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Assessing hypogonadism in men

How helpful are current testosterone assays?

Case study

Steven, 28 years of age, had a stem cell transplant 2 years ago for treatment of acute myeloid leukaemia. He is currently in complete remission. He presents to his general practitioner with lethargy, low libido, low mood and feeling weak. While there are many potential causes, you consider low testosterone as one differential. Perhaps a blood test will provide the answer?

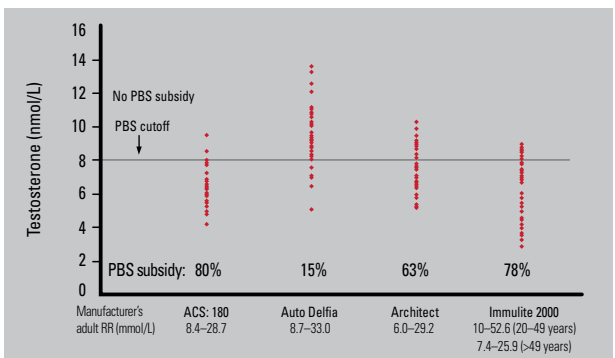
In recent years, hormone therapy (HT) with testosterone has gained increasing prominence and popularity in aging men.[1,2] It has a demonstrated ability to decrease fat mass and increase lean body mass in men with initial 'low' testosterone levels.[3] The Australian Pharmaceutical Benefits Scheme (PBS) only allows subsidisation of male HT if two morning testosterone values are <8.0 nmol/L (or 8–15 nmol/L with elevated luteinising hormone [LH]). The scientific basis of this 'cut off' for testosterone replacement is unclear but it is close to the lower limit of normal for some laboratories. There is well documented diurnal variation (and even seasonal variation) of testosterone. However, the PBS requirements avoid such diurnal variation by requiring two morning blood samples. The underlying assumption is that all laboratories obtain similar results.

Discussion

While performing testosterone comparisons at Southern Cross Pathology Australia, we became concerned when we discovered an inconsistency in testosterone results obtained using different testosterone immunoassay methods. This variability influenced whether subsidised HT could be administered to certain men. We carried out a formal comparison of testosterone results obtained by four routine methods used in accredited pathology laboratories.

The study comprised routine clinical specimens with testosterone requests from 40 men aged 21–81 years who had testosterone results 4.0–9.5 nmol/L (from an initial study of 91 males aged 11–81 years with testosterone results 1.0–35.0 nmol/L) measured using the laboratory's Siemens (formerly Bayer) ACS:180 analyser. Comparisons using three other immunoassay analysers (Perkin Elmer AutoDelfia, Abbott Architect and Siemens [formerly DPC] IMMULITE 2000) demonstrated the results were subject to method specific biases (*Figure 1*). The potential impact of these biases on eligibility for PBS subsidised testosterone therapy is dramatic; 80% of patients assayed by the laboratory using the ACS:180 method would qualify while only 20% of the same patients would qualify if their samples were analysed at a laboratory using the AutoDelfia method. Sikaris et al[4]

Figure 1. Testosterone results from men aged 21–81 years (n=40) analysed by four method technologies. The Australian PBS cut off of 8.0 nmol/L for subsidisation of HT is indicated



have also shown wide differences in lower reference limits for seven commercial methods.

Taieb et al[5] have shown that immunoassays in general underestimate testosterone concentrations in samples from men, with mean results 12% lower than those obtained by the reference method isotope dilution gas chromatography mass spectrometry (GC-MS). There is reasonably close correlation between immunoassay and mass spectrometry for testosterone values >8–10 nmol/L, therefore 'normal' values may be informative. There is more divergence between immunoassay and mass spectrometry testosterone values in the low end of the range; meaning results for hypogonadal men are more difficult to interpret and need to be scrutinised carefully.[6] In addition, significant bias in this range was demonstrated between automated immunoassays (Vitros ECI, Abbott Architect, Bayer ACS:180, DPC IMMULITE 2000, Coat-a-Count and Immunotech, Vidas, Bayer Immuno I, Biomerieux Vidas and AutoDELFIA). Wang et al[7] have also demonstrated significant biases for Roche Elecsys, Vitros ECI, Bayer Centaur, DPC IMMULITE 2000 and Coat-a-Count RIA.[7] Such differences did not correlate with the manufacturers' guidance for reference intervals. This reflects the well accepted fact that steroid immunoassays (particularly testosterone and estradiol), are notoriously subject to antibody specificity because of the many steroids, steroid metabolites and interfering substances in normal serum.

Currently cost prevents the use of the reference GC-MS method in most pathology laboratories. However there is a pressing clinical need for the pathology industry to invest in improved analytical methods to ensure results reflect true levels as determined by such reference methods. This is the key recommendation of the recent position statement by the USA Endocrine Society.[6]

The clinical implication is that it is imperative for methodological bias to be considered when defining low testosterone in males. We acknowledge that using method related normal ranges should be a temporary solution while awaiting true standardisation of the methodology as suggested by Rosner et al.[6] It is of interest that our study showed highest testosterone results using the Abbott Architect test which has the lowest reference limit of the assays in this study. This implies that manufacturer reference intervals are often a crude or inadequate indication of the relevant range in a given clinical setting. Whether age related ranges should also be taken into account is a further debatable issue. At the very least it is important for pathology laboratories to validate their own reference intervals. Assays of free testosterone have theoretical appeal but are not routinely available. A recent study suggests that in older men free testosterone may decrease even with total testosterone remaining stable due to age related increases in sex hormone binding globulin.[8] When this is of physiological significance one would expect a rise in LH.

Conclusion

The current rigid PBS cut off of 8 nmol/L appears inconsistent with the reality of currently available testosterone assays. We therefore urge the diagnostics industry to improve the accuracy of currently available immunoassays and to collaborate with a view to harmonising the results. In the meantime we recommend that the PBS criteria for testosterone subsidy in patients with clinical symptoms consistent with androgen deficiency be based upon:

- two morning testosterones which are low, interpreted with respect to a validated method based reference interval, or
- two morning testosterones which are intermediate, with accompanying LH >1.5 times the upper limit of the eugonadal reference interval for young men.

An additional, and more contentious, criterion would be to require GC-MS confirmation of deficiency as the only basis for PBS subsidisation of HT.

We believe it is imperative for clinicians, pathologists and the diagnostics industry to confront and resolve this long standing issue. With appropriate collaboration and current technology we believe this can be done. In the meantime, GPs confronted by borderline testosterone results should not hesitate to enquire whether the laboratory's reference intervals have been established using morning samples from healthy eugonadal men analysed by the current testosterone method.

Conflict of interest: none declared.

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