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Comparison of different methods for the measurement of serum testosterone in the aging male

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Summary

Background: Circulating testosterone (T) levels, mainly non-SHBG bound fractions, decline with advancing age. Free (FT) and bioavailable (BT) testosterone levels have been suggested to represent more reliably the bioactive hormone at the tissue level as compared to total testosterone (TT) levels. However, there is an ongoing controversy as to which T assay is most appropriate in defining T deficiency in the aging male.

Methods: In a prospective observational study, TT, FT and BT levels were measured in 51 elderly men (55 to 70 years). TT levels were determined on two different days using 2 different assays. FT was calculated using TT, albumin and SHBG levels as well as measured directly by radioimmunoassay. The free androgen index (FAI) was calculated using the formula 100 × TT/SHBG. BT was obtained by precipitation of SHBG-bound T with ammonium sulphate.

Results: We found good correlations within different assays for TT (r = 0.87 to 0.91, p <0.001) and between TT and calculated FT (r = 0.66 to 0.84, p <0.001). In contrast, the correlation between TT and BT was poor (r = 0.17 to 0.19, p = ns). TT was equivalent to calcFT and was better in mirroring clinical sings of androgen deficiency in elderly men as compared to BT. The intra-individual day-to-day variance of TT levels was 14.8% (range 0.1–79%) with a coefficient of variance of 12.6%.

Conclusion: Serum TT and the calculated FT fraction correlated well with each other and were superior in defining a group of elderly men with suspected androgen deficiency. In contrast, FT measured by direct RIA and BT reflected gonadal function poorly. Our data favour the repetitive use of TT when screening for androgen deficiency in elderly men.

Key words: testosterone; assay; aging

Introduction

Testosterone (T) circulates in plasma non-specifically bound to albumin, specifically bound to sex hormone-binding globulin (SHBG) and, in a small percentage, in an unbound, bioactive form. The sum of free (FT) and non-SHBG bound T levels are generally referred to as "bioavailable T" (BT). In men, T levels decline continuously after the age of 30 years [1]. It is controversial which T assay is most appropriate in the diagnosis of low T levels in the aging male, also referred to as the "andropause" or partial age-related androgen deficiency ("PADAM"). Although both FT and BT only partially reflect the hormone available at the cellular level in specific tissues, they have been thought to mirror the clinical status more accurately than the total circulating hormone levels [2, 3]. Despite the clinical importance, there are no well designed, controlled, prospective clinical trials that have clarified which androgen measure is superior in defining a group of men who are allegedly androgen deficient [4]. At present, several different methods are available to measure circulating levels of FT or BT in plasma. FT can be measured by equilibrium dialysis, referred to as the gold standard method, or by direct radioimmunoassay. In addition, FT and the free androgen index (FAI) can be calculated [5]. The gold standard for the measurement of BT is the measurement by precipitation of SHBG-bound T with ammonium sulphate [6, 7].

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In view of the high prevalence of elderly patients with hypogonadal symptoms, it would be most useful for the clinician to know which androgen measure should be used to quantify the hormonal status. Therefore, this study aims to evaluate the ability of different methods of TT, FT and BT measurement in defining a group of elderly men with clinical signs and symptoms associated with the "andropause".

Methods

Study subjects

Participants were men living in the community aged 55 years or older enrolled to evaluate the relationship between T levels and clinical symptoms in the aging male. Study subjects were recruited either through newspaper advertisements (n = 29) or after presentation to the medical and urological outpatient clinics for routine, non-disease specific check-ups (n = 22). All men were in good general health and free of any serious medical conditions (acute or chronic) and were examined in the Research Unit of the Division of Endocrinology, Department of Medicine at the University Hospital, Basel.

Subjects with chronic medical conditions, severe depression or psychosis, body mass index (BMI) >35 kg/m^2, acute illness during the preceding six months or any hormonal treatment during the previous six months were excluded. Men who agreed to participate in the study underwent complete medical history and physical examination.

The study was approved by the local Ethics Committee for Human Studies. All patients gave their written informed consent to participate in the study.

Hormone measurements

Blood samples were collected after an overnight fast. Serum concentrations of TT (normal reference range, 9.9–28.0 nmol/L) were measured using the Elecsys-System (Roche Diagnostics, Rotkreuz, Switzerland) as well as the Centaur-System (Bayer Diagnostics, Zürich, Switzerland). To evaluate the inter-laboratory variability, TT levels were batch-assessed by the Elecsys-System in two different laboratories (University Hospitals Basel and Bioanalytica, Lucerne, respectively), referred to as TT Elecsys I and II. To evaluate the day-to-day variance, TT was measured by the Elecsys-System on two different days within one month between 8 and 9 am in the fasting state. FT was calculated from TT, albumin and SHBG capacity according to the formula of Vermeulen [5]. In addition, FT was assessed by a direct RIA method (Diagnostic products Company (DPC), Bühlmann Laboratories, Allschwil, Switzerland). The FAI was calculated from TT and SHBG according to the formula FAI = (100 × TT/SHBG). BT was obtained by precipitation of SHBG-bound T with ammonium sulphate [6, 7]. At 4 °C, a saturated ammonium sulphate solution was added drop wise to the serum sample with continuous gentle shaking until a 1:1 dilution was obtained. The sample was then immediately centrifuged (4 °C refrigerated centrifuge, 3000 g, 30 minutes) and the supernatant was used to measure T by the Elecsys system. Intra-assay coefficients of variation for all assays were less than 5% and inter-assay coefficients of variation were less than 10%.

Albumin (35–52 g/L) and SHBG (10–70 nmol/L) levels were measured with the Immulite analyser (Diagnostic Products Corporation, Los Angeles, CA, USA). Total cholesterol (3.0–5.2 mmol/L) and high-density lipoprotein cholesterol (HDL-C, 0.9–2.2 mmol/L) were assayed enzymatically by automated procedures (Roche, Rotkreuz, Switzerland). Low-density lipoprotein cholesterol (LDL-C, 1.6–3.4 mmol/L) was calculated using the formula of Friedewald. Apolipoprotein A1 (ApoA1, 1.0–2.0 g/L) and B (ApoB, 0.5–1.4 g/L) were measured on the Immage-System (Beckman Coulter, Inc. Fullerton CA, USA).

Bone mineral density in the lumbar spine and hip was evaluated by dual X-ray absorptiometry (DXA) using a Lunar Expert densitometer (Lunar, Madison, WI). Body composition was measured using tetrapolar bioelectrical impedance analysis (BIA) [8].

The following self-assessment questionnaires were completed.

The Beck-Depression-Inventory (BDI) is a 21-item self-report questionnaire measuring severity of depressive symptoms over the past week [9]. The ADAM-questionnaire is a previously published questionnaire for identifying middle-aged and older men with T deficiency [10]. The International Index of Erectile Function (IIEF) questionnaire is a 15 items self-administered questionnaire scale and a brief and reliable measure of erectile dysfunction [11]. The Dalbert questionnaire evaluates the subjective well being as part of general mood level and satisfaction with life [12].

Statistical analyses

All data are expressed as means ± standard deviation (SD) in text and tables. Two group comparisons were performed by Student's t-test or by Mann-Whitney U test in nonparametric distribution.

Correlations between variables were assessed using Spearman correlation analysis. Since univariate analysis showed a significant correlation between age and T levels, we corrected the influence of age on T levels according to the formula $F_c = F - (\alpha[X - X_{mean}])$ where F_c is the corrected factor, F is the measured factor, X is the actual age, X_{mean} is the mean age of the population and α is the slope of the regression line between F and X.

P values <0.05 were considered statistically significant. Data were analysed using Statistica for Windows (version 6.0, StatSoft, Inc., Tulsa, OK).

Results

Comparison of the various testosterone measurements

Mean values and standard deviations of different T values are shown in table 1. As regards TT levels, the two measurements with the Elecsys system revealed similar mean values (p = 0.43). In contrast, TT measured by the Centaur system showed significantly increased levels as compared to levels

measured by the Elecsys system ($p < 0.01$). The correlations between different T measurements are shown in table 2. We found strong correlations between different TT assays, between TT measured in different laboratories with the same assay, as well as between TT assays and the calcFT, respectively. In contrast, the correlation between TT and BT was weak. Calculated FT showed a fair correlation with FT measured by direct radioimmunoassay or the FAI, whereas the respective correlations were relatively poor for BT.

Table 1

Mean values, standard deviation (SD) and total range of different testosterone measurements.

Variable	Mean ± SD	Min – Max
TT Elecsys I	13.5 ± 5.1	4.7 – 29.5
TT Elecsys II	14.2 ± 5.0	7.0 – 28.3
TT Centaur	19.3 ± 6.3	8.9 – 34.0
calcFT	0.27 ± 0.10	0.12 – 0.50
FT	40.1 ± 11.2	22.2 – 67.7
FAI	38.7 ± 16.8	15.2 – 125.2
BT	3.8 ± 1.5	1.2 – 8.8

Day-to-Day variance of total testosterone levels

Overall, the intra-individual day-to-day variance of TT levels, measured on two different days in the fasting state in the morning, was 14.8% (range 0.1–79%). The coefficient of variance (CV) for TT is 7.4% for a mean TT value of 0.85 nmol/L, 2.2% for a TT value of 9.55 nmol/L and 1.7% for a TT value of 24.3 nmol/L.

Correlation of various testosterone measurements with clinical symptoms

The correlations of the different T assays with clinical signs and symptoms of the aging male are shown in table 3. TT and calcFT showed the strongest correlation with the testicular volume, whereas testicular volume did not correlate with

Table 2

Correlation coefficients (R) between different testosterone levels.

Variable	TT Elecsys I	TT Elecsys II	TT Centaur	calcFT	FT	FAI	BT
TT Elecsys I		0.91***	0.87***	0.84***	0.65***	0.43**	0.19
TT Elecsys II			0.91***	0.71***	0.58***	0.30*	0.19
TT Centaur				0.66***	0.57***	0.26	0.17
calcFT					0.79***	0.80***	0.48
FT						0.69***	0.51***
FAI							0.67***
BT							

* p <0.05, ** p <0.01, *** p <0.001

Table 3

Correlation coefficients (R) between testosterone levels and clinical signs.

Variable	TT Elecsys I	TT Elecsys II	TT Centaur	calcFT	FT	FAI	BT
BMD lumbar spine	–0.14	–0.18	–0.33*	–0.06	–0.14	0.11	–0.12
BMD femoral neck	–0.05	–0.04	–0.14	0.02	0.04	0.15	–0.11
Fat mass	–0.18	–0.15	–0.15	–0.26	–0.01	–0.18	0.02
Lean body mass	0.04	0.07	0.04	0.01	0.01	–0.01	–0.01
Muscle mass	0.20	0.24	0.20	0.06	0.15	–0.05	–0.16
BMI	–0.12	–0.11	–0.10	–0.19	0.01	–0.14	–0.12
Testicular volume	0.41***	0.41***	0.37*	0.42***	0.29**	0.40**	0.19
LDL-Cholesterol	0.09	0.11	0.15	0.05	0.12	–0.04	–0.19
HDL-Cholesterol	0.33*	0.34**	0.36*	0.36*	0.20	0.21	0.22
ApoA1	0.24	0.17	0.26	0.10	–0.09	–0.04	–0.08
ApoB	–0.12	–0.13	–0.05	–0.26	–0.09	–0.35*	–0.35*
ApoB/A1	–0.28	–0.27	–0.23	–0.31*	–0.11	–0.29*	–0.26
BDI	–0.09	–0.16	–0.09	0.08	0.09	0.22	0.14
Dalbert	–0.06	–0.03	0.01	–0.13	–0.12	–0.18	–0.07
IIEF							
– Erectile function	0.11	0.17	0.16	0.18	0.21	0.16	–0.15
– Intercourse satisfaction	0.13	0.19	0.21	0.17	0.16	0.12	–0.14
– Orgasmic function	0.23	0.31*	0.31*	0.26	0.25	0.21	–0.09
– Sexual desire	0.19	0.28	0.24	0.16	0.14	0.02	–0.02
– General well-being	0.28	0.37**	0.36*	0.27	0.33*	0.18	–0.02

p <0.05, ** p <0.01, *** p <0.001

BT. In contrast, only weak or even absent correlations were obtained between all T assays and clinical signs and symptoms. Specifically, we found no correlations between different T assays and BMI, bone mineral density, body composition and depressive symptoms. Correlations with lipid levels and erectile dysfunction were weak or absent. The positive predictive value of a testicular volume of <40 ml to indicate hypogonadism was 25% and <35 ml 73%, respectively. The positive predictive value for a HDL-cholesterol level of <1.0 mmol/L was 60%.

Discussion

According to the results of this study, FT or BT levels are not superior to TT levels in defining a group of elderly men who are androgen deficient. Since the measurement of TT is less expensive and time consuming compared to all other measurements of serum T, we consider TT as the most appropriate test to screen for androgen deficiency in older men. TT and calcFT showed the best correlation with clinical signs of hypogonadism and especially testicular volume.

A limitation of our study is that we did not measure FT by equilibrium dialysis, which is regarded to be the physiologically most representative method for estimating FT [5]. However, according to the literature, calcFT shows a very good correlation with FT measured by equilibrium dialysis [5]. Our study shows good, albeit not excellent correlations, between calcFT with other measurements of the FT index, i.e. direct measurement by RIA and the FAI. Thus, our results indicate that direct measurement of FT levels as well as the FAI are not a reliable index of FT. Conversely, although the direct measurement of FT levels as well as the calculation of FAI appears to be an attractive and simple alternative for estimating FT levels, they should not be used to determine androgen deficiency in elderly men.

BT has been suggested to be the assay of choice in older persons, where SHBG increases and substantial variation of albumin levels may occur [2, 13]. The gold standard method used to measure BT is the utilization of the ammonium sulphate precipitation technique [5]. In our study, BT levels measured by ammonium sulphate precipitation did not show a good correlation with clinical signs of the aging male. The correlations were even weaker when compared to the correlations with calcFT or TT levels. Thus, according to our data, BT levels are not a reliable marker of androgen deficiency in elderly men.

With aging there is a marked decline in the circadian variation of T levels [14]. This has led to the suggestion that in older persons a single T sample is sufficient to make the diagnosis of hypogonadism, independent of the sampling time. However, the marked intra-individual day-to-day variance of TT levels in our study suggests the need to obtain a second confirmatory sample in older men with symptoms of hypogonadism. The same has recently been shown for BT levels [2].

Overall, our data show only weak or even absent correlations of various T measurements with clinical signs in the aging male. This emphasizes the difficulty in assessing androgen status, since there is no good independent marker of androgen action that can be used in vivo. Clinical parameters cannot predict a low T level in elderly men and the positive predictive value even of significantly correlated parameters like low testicular volume is disappointing, as has been stated previously in the literature [15]. According to our findings, a low testicular volume in elderly men can contribute to the diagnosis of hypogonadism, but this criterion has low predictive value for detecting decreased T production. Thus, the measurement of TT should be performed in suspected hypogonadism, independent of specific clinical findings or symptoms. In addition, there is controversy as to whether the normal laboratory range for T is valid in the older man or if there are changes in T dynamics with age that may affect interpretation of T levels. Our data, as well as other well-designed clinical trials, have indicated that no method of T measurement is better than any another in defining a group of men who are androgen deficient. Therefore, we conclude that until appropriate clinical studies are published or until a good marker of T action becomes available, measurement of TT is appropriate and less expensive when compared to a more complex and labour intensive measurement of FT or BT. Importantly, there is a marked intra-individual day-to-day variance. In addition, in low TT levels, the precision of the assay decreases. Thus, all T levels should be measured at least twice to make the diagnosis of hypogonadism in the aging male.

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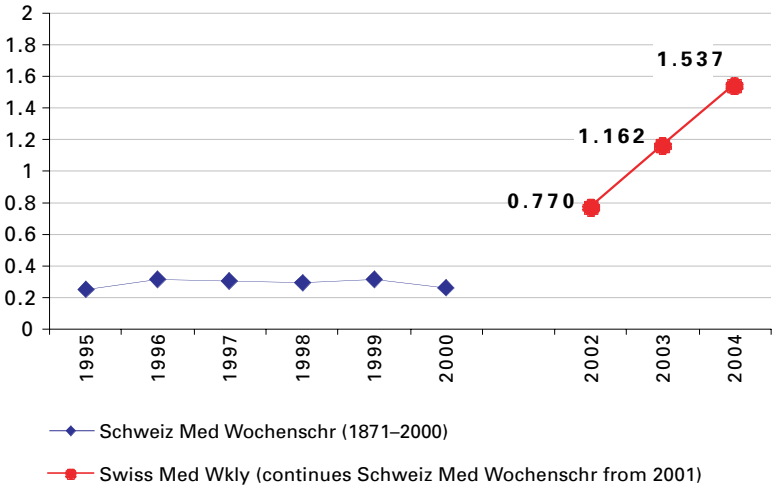

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