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Challenges in the measurement of serum testosterone concentrations as a biomarker of men's health[1]

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Abstract

Male testosterone concentrations gained recent attention in the field of andrology. Various epidemiological cohort studies presented associations of low serum testosterone concentrations with incident cardiovascular risk factors and increased mortality risk. Furthermore, low serum testosterone concentrations are integral part for the diagnosis of late-onset hypogonadism. Because of their high validity and throughput, mass spectrometry assays have replaced conventional immunoassays for testosterone measurement, and are believed to become the "gold standard" for sex hormone quantitation. This article will focus on challenges in the measurement of serum testosterone concentrations and will provide practical information on the interpretation of serum testosterone measurements.

Keywords: androgens; biomarker; immunoassay; mass spectrometry; testosterone.

Introduction

In recent years increasing interest in the definition of hypogonadism or the aging male has developed in the field of andrology. The terms "aging male", PADAM (partial androgen deficiency in the aging male), climacterium virile or andropause describe hormonal changes caused by reduced testosterone secretion and the resulting physical and psychological symptoms.

Although there are no specific symptoms of androgen deficiency, there is a discussion that physical complaints like erectile dysfunction, decreased libido, muscle weakness or osteopenia as well as psychosocial aspects like fatigue or depression are testosterone-associated [1]. In addition, numerous observational studies presented during the last few years showed an inverse correlation between testosterone concentration and known cardiovascular risk factors like adiposity [2], lipid metabolism disorders [3] and hepatic steatosis [4]. Prospective observational studies produced evidence that a low testosterone concentration in serum can be considered a biomarker for the development of dyslipidemia [3], metabolic syndrome [5], Type 2 diabetes [6] or hypertension [7] or that a low testosterone concentration is a potential risk factor of cardiovascular end points [8] and increased mortality [9] in males (Figure 1). Evaluating the soundness of testosterone measurements and their clinical implications, however, requires knowledge of various biological, pre-analytical and analytical factors of influence (Table 1).

Biological influences on testosterone measurement

Testosterone is the dominant male sexual hormone and is produced in the testes throughout life [10]. In serum, testosterone is bound predominantly to sex hormone-binding globulin (SHBG) and albumin, while only about 2% are present in free form. Besides minor but detectable seasonal variations [11], the mean testosterone concentration is subject to distinct circadian rhythms with higher concentrations in the morning and 30% to 50% lower evening concentrations [12, 13]. Therefore, current guidelines for diagnosing testosterone deficiency invariably recommend that blood samples should be collected repeatedly at the same day time between 7 a.m. and 11 a.m. [14]. The fasting state at the time of blood col-

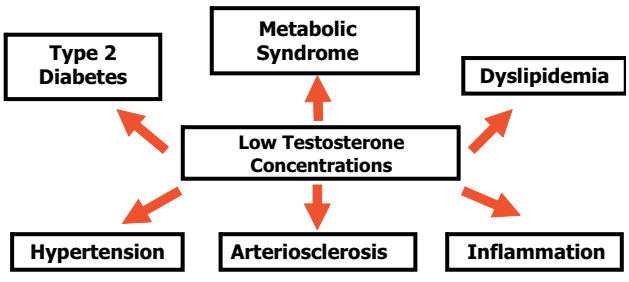

Figure 1 (online only) Low male serum testosterone concentration as biomarker for various cardiometabolic risk factors.

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Table 1 Criteria of the analytical capacity of testosterone measurements.

Influence factors of testosterone measurement		
Biologic factors	Pre-analytic factors	Analytic factors
Continuous physiologic decline of male testosterone concentrations	Standardized blood collection technique	Cross-reactivities
Intra-individual variability caused by general physical condition and co-morbidities	Sample management	Variation coefficient (sensitivity / specificity)
Distinct circadian rhythm	Sample storage	Assay platform
Fasting status	Reagents	

lection is yet another biological factor of influence [15]. An intervention study showed that the standard oral dose of 75 g glucose reduces the fasting testosterone concentration by 15% for up to three hours [16]. Chronic stress and/or a negative subjective assessment of health could also lower testosterone concentrations [17].

Hence, the individual testosterone concentration is to a considerable degree subject to lifestyle influences. Healthy nutrition, weight control, as well as physical activity could positively influence the age-related, secular decline of male testosterone concentrations [18]. Beginning at the age of forty this decline amounts to approx. 1% to 2% per year [19]. With the addition of an accompanying illness or medication, the decline of testosterone concentration is clearly more pronounced [20]. The physiologic testosterone concentration is therefore age-dependent and subject to fluctuations ranging from 6.2 to 32.2 nmol/L [21]. Particularly the free testosterone fraction in serum declines even more with advancing age, caused by the increase in SHBG. Generally, the testosterone concentration of an individual shows a random distribution in the range of the subject's individual, diagnostically relevant mean level. This intra-individual variability is approx. 9% [22]. Since the intra-individual variability cannot be changed, the potential for a quality improvement of analytical precision lies in the optimization of pre-analytical and analytical factors.

Pre-analytical influences on testosterone measurement

To minimize pre-analytical factors of influence, a standardized method of blood collection is absolutely essential. Prior to venipuncture the patient should lie or sit for 15 minutes and torniquet application should not exceed one minute. Generally the sample material used should be serum. The Siemens Centaur Assay (Siemens Medical Solutions Diagnostics), for example, is validated solely for testosterone measurements in serum [23]. Even the immediate transport of the sample has some influence. Samples that have been stored at room temperature for more than 24 hours show a higher testosterone concentration [24], whereas testosterone concentrations remain nearly unchanged when samples are stored at room temperature for up to six hours or at 4°C for up to 48 hours [23]. Even long-term storage of more than 40 years at −25 °C has no appreciable influence on the reliability of subsequent measurements [25, 26]. Although manufacturers do not recommend the repeated thawing and freezing of any serum samples to be measured, a study demonstrated stable testosterone concentrations even after twelve freeze-thaw cycles [27].

Analytical influences on testosterone meansurement

The extensively automated procedures for analyzing serum testosterone concentrations presently used in routine diagnostics are based on immunologic methods that differ only in their various principles, e.g. homogeneous or heterogeneous preparation, and in the measurement technique for quantifying the immunologic reaction. The detection markers used are isotopes (RIA), enzymes (EIA, ELISA), fluorescence- or chemiluminescent compounds (e.g. ECLIA) [28]. The central quality criteria for evaluating the diagnostic capacity of a testosterone measurement are their selectivity, which in immune procedures is essentially determined by the specificity of the antibodies used. However, even with modern immunoassays there is not absolute selectivity for measuring testosterone and cross-reactivities between 1.9% and 5.4% are of little practical importance [29].

The variation coefficient, as a measure of the reproducibility of the result of an analysis, lies between approx. 2% in the upper and 7.5% in the subnormal measuring range of testosterone concentration, while in the diagnostic range of 12 nmol/L immunologic procedures achieve a variation coefficient of 5% to 7% [29, 30]. At present, the internationally recognized reference method for the reference value standardization of immune procedures for testosterone detection is the isotope dilution mass spectrometry. According to the German Medical Association's guidelines for quality assurance in quantitative medical laboratory tests the maximum allowable deviation of a measured testosterone value from the reference method value is 42%, a target missed by 20% of the participants in the most recent interlaboratory tests of January 2006 [29]. The interlaboratory test conducted by the College of American Pathologists (CAP) in 2008 also demonstrated a significant lack of specificity of the current standard immune procedure for testosterone detection. The

lowest mean value measured (52.6 ng/dL) differed from the highest mean value measured (148.7 ng/dL) by a factor of 2.8. In contrast, the factor measured by means of mass spectroscopic methods was 1.4 (low pool) or 1.2 (high pool) [31]. Another comparison study showed that up to 50% of testosterone concentrations detected through single immunoassays were outside the ±20% range of the exact value measured by means of mass spectrometry [32].

However, objections concerning the validity of immunologic testosterone measurements in population studies [33], expressed in view of these marked differences, were disproved through several comparison studies [30, 34]. But because of the sometimes substantial differences in measured absolute testosterone concentrations [30, 34] immunologic procedures for the diagnostically interesting low measurement range are insufficient [32, 35, 36]. In addition, when compared to immunologic testosterone measurements, the more precise mass spectroscopic procedures demonstrate considerably lower intra- and interlaboratory variability [37], for what reason they are increasingly considered to be the gold standard for clinical and epidemiologic testosterone measurements [14, 32, 38–40]. The recently started "steroid hormone standardization project" performs various activities to standardize testosterone measurements, establish reference ranges, and improve the comparability of testosterone measurements independent of method, time, and place [41].

The detection of free or bioavailable testosterone could support a diagnosis of testosterone deficiency. However, the measurement of free testosterone by means of equilibrium dialysis, of bioavailable testosterone by means of ammonium sulfate precipitation, or of both androgens by means of mass spectrometry, is too costly or impracticable for routine diagnostics and up to now has been used mainly for research purposes or in reference laboratories [42]. Because of the their unreliable measurement, so-called analog immunoassays for the direct measurement of free testosterone should not be used [43, 44]. However, assuming a constant albumin concentration, bioavailable and free testosterone can be calculated cost- and time-effectively by means of various mathematical algorithms using measured values of total testosterone and SHBG [45]. Although a comparison study between the currently established algorithms showed that the calculated free testosterone values correlate well with the measurements from equilibrium dialysis [28, 46], the calculated values should be very critically evaluated when there is no laboratory-specific "in-house" validation [42].

Conclusions

In summary, immunologic testosterone measurements, in spite of their accuracy in the target concentration range of healthy males, must be cautiously evaluated in the low concentration range (especially among comorbid males, females, and children) for reasons of insufficient diagnostic and analytic quality. In addition, various biologic and pre-analytic factors of influence should be considered when interpreting measured testosterone concentrations. Since a diagnosis of late-onset hypogonadism is based on at least three sexual symptoms and repeatedly detected low serum testosterone concentrations [1], the interpretation of these measured testosterone concentrations is a crucial diagnostic criterion. To be able to make a valid clinical diagnosis, Table 1 provides an overview of the various influence factors of testosterone measurement (see Table 2 in [23] or [47]).

Whenever testosterone therapy is under consideration, the advantages and disadvantages, as well as risk factors concerning the prostate [48] and other organs [49], must be discussed with the patient. The effects of testosterone therapy must be monitored. When there is no improvement in any of the symptoms of testosterone deficiency under testosterone substitution, treatment should be discontinued and the patient should be re-examined for other possible causes of his clinical symptoms [50]. Besides the question of which testosterone concentrations are clinically relevant [51], the overall effectiveness of a testosterone substitution must be considered questionable at present [52]. Since long-term results (i.e. >3 years) about potential benefits and risks of testosterone substitution do not exist to date, future long-term clinical studies are much-needed.

In contrast, evidence from different prospective observational studies suggest low testosterone concentrations as a biomarker for the general health of men and/or off-balance metabolic processes. Thus, testosterone measurements together with already existing prevention strategies could help to prevent the development of subclinical and manifest cardiovascular diseases and more specifically, to motivate and empower men to adopt a healty lifestyle.

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