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WORKSHOP REPORT

Functional testosterone: Biochemical assessment of hypogonadism in men – Report from a multidisciplinary workshop hosted by the Ontario Society of Clinical Chemists

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Abstract

Objectives. In 2004, the Ontario Society of Clinical Chemists (OSCC) held an invitational multidisciplinary workshop to establish the most reliable, cost-effective approach to the biochemical assessment of hypogonadism in men.

Methods. Specialists across Canada in clinical biochemistry, endocrinology, family medicine and urology were invited to participate in this workshop which included individual presentations and a consensus component addressing two challenge statements: 1) 'Determinations for total testosterone (TT) are equivalent to those for bioavailable testosterone (BAT) or calculated BAT (cBAT) or free testosterone (FT) (by analogue radioimmunoassay or equilibrium dialysis) or calculated FT (cFT)'; 2) 'There is no good evidence that borderline low testosterone concentrations in men should be treated'. The main outcomes were to identify what agreement exists in Canada, what issues were still controversial, and what research remains to be addressed.

Results. Six recommendations based on expert opinion addressed these main themes: investigate with morning total testosterone (TT) followed by repetition and reflexive testing of sex hormone binding globulin (SHBG) if testosterone is 8–15 nmol/L with automatic calculation of cBAT; discontinue the use of analogue free testosterone assays; and definitive methods and standards must be available to ensure standardized results.

Conclusions. Total testosterone is a reliable marker for the initial investigation of men presenting with symptoms of hypogonadism; cBAT is a reasonable follow-up test in patients with equivocal biochemical or consistent symptomatic findings.

Keywords: *Position statement, expert opinion, hypogonadism, men, testosterone, calculated bioavailable testosterone*

Introduction

Symptomatic hypogonadism is frequently observed in the aging male population [1]. Since some of the clinical manifestations are non-specific and overlap with other common conditions such as depression, hypothyroidism, and even healthy aging, definitive diagnosis on a clinical basis alone is often impossible [2–4]. Presenting symptoms may include: erectile dysfunction, loss of libido, depression, lethargy, inability to concentrate, sleep disturbance, irritability, fatigue, anemia, osteoporosis, loss of muscle strength, regression of secondary sex characteristics, increase in visceral fat and decreased interest in daily activities [5–7]. Men investigated for low testosterone are in an age group where a significant proportion will be on medication that can alter testosterone levels or have side effects suggestive of androgen deficiency [8]. These include opioid analgesic, antidepressant, antihypertensive, gastrointestinal and antifungal drugs. There is a high incidence of hypothyroidism, obesity and type 2 diabetes in elderly men that can affect testosterone and sex hormone binding globulin (SHBG) concentrations, while diet itself is a further

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consideration that may influence SHBG concentrations [8,9]. It is therefore important to be aware of the patient's clinical and medication history when interpreting results. Equally challenging is the biochemical diagnosis of hypogonadism given the physiological milieu associated with aging and other co-morbidities; and the biological variation associated with the biochemical markers themselves [4,10]. Primary considerations include the potential decrease in testicular production of testosterone or increase in the hepatic production of SHBG in men as they age, and the large inter-individual variation in SHBG concentrations [3,10–13]. Interpretation of total testosterone and BAT results are complicated by significant diurnal variation (which may decrease with age), biological variation (week to week variation) and potential seasonal variation [10,14–16]. The ultimate consideration is probably that no currently available test reflects tissue androgen activity [4,11]. Indeed, it is unknown whether tissue responses differ across organs (brain, bone, prostate, muscle, etc.), or change in parallel over time. Thus, androgen deficiency may become clinically apparent at different set points within an individual or a population.

Over the last decade, laboratory investigation of hypogonadism in men has been approached in a variety of different ways [3,4,17]. Total testosterone measurement has evolved from radioimmunoassay (RIA) testing after serum extraction, to direct automated immunoassay testing available in most laboratories, and recently to mass spectrometry methods in several reference laboratories. In a few laboratories, bioavailable testosterone (BAT = free and albumin-bound testosterone) is measured by 'selective' ammonium sulphate precipitation of sex hormone binding globulin (SHBG). Different laboratories have provided calculations such as the free androgen index (FAI = testosterone/SHBG), and calculated versions of FT and BAT (cFT and cBAT) as SHBG immunoassays have become available. BAT, cBAT and cFT are often reported to correlate well with each other [3,4], as well as with FT, age and even total testosterone, suggesting that parallel testing in every patient may result in redundant information [17].

Given the minimal amount of random control trial (RCT) evidence in this area, and the variable approaches and expert opinions held by different specialists including those in laboratory medicine, the Ontario Society of Clinical Chemists (OSCC) organized a multidisciplinary expert panel to discuss the issues of what testing approach to recommend, based on assay performance and the evidence currently available. These considerations were then to be extended to treatment implications and recommendations for necessary future research.

Methods

In 2004, the OSCC invited professionals from clinical biochemistry, endocrinology, family medicine and urology departments across Canada who have had long-term clinical practice and research interests in testosterone and hypogonadism to participate in an invitational multidisciplinary workshop on 'functional testosterone'. Those who were able to attend the one-day event at Queen's University in Kingston, Ontario were invited to provide specific references for a pre-meeting bibliography. The workshop included individual presentations and a consensus component. Each participant was allotted 15 minutes in the morning to address either or both of these challenge statements: 1) 'Determinations for total testosterone (TT) are equivalent to those for bioavailable testosterone (BAT) or calculated BAT (cBAT) or free testosterone (FT) (by analogue radioimmunoassay or equilibrium dialysis) or calculated FT (cFT)'; 2) 'There is no good evidence that borderline low testosterone concentrations in men should be treated'. The purpose of these brief introductions was to allow presentation of the participants' expert opinion and actual clinical practices. Dr M. Godwin, an expert in evidence-based medicine, chaired the session, with the specific purpose of identifying what agreement there was among experts in Canada, what issues were still controversial, and what research remains to be addressed in order to establish a reliable, cost effective, biochemical assessment of hypogonadism in men.

The recommendations from this multidisciplinary workshop are at the 'expert opinion' level of evidence. The conference report was drafted by two of the authors (CC and AC) with review and further discussion by the participants on salient points.

Results

Six consensus recommendations were developed from the multidisciplinary workshop:

1) A morning total testosterone (TT) measurement (before 11:00 am) should be used as the first line test for investigations of male hypogonadism.
2) If the total testosterone is less than 8 nmol/L, there is a high probability of hypogonadism. Repetition of the total testosterone measurement along with baseline investigations for luteinizing hormone (LH) and prolactin, are recommended to support the clinical diagnosis of primary or secondary hypogonadism.
3) If the total testosterone is greater than 15 nmol/L, there is limited probability of clinically significant hypogonadism. Repetition of the total testosterone measurement along with baseline investigations for LH and prolactin, are recommended if clinical symptoms of hypogonadism persist.
4) If the morning total testosterone is between 8 to 15 nmol/L, repeat the total testosterone (before 11:00 am) with a request for automatic reflexive

testing of sex hormone binding globulin (SHBG) if the total testosterone is still in the 8 to 15 nmol/L range. Calculation of bioavailable testosterone (cBAT) should be automatically provided by the laboratory. Baseline investigations for LH and prolactin are also recommended.

5) The analogue free testosterone assay has no place in the diagnosis of hypogonadism in men. It should be replaced with SHBG and total testosterone for the calculation of bioavailable testosterone (cBAT).
6) Provincial governments should support the development or use of an isotope dilution gas/liquid chromatography mass spectrometry (ID-MS) reference testing laboratory to assure the accuracy and reliability of testosterone assays.

Discussion

Six recommendations were developed by the multidisciplinary experts. The first recommendation is that total testosterone be used as the first line biochemical test for the investigation of hypogonadism in adult men. Total testosterone assays are widely available and relatively inexpensive; and, analytical variation (precision) in most laboratories is usually about 4% to 10%, meaning that most results would have an analytical 95% confidence interval of $\pm 8\%$ to 20%. This approach is reasonable because of the close correlation between testosterone, cBAT and FT in most studies and because approximately 56% of patients will be definitively classified as either normal or testosterone-deficient based on the first testosterone result [18]. Note that this recommendation emphasizes that the sample ideally should be obtained in the morning, i.e. before 11:00 a.m., because testosterone is considered to have a significant circadian variation in healthy men although, it is recognized that this variation may be less pronounced in the elderly [6,10,14,16]. It is interesting to note that this approach of using total testosterone as the primary entry marker was advocated by several groups and reports released about the same time or shortly after the OSCC multidisciplinary workshop, heralding a shift from FT or BAT which had been the previously favoured marker [3,4,10,11,18–20].

In the second recommendation there was agreement for further investigation of men with a total testosterone less than 8 nmol/L. Repetition of total testosterone along with baseline investigations for luteinizing hormone (LH) is recommended to support the clinical diagnosis of either primary or secondary hypogonadism [11]. Inclusion of follicular stimulating hormone (FSH) in these investigations may be helpful, especially if increased, however a result in the normal range is considered inconclusive. Prolactin (PRL) measurement is also commonly included in investigations of secondary hypogonadism.

The third recommendation states that testosterone results greater than 15 nmol/L are indicative of normal gonadal function and further investigations are usually not warranted. However, as with any result, if clinical symptoms are not consistent with the biochemical results or if clinical symptoms persist, repeat or further biochemical testing is appropriate.

Recommendation four addresses total testosterone results within the borderline range of 8 to 15 nmol/L. In order to account for both biological and analytical variation, the recommended follow-up for a result in this range is to repeat the total testosterone using a morning sample [10,15,16] with a request for reflexive testing of SHBG (and albumin) if the total testosterone concentration remains in the borderline range [18]. A calculated bioavailable testosterone (cBAT) should be automatically provided by the laboratory, and baseline investigations for LH and PRL are recommended for persistently borderline results. Again, biochemical results need to be interpreted in light of the patient's clinical symptoms and health status. Symptoms are generally non-specific, and other conditions, medications and co-morbidities (especially in this commonly presenting age group) may need to be ruled out [8]. It should be noted that as most hormone assays are based on antibody reactions, unexpected results may occasionally be obtained [10,19]. For patients with atypical presentations or inconsistent findings, family physicians may consider discussing potential analytical issues with a clinical chemist, or referring the patient to a specialist.

Recommendation five states that the analogue free testosterone assay should be replaced by cBAT for hypogonadal investigations in men. In fact, analogue free testosterone assays were approved only for investigation of hirsutism in women. The analogue free testosterone assay has been dismissed in the literature since 1997 as 'seriously inaccurate, underestimating the true concentration (as estimated by the equilibrium dialysis method) by many fold' [2,10,15,19,21].

Finally, recommendation six addresses the need for access to a definitive 'gold standard' reference method. At the present time, external quality assurance programmes and method comparison papers have demonstrated that inter-laboratory performance of total testosterone is less than desired for routine clinical use [10,19,22,23]. It is hoped that regulatory agencies and governments will support the development or use of an ID-MS reference method to assure the accuracy (trueness) of testosterone assays [17]. Proficiency testing programmes should use ID-MS as their reference method as opposed to an all-methods mean comparison whenever possible [17]. At the present time, such assays are available at several commercial or reference laboratories in the United States. Assay standardization is necessary whenever method-independent population reference

ranges and decision limits are advocated. Thus, the borderline range of 8 to 15 nmol/L recommended in this report should be considered a 'proposed global range' applicable to methods with insignificant bias.

Challenge statement number 1 was that 'Total testosterone is equivalent to bioavailable testosterone (BAT) or calculated BAT (cBAT) or free testosterone (FT) (by analogue RIA or equilibrium dialysis) or calculated FT (cFT)'. The recommendations reflect the majority opinion that the current evidence does not support the use of FT by analogue RIA, that total testosterone is a reliable indicator of 'functional testosterone' for the initial investigation of men presenting with symptoms of hypogonadism, and that cBAT is currently a reasonable follow-up test in patients with equivocal biochemical or consistent symptomatic findings. We introduce the term 'functional testosterone' in this report to emphasize the issue that a reliable biochemical indicator should have a direct relationship with the physiological function it is supposed to represent, and yet this does not always appear to be the case for the different estimates of testosterone function that are variably advocated in the literature.

Comparisons of the ammonium sulphate precipitation method for BAT versus the calculation of cBAT from testosterone, albumin and SHBG were reviewed [19,24]. The ammonium sulphate precipitation method for BAT is a labour-intensive assay, which has several critical steps that may result in inter-technologist or intra- and inter-laboratory variation. It is unlikely that it will achieve the status of a routine test as automation would be difficult. In contrast, cBAT calculation requires measurement of SHBG concentrations, which is increasingly available on automated immunoassay analysers. It should be noted that as neither total testosterone (TT) nor SHBG assays are standardized across manufacturers at the present time [15], results from different laboratories may have significant but consistent biases that will also be translated to cBAT, making it important to perform serial monitoring at one laboratory only [24].

Both free testosterone and bioavailable testosterone can be calculated (cFT, cBAT), and as such, they are directly linked to each other [20,25]. The International Society for the Study of the Aging Male (ISSAM) has a calculator on its website (http://www.issam.ch/freetesto.htm) that provides these calculations in either SI or non-SI units, reporting absolute results and the percentages for both cFT and cBAT [25]. In order to minimize the amount of redundant information on patient reports, it is recommended that only cBAT concentrations be reported. The reasons for not choosing cFT include the different units of 'pmol/L' compared to 'nmol/L' for total testosterone, and the preference for leaving FT to refer to the equilibrium dialysis method or any future 'gold standard' method that might be developed. Equilibrium dialysis is a manual method that is labour intensive and hence available almost exclusively on a research basis.

It is generally accepted that testosterone replacement therapy is clinically effective in men with unequivocal 'classical' hypogonadism [26–28], however, some patients present with a request for androgen replacement to improve their quality of life. Not only may non-specific symptoms be ameliorated by supplementation, but protective effects of testosterone on bone metabolism and atherosclerosis need to be considered [29]. As with any therapeutic intervention the benefits and risks must be carefully considered by each individual patient and their physician.

Challenge statement number 2 was that 'There is no good evidence that borderline low testosterone concentrations in men should be treated'. It was noted that it is not uncommon for clinicians to provide a therapeutic trial of androgen replacement in symptomatic men with borderline testosterone levels in the hope that this might provide another piece of evidence to support the use of long-term replacement therapy in individual patients [2,6,11,12,30–33]. This challenge statement is deemed important as the purpose of most testing is to either determine the need for treatment or to evaluate the efficacy of treatment. Although the workshop made a significant step towards addressing this challenge question by defining what concentration of testosterone should be considered decreased or borderline, most researchers recognize that a large men's health initiative study is the only way to provide the necessary evidence to resolve this issue [2,11,12,15,32,33].

Recently, various groups and organizations have addressed this challenging area producing guidelines and algorithms that highlight the need for definitive research [6,17,31]. At the present time evidence in this area is scarce necessitating that recommendations, including these current ones, are essentially expert-based [17]. In June 2006, the Endocrine Society published their clinical practice guideline entitled 'Testosterone therapy in adult men with androgen deficiency syndromes' [31]. The recommendation for diagnosis of androgen deficiency in men was: 'consistent symptoms and signs and unequivocally low serum testosterone' based on 'measurement of morning total testosterone by a reliable assay as the initial diagnostic test' and 'confirmation of the diagnosis by repeating the measurement of morning total testosterone, and in some patients by measurement of free or bioavailable testosterone using accurate assays'. In addition, there were also recommendations 'against screening for androgen deficiency in the general population', and against the 'use of available case-finding instruments [questionnaires] for detection of androgen deficiency in men receiving health care for unrelated reasons'. Instead, 'case detection by measurement of total testosterone in men with

certain clinical disorders in which the prevalence of low testosterone is high [significant] or for whom testosterone therapy is suggested is recommended' (see Table 3 and Section 2 of the guideline [31]). In summary, current best practice suggests the use of both clinical and biochemical parameters for the diagnosis, treatment and monitoring of symptomatic hypogonadism in men.

The OSCC multidisciplinary workshop was convened because of the perceived lack of diagnostic sensitivity and diagnostic specificity observed for the tests commonly used to assess functional testosterone status. Studies on the relationships between the different biological tests and symptom screening questionnaires have varied from poor to good concordance for some or all of the parameters [33]. This lack of consensus between literature reports and current clinical practices attests to the need for evidence-based direction in this area. Specific, well-developed, adequately powered research protocols need to be performed. It is important to also coordinate a multidisciplinary approach for critical literature updates and future consensus efforts.

One of the purposes of this conference was to identify, based on actual clinical practice, what future research might provide better direction of patient care. A number of future research steps and initiatives were identified:

1) Assess how the total testosterone (TT) methods currently employed by routine laboratories compare to definitive isotope dilution mass spectroscopy results [15,17].
   a) Decide what sort of population reference interval studies need to be conducted within or across laboratories to validate the ranges currently in use, such as the proposed global borderline range of 8 to 15 nmol/L.
   b) Develop an international TT reference method and calibration standard for use by manufacturers and proficiency testing programmes [15,17].
2) Standardization also needs to be achieved for SHBG assays so that cBAT is consistent across laboratories.
   a) Can a challenge set of definitive low, borderline and normal clinical samples be developed to facilitate the current state of assays in the interim?
3) Determine the biological variation of the different parameters of functional testosterone (TT, SHBG, cBAT) in healthy and hypogonadal patients, and identify what a 'significant change' would be for these tests (season-to-season, day-to-day, and within-day circadian variation).
   a) If the circadian variation is muted in older men, can reliable samples be obtained in the afternoon?
   b) Determine the number of repeat samples needed for a reliable estimate of total testosterone (TT) concentration prior to the initiation of treatment [15,17].
4) Should the calculation for cBAT include age (i.e. similar to the new calculations used to estimate glomerular filtration rate, eGFR)? Should any of the tests for functional testosterone have age specific reference intervals?
5) Is there definitive evidence that cBAT provides added information in the investigation of hypogonadism? What is the physiological basis for this, and when and how should it be specifically incorporated for patient care?
6) Is salivary testosterone a reliable indicator of functional testosterone?
7) Can an algorithm for the diagnosis and monitoring of hypogonadism be developed? For example: to include issues of *in vivo* and *in vitro* drug effects, to address co-morbidities, to inform standardized testing progression, to identify reflexive testing options, to differentiate the interpretation and approach during diagnosis versus replacement monitoring.
   a) For patients receiving supplemental testosterone, what is the ideal time to measure the biochemical response: i) at nadir, or ii) at mid-course?
8) Other hormones (e.g. thyroid) change with ageing, resulting in similar symptoms as hypogonadism. Would the analysis of other hormones be useful in the assessment of difficult patients? (e.g. symptomatic patients with minimal biochemical evidence?)

For many of these questions a significant amount of research has already been published. However, it is necessary to ensure that these and future results meet minimal evidence-based standards to assure standardized clinical practices and optimal patient care in the future.

Conclusion

The lack of a standard approach to the investigation of hypogonadism in men is due to the ambiguity of clinical presentation and the apparent poor diagnostic performance of testing currently used to estimate functional testosterone status. This workshop was convened because there is general agreement that current practices are not cost efficient, and that research to determine appropriate evidence-based practice is urgently needed. Health care funding requires this sort of critical inquiry to achieve both efficient medical practice and improved patient care.

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