ORIGINAL RESEARCH—ENDOCRINOLOGY

Wide Variability in Laboratory Reference Values for Serum Testosterone

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ABSTRACT

Introduction. The laboratory determination of testosterone levels consistent with a diagnosis of hypogonadism is complicated by the availability of multiple testosterone assays and varying reference ranges.
Aim. To assess current laboratory practices regarding availability of testosterone assays and use of reference values.
Methods. A telephone survey of 12 academic, 12 community medical laboratories, and one national laboratory.
Main Outcome Measures. Types of androgen assays offered and determination of reference values.
Results. All of the academic and eight of the community centers performed total testosterone testing. Free testosterone was performed in-house by six of the 12 academic and one community center. Testing for bioavailable testosterone, free androgen index, and percent free testosterone was performed in-house by no more than two centers. There were eight and four different assays used for total and free testosterone, respectively. One national laboratory offered equilibrium dialysis measurement of free testosterone. Of the 25 labs, there were 17 and 13 different sets of reference values for total and free testosterone, respectively. The low reference value for total testosterone ranged from 130 to 450 ng/dL (350% difference), and the upper value ranged from 486 to 1,593 ng/dL (325% difference). Age-adjusted reference values were applied in four centers for total testosterone and in seven labs for free testosterone. All reference values were based on a standard statistical model without regard for clinical aspects of hypogonadism. Twenty-three of the 25 lab directors responded that clinically relevant testosterone reference ranges would be preferable to current standards.
Conclusions. Laboratory reference values for testosterone vary widely, and are established without clinical considerations. **Lazarou S, Reyes-Vallejo L, and Morgentaler A. Wide variability in laboratory reference values for serum testosterone. J Sex Med 2006;3:1085–1089.**

Key Words. Testosterone; Reference Values; Hypogonadism; Measurement

Introduction

An estimated 2–4 million men in the United States have clinical hypogonadism, and its prevalence increases with age [1,2]. Testosterone replacement therapy (TRT) results in a demonstrable improvement in symptoms of hypogonadism, including diminished libido, energy and well-being, impaired cognition, decreased muscle mass/strength, anemia, osteoporosis, erectile dysfunction, and visceral obesity [3–14].

Yet only about 5% of affected men currently receive treatment [3]. In part, this may be due to confusion as to what serum level of testosterone is consistent with hypogonadism. Because the diagnosis of hypogonadism requires laboratory confirmation of a low level of testosterone, clinicians rely heavily on laboratory reference ranges to determine whether a patient may be a candidate for TRT. However, a wide variety of testosterone assays are in use with varying reference ranges. Anectodal experience reveals that a man may have his serum testosterone categorized as "low" by one lab and "normal" by another. Moreover, the availability of a variety of testosterone assays often leads to conflicting results.

Table 1 Institutions surveyed

Institution	City, state	Center
Boston University Medical Center	Boston, MA	Academic
New England Medical Center-Tufts	Boston, MA	Academic
University of Massachussetts	Worcester, MA	Academic
Beth Israel Deaconess Medical Center	Boston, MA	Academic
University of Connecticut	Farmington, CT	Academic
Lahey Clinic	Burlington, MA	Academic
University of Vermont Medical Center	Burlington, VT	Academic
Maine Medical Center	Portland, ME	Academic
Dartmouth-Hitchcock Medical Center	Lebanon, NH	Academic
Massachussetts General Hospital	Boston, MA	Academic
Brigham and Women's Hospital	Boston, MA	Academic
Rhode Island Hospital	Providence, RI	Academic
Springfield Hospital	Springfield, VT	Community
Newport Hospital	Newport, RI	Community
St. Joseph Hospital	Nashua, NH	Community
Stamford Health	Stamford, CT	Community
Kent Hospital	Warwick, RI	Community
Roger Williams Hospital	Providence, RI	Community
Morton Hospital	Taunton, MA	Community
Metrowest Hospital	Framingham, MA	Community
New England Baptist Hospital	Boston, MA	Community
Newton-Wellesley Hospital	Newton, MA	Community
St. Elizabeth's Hospital	Brighton, MA	Community
Caritas Norwood Hospital	Norwood, MA	Community
Quest Diagnostics Laboratory	Teterboro, NJ	National

The purpose of this study was to assess the state of current laboratory diagnosis of hypogonadism by surveying clinical labs in New England with regard to their use of testosterone assays and reference ranges.

Methods

A telephone survey was conducted in September 2004 of academic and community medical centers. Twenty-five clinical lab directors or codirectors in six states completed the questionnaires, consisting of 12 academic, 12 community, and one national laboratory (Table 1). Data regarding the types of androgen assays offered, the manufacturers of the assays, and the reference ranges utilized at their institutions were collected. We further inquired as to how the reference ranges were established and applied, and whether the labs had performed independent validation of reference ranges supplied by the manufacturers. Finally, the lab directors were asked for their opinions regarding the clinical utility of current testosterone assays and reference ranges.

Table 2 TT and FT assays

	Type of assay	Number of centers using assay
TT		
Bayer Advia Centaur™	Chemiluminescence	11
Beckman Coulter™	Chemiluminescence	3
Roche Elecsys 170™	Chemiluminescence	3
DPC Immulite 2000™	Chemiluminescence	2
DPC Coat-A-Count™	Radioimmunoassay	2
Bayer ACS 180™	Chemiluminescence	2
Ortho™	Chemiluminescence	1
Diagnostic Systems Laboratories	Radioimmunoassay	1
FT		
Mathematical calculation based on TT, albumin, and SHBG		14
DPC Coat-A-Count™	Radioimmunoassay	7
Diagnostic Systems Laboratories	Radioimmunoassay	2
Direct analog	Radioimmunoassay	2

FT = free testosterone; SHBG = sex hormone-binding globulin; TT = total testosterone.

Results

All of the academic and eight of the 12 community centers offered in-house total testosterone (TT). Six of the 12 academic centers but only one of the 12 community hospitals offered in-house free testosterone (FT). Eight different assays were used to measure TT and four for FT (Table 2). All TT and FT tests not performed in-house were sent to outside diagnostic laboratories. Other testosterone-related assays performed in-house included bioavailable testosterone in two laboratories, sex hormone-binding globulin (SHBG) in five, free androgen index in two, and percent FT in one lab.

Of the 25 surveyed laboratories, there were 17 different TT reference ranges. For FT, there were 13 different reference ranges. Although there was a grouping of laboratories using a TT reference value of 251–300 ng/dL as the low reference value, there remained a considerable variation for this value (Table 3).

Table 3 Reference ranges utilized among surveyed New England labs

Lowest threshold TT value (ng/dL)	Number of institutions	Highest threshold TT value (ng/dL)	Number of institutions
401–450	1	401–600	1
351–400	1	601–800	4
301–350	2	801–1,000	15
251–300	10	1,001–1,200	4
201–250	6	>1,200	1
151–200	4		
101–150	1		

TT = total testosterone.

The reference value for low TT ranged from 130 to 450 ng/dL, a variation of approximately 350%. The highest end of normal ranged from 486 to 1,593 ng/dL, a variation of approximately 325%. The widest interval for normal TT values from a single laboratory was 262 to 1,593 ng/dL, and the narrowest was 180–486 ng/dL. For FT, the low reference value ranged from 5.0 to 13.5 pg/mL, a variation of approximately 270%. The highest reference values ranged from 19.0 to 54.7 pg/mL, a variation of approximately 290%.

One outlier lab applied age-adjusted values for 16- to 19-year-old men as their reference values. A second lab used a TT value of 200 ng/dL as the low reference value based on the recommendation of the insitution's chief of endocrinology. A third lab reported that reference values were determined using unpublished data provided by the assay vendor.

Age-adjusted reference values for TT were applied in four of the 12 academic centers but none of the community hospitals. Age-adjusted reference values for FT were used in seven of the 12 academic and community centers, respectively. In all cases, the use of age-adjusted reference ranges arose from the recommendation of the assay manufacturer.

However, even laboratories that used the same kits employed different reference ranges. For example, two different TT reference ranges were used for the eight labs using the same kit made by Diagnostic Products Corp. (DPC, Los Angeles, CA), four reference ranges for TT were used among the eight labs using the Bayer Advia Centaur kits, and all three labs that used the Beckman Coulter kits had different TT reference ranges.

All but three TT assays were performed using chemiluminescence. Three labs at the academic medical centers used radioimmunoassays. Traditional radioimmunoassays are conducted through automated immunoassays using testosterone analogs as standards, proprietary reagents, and instrumentation. Equilibrium dialysis was not offered at any of the various medical centers and was available from the national reference laboratory only upon special request.

No laboratory performed independent validation of the manufacturer's reference values. Reference values were provided by the manufacturers based on a standard statistical model used throughout clinical chemistry, in which the central 95% of values are categorized as normal, with the lowest and highest 2.5% of values (greater than two standard deviations from the mean) categorized as abnormal, without regard for clinical correlation. One of the most widely used assays (DPC) based their reference values on a published study of inpatients and outpatients [16].

There were eight different assays used to measure TT, and 17 different reference values among the 25 labs. Four different assays were used for FT, with 13 different reference values. Several labs applied different reference values for TT or FT despite use of the same test kit.

Furthermore, no laboratory used a clinically determined target, or threshold values (e.g., <300 ng/dL), to identify men with low TT or FT, as is commonly performed in clinical trials for hypogonadism.

When asked for their opinion, 23 of the 25 (92%) lab directors indicated that clinically relevant threshold values for testosterone would be preferable over current reference values. Those in favor of such a change would look to national panels or specialty societies to recommend appropriate reference values for testosterone testing.

Comment

It is now well recognized that there are significant issues pertaining to the performance and interpretation of testosterone assays [15]. The results of this survey reveal enormous variability in the use of testosterone assays and reference ranges by academic and community clinical laboratories. Numerous testing kits made by different manufacturers are in use, with an uncertain correlation of results from one to another. Reference values used to categorize values as high or low differed as much as threefold between laboratories. Perhaps most importantly, in no case was any attempt made to link reference values to clinically relevant considerations for hypogonadism. It is therefore no wonder that the diagnosis of hypogonadism, which is based in part on laboratory confirmation of low testosterone levels, remains a confusing issue for clinicians and patients alike. The significance of this is that men who might benefit from TRT may not be offered treatment.

All the institutions offered the two most commonly used testosterone assays, for TT and FT, either performed as an in-house test, or by sending out specimens to a central diagnostic laboratory. Minor differences were noted between the community and academic hospitals. Nearly all the labs performed TT testing in-house, whereas half of all the academic centers but only a single community hospital performed in-house testing for FT. A

small number of the academic institutions offered additional testosterone-related assays, such as bioavailable testosterone, SHBG, or free androgen index. As a side-note, despite recommendations promoting the use of equilibrium dialysis as the assay of choice for measurement of FT, only the national reference laboratory offered equilibrium dialysis at the time of the initial survey, and only when specifically requested. This is consistent with the use of equilibrium dialysis primarily as a research tool [17,18].

One of the most remarkable findings in this study was that there were 17 different sets of TT reference ranges among 25 laboratories. Not only did reference ranges differ between different manufacturers of TT assays, but they even differed for labs using the same kits. In addition, the reference value used to categorize individual test results as low varied by approximately 350%, meaning that the TT results for one man might be low at one laboratory, and well within the normal range according to another. For instance, a TT value of 251 ng/dL would be categorized as hypogonadal by 14 of the labs we surveyed, and normal by the other 11 labs. Similar results were noted for FT.

Variability of the upper reference values given for TT and FT presents a slightly different problem. This upper value is frequently used to monitor treatment levels for men undergoing testosterone supplementation. When upper reference ranges are inappropriately low, clinicians may become unnecessarily concerned that therapeutic doses of testosterone treatment are excessive if TT or FT results are higher than the reference values.

However, in our opinion, the largest problem is that none of the institutions offered reference values linked to clinical considerations regarding hypogonadism. All the manufacturers represented in this study used a standard statistical model for reference values that categorizes only the lowest and highest 2.5% of values as abnormal. If the prevalence of hypogonadism affects 15–35% for men over the age of 50 years [19] and if only 2.5% of values are categorized as "low," this means that a large majority of affected men will fail to be properly identified as hypogonadal.

The use of age-adjusted reference values compounds this problem by downshifting the lower and upper ends of reference ranges by as much as 50%. Because TT and FT values decline with age, this means that older men must have exceedingly low test results in order to be categorized as hypogonadal when age-adjusted values are applied. The use of age-adjusted values may make sense for a statistical representation of a population, but has no clinical justification for the diagnosis of hypogonadism. As stated by Ooi et al., which forms the basis for the reference values provided with the DPC assay, age-adjusted reference values "decrease the test sensitivity for detecting androgen deficiency in aging males" [16].

When clinicians obtain a blood test to determine whether hypogonadism is present, they expect that reference ranges provided by the laboratory report will help guide them to a proper diagnosis. As demonstrated herein, currently used reference ranges are inadequate in this regard, and may contribute to the underdiagnosis of hypogonadism. A more useful approach may well be the application of a clinically relevant threshold, or target levels, for TT and FT, as has been used in numerous clinical trials. This approach is universally used for several tests, such as serum cholesterol, glucose, and prostate-specific antigen, in which a clinically determined threshold value provides greater guidance than identification of values that fall within the lowest or highest 2.5% of the population.

Our survey of laboratory directors indicated that a strong majority (92%) favored clinically relevant threshold values for testosterone testing rather than the reference ranges used in current practice. Although national and international specialty organizations have suggested thresholds for the laboratory diagnosis of hypogonadism, none of the 25 labs participating in this study referred to such values. Recommended values also lack consensus as evidenced by TT thresholds values ranging from 231 to 400 ng/dL [20,21].

In summary, these results indicate that the current use of testosterone reference values is confusing and inadequate. There is a clear need for greater standardization and more clinically relevant reference values to guide clinicians in the diagnosis and treatment of hypogonadism.

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