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Standardising the biochemical confirmation of adult male hypogonadism; a joint position statement by the Society for Endocrinology and Association of Clinical Biochemistry and Laboratory Medicine*

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Abstract

Background: Inter-assay variation between different immunoassays and different mass spectrometry methods hampers the biochemical confirmation of male hypogonadism. Furthermore, some laboratories utilis eassay manufacturer reference ranges that do not necessarily mirror assay performance characteristics, with the lower limit of normality ranging from 4.9 nmol/L to 11 nmol/L. The quality of the normative data underlying commercial immunoassay reference ranges is uncertain.

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Design: A working group reviewed published evidence and agreed upon standardised reporting guidance to augment total testosterone reports.
Results: Evidence-based guidance on appropriate blood sampling, clinical action limits, and other major factors likely to affect the interpretation of results are provided.
Conclusions: This article aims to improve the quality of the interpretation of testosterone results by non-specialist clinicians. It also discusses approaches for assay harmonisation which have been successful in some but not all healthcare systems.

Keywords
Male hypogonadism, testosterone, assay

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Problem statement

Male Hypogonadism comprises clinical features and laboratory evidence of impaired testosterone secretion and reduced fertility.[1] The diagnosis poses challenges to the clinician due to its non-specific symptomatology in some and, more importantly in relation to the UK healthcare environment, due to surprising uncertainties in relation to the biochemical diagnosis. Total testosterone, the most frequently used biochemical marker to diagnose male hypogonadism, like many hormones, is influenced by several biological factors including diurnal variation, circannual rhythms, food intake, acute illness and medications. Moreover, total testosterone measurement has limitations in male with sex hormone–binding globulin (SHBG) levels outside the reference range.

Total testosterone measurement is performed using immunoassays or mass spectrometry (MS) in most UK healthcare facilities. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is considered the method of choice with potentially higher sensitivity and specificity;[2] however, some MS assays are outperformed by the best immunoassays (Unpublished data from the United Kingdom National External Quality Assessment Service-UKNEQAS). A significant inter-assay variation has been observed between different immunoassays and different MS units in the UK,[3] with intra-assay variability being another technical limitation. Superimposed on this is a wide variability in the reference ranges provided by laboratories that do not necessarily mirror assay performance characteristics, with the lower limit of normality ranging from 4.9 nmol/L to 11 nmol/L.[3] The quality of the normative data underlying commercial immunoassay reference ranges is uncertain in respect of the population sampled and pre-analytical factors affecting testosterone levels outlined above. Therefore, commercially produced reference ranges have no discernible clinical value for the management of male hypogonadism.

These biological and analytical variables have led to a substantial variation in the practice pertaining to the diagnosis of male hypogonadism. Having noticed these variations, the Society for Endocrinology and Association for Clinical Biochemistry and Laboratory Medicine commissioned this joint position statement. This aims to provide evidence-based recommendations as a guide to clinicians assessing men with possible hypogonadism. Clinicians have a responsibility for ensuring the correct classification of the type of hypogonadism, identifying the aetiology, appropriate prescription and monitoring of testosterone for men with hypogonadism in line with current guidelines; these aspects are beyond the purview of the current position statement.

Recommendations

Total testosterone levels should be tested in men with symptoms of adult male hypogonadism, using morning, fasting blood samples when the patient is not acutely ill. Testosterone levels vary during the day with a pattern showing higher levels in the morning and lower levels in the afternoon.[4,5] However, this pattern does not occur in nightshift workers because the diurnal rhythm is primarily driven by sleeping patterns, and not endogenously by circadian factors.[6] Since testosterone levels decrease after meals and with restricted sleep,[7] clinicians and laboratory staff should generally advise patients to obtain morning-fasting samples after a good night's rest.[8,9] Laboratory confirmation of hypogonadism in male shift workers is complicated and warrants specialist referral. Testosterone levels drop during acute illness; therefore, it is not recommended to test during acute illness.[10,11] When testosterone levels were followed up longitudinally in the same person, a marked day-to-day variation also had been observed;[4] therefore, a low total testosterone value generally requires confirmation with a repeat measurement.

As of now, a single reference range or cut-off value is not appropriate in the UK due to the variability of the immunoassays used and their associated reference ranges. However, the following threshold values will function as action cut-off values for

clinical practice rather than reference ranges. Patients with suggestive clinical features and two consecutive morning fasting levels <8 nmol/L are likely to have hypogonadism. Though there are no studies directly comparing different testosterone cut-off levels for intervention, total testosterone <8 nmol/L correlates well with sexual symptoms of male hypogonadism and there is strong evidence in this group for a high prevalence of complications of hypogonadism and symptomatic improvement with treatment.[12–17] Most of the current guidelines also agree with this action limit.[1,18] Further assessment for the aetiology of hypogonadism is required in these men.

Morning, fasted levels of 8–12 nmol/L may be seen in eugonadal or hypogonadal men, and so require careful clinical correlation.[15] Additionally, discussing with the local biochemistry provider would be helpful in borderline cases to understand whether the assay used is a more positively or negatively biased one. Guidelines agree that total testosterone >12 nmol/L is unlikely to represent hypogonadism.[1,19] One exception would be when the luteinising hormone (LH) level is raised and there is a concern about subclinical/compensated primary hypogonadism, or androgen receptor cytosine-adenine-guanine (CAG) repeat polymorphism.[20–22] Even then, there is little evidence for benefit of testosterone replacement therapy in men with serum testosterone >12 nmol/L.

When SHBG is in the reference range, calculated free testosterone has no diagnostic value beyond total testosterone. When SHBG is above the reference range, calculated free testosterone may help diagnose hypogonadism despite normal total testosterone levels.[23] When SHBG is below the reference range, calculated free testosterone may help exclude hypogonadism despite low total testosterone levels. Therefore, it is recommended to check SHBG in men with conditions likely to cause abnormal SHBG and men with borderline total testosterone.[24]

Direct measurement of free testosterone using equilibrium dialysis followed by MS is considered the reference method for estimating free testosterone.[2] This method is not available in the UK for routine clinical practice and other direct measurement methods tend to be inaccurate and are not recommended.

The above recommendations are summarised in panel 1.

Panel 1: Standard comments for aiding interpretation of serum testosterone reports

Patients with suggestive clinical features and two consecutive morning, fasted levels <8 nmol/L are likely to have hypogonadism. Morning, fasted levels of 8–12 nmol/L may be seen in eugonadal or hypogonadal men, and so require careful clinical correlation. Levels >12 nmol/L are not usually consistent with hypogonadism. One reading >12 nmol/L usually excludes hypogonadism, even if other readings are lower.

Measurements in a non-fasted state, during acute illness, or later than 11 a.m. cannot be used to diagnose male hypogonadism. Laboratory bias or nightshift working may affect the results.

When SHBG is within the reference range, calculated free testosterone has no diagnostic value beyond total testosterone. When SHBG is above the reference range, calculated free testosterone may help diagnose hypogonadism despite normal testosterone levels. When SHBG is below the reference range, calculated free testosterone may help exclude hypogonadism despite low testosterone levels.

Clinicians take responsibility for ensuring the appropriate prescription and monitoring of testosterone for men with hypogonadism in line with current guidelines.

Future directions

Developing harmonised reference ranges for testosterone in all the laboratories in the UK would minimise variation among clinicians and centres in the diagnostic approach and make treatment thresholds more robust. Generating harmonised reference ranges using a reference LC-MS/MS method has shown that most of the inter-cohort variation in reference ranges in large population studies was related to assay variation.[25]

Another option is developing method-specific reference ranges for total testosterone using a reference method such as LC-MS/MS similar to cortisol cut-off values in the cosyntropin stimulation test.[26] However, these solutions need intensive technical and human resources, limiting their implementation in near future.

The place of age-specific reference ranges and the need for cut-off values for men with and without obesity still remain unanswered.[25] We recommend following the above guidance until more data appears in this field.

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Ethical approval

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Guarantor

CJ.

Contributorship

CJ and RQ chaired the "Thresholds and Reporting of Testosterone of Male Hypogonadism group" appointed by the Society for Endocrinology. MR, FM, RM, HJ, ML, PD, GH, SR, JT, JD, CB, MP, JT, and FW were members of the group. NS wrote the initial draft and all the authors revised the article. All authors agreed on the final article.

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