Using database mining on residual samples to establish healthy reference intervals for testosterone measured by LC-MS/MS

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Background

The adoption of LC-MS/MS by clinical laboratories, availability of reference material and CDC standardization program have improved the accuracy of measurement of total testosterone. The availability of a secondary reference material enables traceability to primary calibrator, comparability among clinical laboratories, and potentially, the ability to define standardized reference intervals. Clinical laboratories will always need to verify that reference intervals are appropriate in their patient populations, and the best practice until standardized reference intervals become available, is to establish these intervals. However, this is a costly process and most clinical laboratories lack the resources necessary to do it properly. The challenge is especially daunting for analytes such as testosterone where age- and sex-specific intervals are necessary. Furthermore, clinical laboratories are divided as whether to account for "andropause" by decreasing the lower limits of normal with increasing age. Decreasing total testosterone has been observed in population studies of aging men (1), and one large volunteer-based reference interval study using LC-MS/MS recently documented decreasing total testosterone at a rate of -20% per decade in purportedly healthy men over 40 years (2). Here we present a suggested algorithm to establish healthy reference intervals for total and free testosterone in healthy adult males and females along with the ultimate reference intervals determined.

Methods

A method to quantitate total testosterone in serum was developed on a 5500 ABSciex triple quad LC-MS/MS with mass transitions, chromatography and sample preparation adapted from French (3). Comparison with the CDC reference method on 40 single-donor samples (Phase 1 of CDC's standardization program) had a slope of 1.02, correlation coefficient of 0.9997 and mean bias of

2.4%. Free testosterone was calculated from total testosterone and sex hormone binding globulin (SHBG; measured on the Siemens Immulite) by the Vermeulen equation (4).

Residual patient samples were used to establish reference intervals after extensive pre-screening using data from the electronic medical record. A bi-weekly automated report was generated to identify recent samples with normal fasting glucose and normal lipids collected between 7 and 9 in the morning. The report automatically rejected subjects who met exclusion criteria: recent use of any of 9 prescription drug classes, 10 clinically relevant laboratory tests and over 20 ICD9 codes from physician encounters. Example exclusion criteria were: decreased eGFR, presence of diabetes, opiate use, and androgen prescriptions. Diagnostic criteria relevant to females included diagnoses of polycystic ovarian syndrome, congenital adrenal hyperplasia, and hirsutism.

Free testosterone data were transformed by optimized Box-Cox transformation (lambda=0.32) in order to improve normality. Fitting of the median and the 97.5th and 2.5th centile was performed according to method of Altman (5). Data was reverse Box-Cox transformed for establishment of reference intervals.

Results and Conclusions

Data from 292 healthy adult males did not demonstrate decreasing levels of total testosterone with increasing age (up to 79 years; Fig. 1A). The slope of total testosterone distributed as a function of age was 0.38 ng/dL per year [95% CI -0.83 to 1.58] and the correlation was not statistically significant (p=0.54). A lower limit of 240 ng/dL (8.33 nmol/L) was established for all males $\geq$ 18 years. However, decreasing levels of free testosterone were observed with increasing age (Fig. 1B), supporting the need for age-dependent reference intervals. Reference intervals were set at 60-170 pg/mL (0.208-0.590 nmol/L) for males 18-39 years, 53-142 pg/mL (0.184-0.493 nmol/L) for 40-59 years, and 39-117 pg/mL (0.135-0.406 nmol/L) for > 60 years.

Data from 142 healthy adult females suggested a total testosterone reference interval of < 44 ng/dL (<1.53 nmol/L) for all ages. Free testosterone was observed to decline with age in adult females. The reference intervals for free testosterone were set at 0.8-5.7 pg/mL (0.00278-0.0198 nmol/L) in females 18-49 years and 0.6-3.9 pg/mL (0.00208-0.0135 nmol/L) for > 50 years.

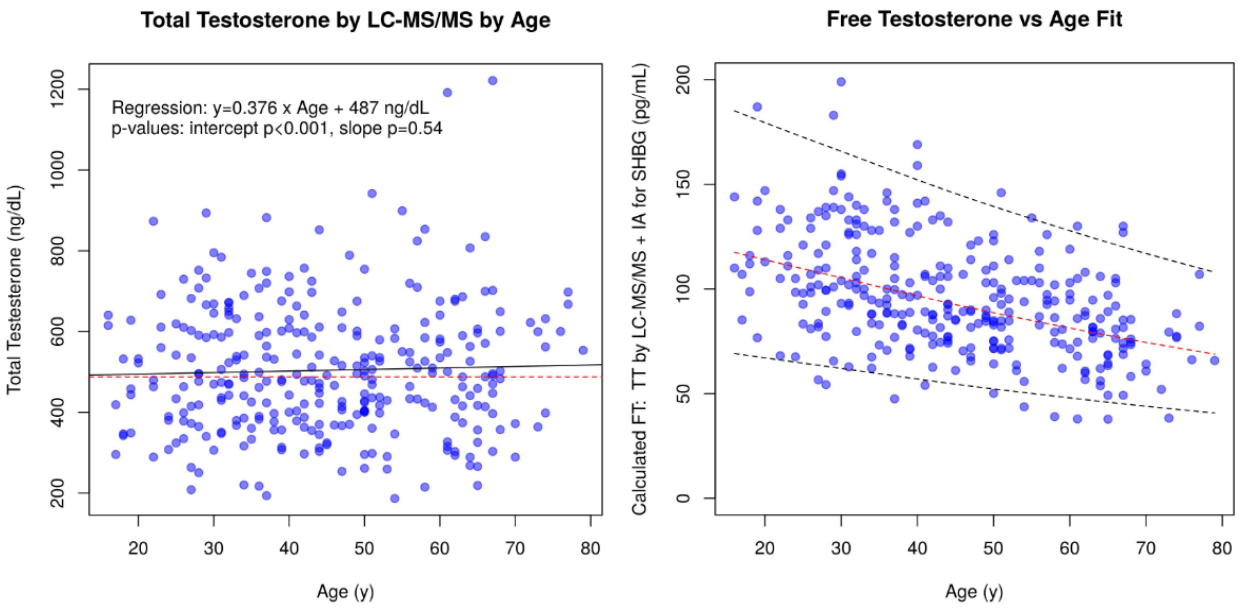

Figure 1. Distribution of total testosterone (A) and calculated free testosterone (B) by age in healthy adult males. In figure 1A, the red dashed line represents the line of 0 slope at the median of the total testosterone irrespective of age. In figure 1B, the red dashed line represents the fitted median while the upper and lower black dashed lines are the fitted 2.5 and 97.5th centiles.

References

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