**Haematopoiesis shows closer correlation with calculated free testosterone in men than total testosterone.**

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Serum total testosterone (tT) includes both free testosterone (fT ; 0.5 to 3% of total and believed to be the biologically active form) and SHBG- and albumin- bound forms [1]. Guidelines for diagnosing and treating male hypogonadism that emphasise measurement of serum total testosterone (tT) [2] may not address the major variations in sex hormone binding globulin (SHBG) and consequent testosterone bioavailability (BT –either calculated or assayed directly) that are associated with age, obesity, anticonvulsant therapy, thyroid and liver disease. For these reasons, recent Endocrine Society of Australia (ESA) guidelines [2] acknowledge the value of measuring SHBG as well as BT, but nevertheless state that “*There is no evidence that free or bioavailable testosterone levels, which are usually not directly measured but calculated…are a better measure of androgen status than total testosterone level. Moreover, reference intervals for these [BT levels] are even less well defined than those for measured testosterone concentrations. Therefore, they [BT levels] are not recommended for clinical decision making.*”

Although commercially available direct fT assays are not always robust, calculated fT (c-fT) values from mass-action formula based on directly-assayed levels of tT and SHBG ±albumin show promise [4]. The *Vermeulen* equation[[1]](#footnote-1) gives much greater weighting to SHBG compared with albumin levels, aiming to reflect the much stronger affinity of SHBG-binding for testosterone, so that in practice variation in albumin concentration within the physiologic range exerts a relatively small effect on c-fT. Hence, for simplicity, many laboratories input a fixed value (46 g/l in our lab) to the calculation, rather than the actual measured serum albumin concentration.

Measuring both serum tT and SHBG, and utilizing these two values in clinical decision-making but without calculating fT (as per ESA guidance) seems counterintuitive. However, it is important to recognize that both total and BT calculated values are surrogates for the various biological end-points of androgen-action (“androgenicity”) that remain difficult to quantify.

To test our hypothesis that c-fT might provide a better surrogate for androgenicity than tT, we identified an androgen-dependent biological parameter that was (a) important to human health, (b) robust and reproducible with low coefficient-of-variation (CV), (c) routinely-measured in the clinical setting so data collection is convenient and inexpensive, (d) showed an association below, within and above the adult male normal range for serum tT and (e) was independent of aromatisation to estradiol. Complete blood count (CBC) was an obvious candidate as stimulatory effects of testosterone on hematopoiesis are well-recognised, albeit poorly-understood. Men have higher mean hemoglobin concentrations (Hb), hematocrit (Hct) and red cell count (rbc) than women, children and hypogonadal males; intentional (*e.g.* competitive sports) and inadvertent/iatrogenic T over-treatment can also cause erythrocytosis.

Our local population is overwhelmingly Causasian and our laboratory processes all samples collected by local primary and secondary care providers. Laboratory screening for hypogonadism and for monitoring testosterone therapy is performed in both primary and secondary care settings, but the Endocrine unit advises on all initiations of testosterone treatment and provides long-term input in respect of dose-adjustments. Thousands of men had undergone same-day testing for CBC, tT and SHBG levels in our laboratory that consisted of morning venepuncture for diagnostic purposes in untreated men and trough venepuncture for monitoring men receiving intramuscular testosterone replacement, as per local guidelines. We extracted biochemical and hematological parameters over four years on 2,529 consecutive men (aged 18+ years) from our database. We hypothesised that large sample size and low CV for parameters measured would allow meaningful correlations to emerge, despite limited data on confounding factors due to de-identification of data as mandated by the Information Guardian. Data collected were for Hb, Hct, rbc, SHBG and tT levels. SHBG (nmol/L) and tT was analysed on the Roche Modular System (Roche Diagnostics, Lewes, UK) by a two-site sandwich immunoassays and electro-chemiluminescence technology. fT was calculated using the *Vermeulen* equation (4)

Mean data (±s.d) were as follows: tT 13.5±7 nmol/l; c-fT 267±156 pmol/l; Hb 14.7±1.6 g/l, Hct 0.44±0.04; age 54±17 yrs. c-fT and tT were non-parametrically distributed and were therefore log-transformed before performing correlation analyses (*Stata Statistical Software 2013 Release*. College Station, TX, USA).

Ln-c-fT correlated (p<0.001) with Hb (rho 0.304 –Figure 1), Hct (rho 0.300) and rbc (rho 0.278). Ln-tT correlated (p<0.001) with Hb (rho 0.168), Hct (rho 0.153) and rbc (rho 0.101) (Table 1). To address the effects of age on testosterone levels, partial correlations of log transformed tT and c-fT, with age and Hb/Hct/rbc as the independent variables were undertaken. When age was included in the analysis, partial correlations (all p<0.001) of Ln-c-fT changed very little; Hb changed to (rho 0.301), Hct to (rho 0.297), rbc to (rho 0.274), and Ln-tT with Hb to (rho 0.167), Hct to (rho 0.152) and rbc to (rho 0.100).

Differences between correlation coefficients for c-fT and tT were statistically significant, with no overlap between their 95% confidence intervals (CI). As anticipated, these correlations were relatively weak, reflecting the multiplicity of environmental and genetic factors affecting hematopoiesis, but they were significantly stronger for c-fT than tT (p-value <0.05).

Clinicians need to avoid misdiagnosing male hypogonadism and, when indicated, deliver safe, effective testosterone treatment. Even following best practice in venepuncture-scheduling, there are always “gray-area” tT results and many clinicians already use c-fT to aid interpretation in obese men with normal serum gonadotropins and “gray area” tT just above or below the lower end of reference range. Moreover, testosterone replacement can be particularly problematic in men older than 65 years, who are both predisposed to testosterone-induced erythrocytosis and at greater underlying risk of vascular events.

We have identified c-fT as a potentially useful surrogate for androgenicity that is slightly but significantly better in this respect than than tT when validated against one objective parameter of T-action: hematopoiesis. Serum T has previously been analysed in respect of a binary correlation with presence/absence of anaemia in a population [5], but not in relation to hematopoiesis metrics presented as continuous variables. Previous investigators have also found better correlations of c-fT than tT with bone densitometry and patient symptom scores [6,7], but unlike CBC, these metrics of androgenicity are far more resource-intensive to capture and have much larger CVs.

In summary, both tT and c-fT show a small but significant correlation with laboratory measures of hematopoeisis, with c-fT performing significantly better in this respect. Using c-fT instead of tT in diagnostics has the potential to reduce misclassification of male obesity as hypogonadism and, in monitoring testosterone therapy, to reduce the risk of testosterone-induced erythrocytosis.

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1. c-fT = {√[(SHBG - tT + albumin)2 + (2 x albumin x tT)] - (SHBG - tT + ½ albumin)} / 0.001 albumin

   [Normal Range in our laboratory: 215-760 pmol/l when tT and SHBG are expressed in nmol/l and albumin in g/l] [↑](#footnote-ref-1)