Aging Male 2009;12:21-8.

3. Buvat, J., Maggi, M., Gooren, L, Guay, AT, Kaufman, J., Morgentaler, A., Schulman, C., Tan HM, Rorees, LK, Yassin A,m Zitzman, M. Endocrine aspects of male sexual dysfunction. J Sex Med 2010;7:1627-56.

4. Diver, MJ, Imtiaz, KE, Ahmad, AM, et al. Diurnal rhythm of serum total, free and bioavailable testosterone and of SHBG in middle-aged men compared with those of young men. Clin Endocrinol 2003; 58:710-7.

5. Bremner WJ, Vitiello MV, Prinz PN 1983 Loss of circadian rhythmicity in blood testosterone levels with aging in normal m

6. Collier, CP, Morales, A, Clark A et al. The significance of biological variation in the diagnosis of testosterone deficiency, and considerations of the relevance of total, free and bioavailable testosterone determinations. J Urol 2010; 183:2294-9.

7. Isidori AM, Lenzi A. Risk factors for androgen decline in older males: lifestyle, chronic diseases and drugs. J Endocrinol Invest 2005;28:14–22.

8. Pugeat, M, Crave, JC., Tourniaire, J., Forest, MG. Clinical utility of sex hormone-binding globulin measurement 1996; 45(3-5): 148-55.

9. Araujo AB, Wittert GA. Endocrinology of the aging male. Best Pract Res Clin Endocrinol Metab 2011;25:303–19.

10. Dandona, P.,Dhindsa, S., Chaudhuri, A et al. Hypogondatrophic hypogonadism in type 2 diabetes, obesity and the metabolic syndrome. Curr Mol Med 2008;8:816-28.

11. Mulligan T, Frick MF, Zuraw QC, Stemhagen A, McWhirter C. Prevalence of hypogonadism in males aged at least 45 years: The HIM study. Int J Clin Pract 2006;60: 762–9.

12. Wehr E, Pilz S, Boehm BO, et al. Association of vitamin D status with serum androgen levels in men. Clin Endocrinol 2010;73:243–8.

13. Morley JE, Perry HM 3rd, Kevorkian RT, Patrick P. Comparison of screening questionnaires for the diagnosis of hypogonadism. Maturitas 2006;53:424–9

14. Morley JE, Charlton E, Patrick P, Kaiser FE, Cadeau P, McCready D, Perry HM 3rd. Validation of a screening ques- tionnaire for androgen deficiency in aging males. Metabolism 2000;49:1239–42.

15. Heinemann LA, Saad F, Heinemann K, Thai DM. Can results of the Aging Males' Symptoms (AMS) scale predict those of screening scales for androgen deficiency? Aging Male 2004;7:211–8.

16. Smith KW, Feldman HA, McKinlay JB. Construction and field validation of a self-administered screener for testosterone deficiency (hypogonadism) in ageing men. Clin Endocrinol (Oxf) 2000;53:703–11.

17. Wang C, Catlin DH, Demers LM, Starcevic B, Swerdloff RS. Measurement of total serum testosterone in adult men: Comparison of current laboratory methods vs. liquid chromatography-tandem mass spectrometry. J Clin Endo- crinol Metab 2004;89:534–43

18. Stanczyk FZ, Cho MM, Endres DB, Morrison JL, Patel S, Paulson RJ 2003Rosner et al. Testosterone Assays Limitations of direct estradiol and testosterone immunoassay kits. Steroids: 68:1173–1178

19. Lepage R. Measurement of testosterone and its subfractions in Canada. Clin Biochem 2006;39:97–108.

20. Morales A, Collier CP, Clark AF. A critical appraisal of accuracy and cost of laboratory methodologies for the diagnosis of hypogonadism: the role of free testosterone assays. Can J Urol 2012;19:6314–18.

21. Moreno SA, Shyam A, Morgentaler A. Comparison of free testosterone results by analog radioimmunoassay and calculated free testosterone in an ambulatory clinical population. J Sex Med 2010;7:1948–53.

22. Kacker R., Hornstein, A, Morgantaler, A. Free testosterone by direct and calculated measurement versus equilibrium dialysis in a clinical population. Aging Male, 2013; 16(4): 164–168

23. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab 1999;84:3666–72.

24. http://www.issam.ch/freetesto.htm

25. De Ronde, W., Van der Schouw, YT., Pols, H., Gooren, L., Muller, M., Grobbee, D., de Jong, F. Calculation of bioavailable and free testosterone in men: a comparison of 5 publishes algorithms. Clin Chem 2006;52(9): 1777-84.

26. Gooren, LJ. A safety study of the oral androgen testosterone undecanoate. J Androl 1994;15:212-5.

27. Fernandez-Balsells MM, Murad MH, Lane M, Lampropulos JF, Albuquerque F, Mullan RJ, Agrwal N, Elamin MB, Gallegos-Orozco JF, Wang AT, Erwin PJ, Bhasin S, Montori VM. Adverse effects of testosterone therapy in adult men: A systematic review and meta-analysis. J Clin Endocrinol Metab 2010;95:2560–75