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REVIEW

Testosterone testing in adult males

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Abstract

The number of requests for testosterone testing in adult males has been increasing in recent years. In this review, the biochemistry and physiology of testosterone in males relevant to the chemical pathologist or clinical biochemist is outlined. The methodology for total testosterone and various laboratory tests associated with the assessment of testosterone status including free testosterone, calculated free testosterone (CFT), bioavailable testosterone (BAT) and free androgen index (FAI) is then summarised. Clinical and laboratory criteria for the diagnosis of late-onset hypogonadism (LOH) in men are critically discussed with particular emphasis on the interpretation of laboratory test results. Finally, other indications for testosterone testing in adult men such as infertility are also reviewed.

Key words: testosterone, androgen index, hypogonadism, infertility

1. INTRODUCTION

In recent years, clinical diagnostic laboratories have seen an increasing number of testosterone test requests. This trend can be partly attributed to an increased awareness of hypogonadism in adult males and partly to the recognition that testosterone concentrations diminish with age even in "healthy" males. Men's health issues are also becoming more frequently discussed in the media. Some of the common clinical features associated with the aging process in men such as loss of libido, erectile dysfunction, tiredness, reduced muscle mass and mood changes are known to be associated with androgen deficiency. In addition, a link between low testosterone status and some diseases commonly found in the elderly population has been suggested; these include osteoporosis, type 2 diabetes, ischemic heart disease, hypercholesterolaemia and hypertension.[1-3] Another common clinical situation where testosterone testing is indicated is in the investigation of couples presenting with infertility in order to exclude hypogonadism as a cause of male factor infertility. This review article is aimed at chemical pathologists, clinical biochemists and laboratory staff who are involved in the testing of testosterone in adult men.

2. BIOCHEMISTRY AND PHYSIOLOGY

2.1 Testosterone biosynthesis and metabolism

Testosterone is synthesized from cholesterol, which is the precursor of all major classes of steroid hormones including androgens, oestrogens, corticosteroids and mineralocorticoids (Figure 1). In the normal male, the testes are the major source of circulating androgens and testosterone is the principal androgen secreted by the testes. Besides testosterone, the testes also secrete small amounts of other sex steroids including androstenedione, 5α-dihydrotestosterone (DHT), and oestradiol. Testicular functions are regulated by two pituitary gonadotrophins, namely follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Upon stimulation by LH, Leydig cells in the testis produce testosterone, which is secreted into the bloodstream. Plasma concentrations of the two gonadotrophins are regulated by gonadotrophin-releasing hormone from the hypothalamus. The testis exerts an influence on the hypothalamic-pituitary axis by negative feedback mechanisms involving androgens, oestrogens and inhibin B.[4]

Besides the testes, another important source of circulating androgens in man is the adrenal

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gland. Major adrenal androgens include dehydroepiandrosterone (DHEA), DHEA-sulphate (DHEAS) and androstenedione. Although these adrenal androgens are weak androgens compared with testosterone, they can be converted to more active steroid metabolites such as testosterone and DHT in peripheral tissues. The most biologically potent androgen naturally found in men, DHT, is believed to be mainly converted from testosterone in peripheral tissues such as prostate and skin by the action of 5α-reductase isozymes (Figure 1).[5] Some actions of testosterone may also be mediated through oestrogen receptors after conversion of testosterone to oestradiol by the enzyme aromatase.[6] It is probable that many of the actions of testosterone may be regulated in target tissues by the enzymes 5α-reductase or aromatase.[7]

2.2 Transport of testosterone in blood

In normal men, approximately 2% of circulating testosterone is free or unbound, whereas the rest is either tightly bound to sex hormone binding globulin (SHBG) or loosely bound to albumin. There is continued debate concerning which components of circulating testosterone are capable of exerting bioactivity on target tissues. The traditional view is that testosterone exerts its effects through interactions with the androgen receptor and only the relatively small fraction of unbound (free) testosterone can enter cells to bind to androgen receptor and produce a cellular response. It has also been assumed that testosterone bound to SHBG does not dissociate at all during its passage through capillaries. However, to account for the finding of significant uptake of steroids by the liver, it was postulated that the fraction of steroid loosely bound to albumin can also be regarded as "free" and thus has potential bioactivity.[8] The combined total of unbound and albumin-bound testosterone fractions is often referred to as 'bioavailable testosterone' (BAT).[9,10] To date, it is unclear whether BAT is a superior marker of androgenicity than free testosterone.

2.3 Testosterone across the life span

Production of testosterone by the human fetal testis begins at approximately 8 weeks of gestation and peaks at between 11 and 14 weeks of gestation.[11] During the first year of life, plasma testosterone concentrations in males generally decrease with time. In a study on 46 normal male infants, mean plasma testosterone concentrations were found to be 7.2 nmol/L at 1-3 months of age, 3.3 nmol/L at 3-5 months and 0.8 nmol/L at 5-7 months, and reached prepubertal levels (0.2 nmol/L) at 7-12 months.[12] Thereafter, testicular testosterone secretion remains very low during childhood until puberty, when production of both adrenal and testicular androgens markedly increases, leading to androgen-dependent changes such as deepening of voice, growth of pubic hair and increase in muscle bulk.

In normal adult males, plasma testosterone remains at peak levels during the third to fourth decades of life. Both cross-sectional[13-15] and

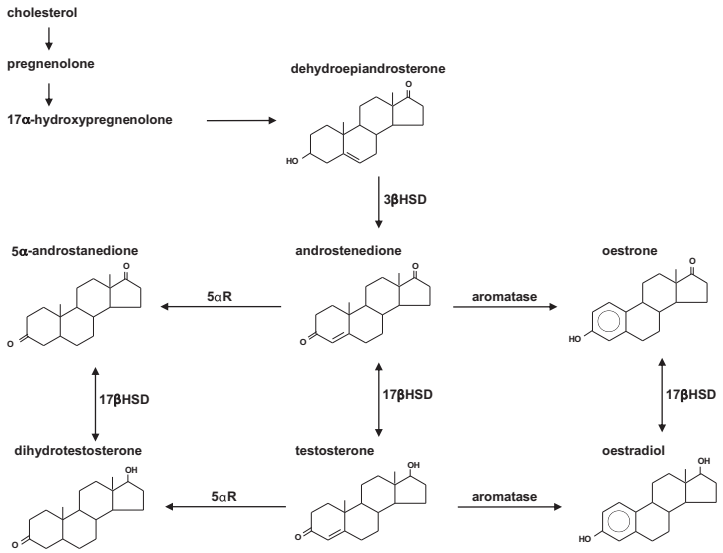

FIG. 1: Biosynthesis and metabolism of testosterone in humans. Abbreviations: 3βHSD, 3β-hydroxysteroid dehydrogenase; 5αR, 5α-reductase; 17βHSD, 17β-hydroxysteroid dehydrogenase.

longitudinal[16,17] studies have shown that total testosterone (total T), free testosterone (free T) and BAT gradually decline with age from the fifth decade. As a result of increasing SHBG concentrations, the fall in free T and BAT with increasing age is generally more marked than that of total T. In the Massachusetts male aging study, after the age of 40 the average age-related reduction in total T is 0.8-1.6% per year, whereas free T declines with age by 1.7-2.8% per year.[17] The age-associated decline in these measures of testosterone occurs in both healthy males and males with chronic illness, although concentrations in healthy males tend to be 10-15% higher than in patients with chronic illness.[17] The reason for the decline in testosterone concentrations with increasing age is not fully understood but multiple mechanisms including primary testicular changes, altered neuroendocrine regulation of Leydig cell function and increase in plasma SHBG binding capacity have been proposed.[18] It is debatable whether the age-related decline in plasma testosterone concentrations should be considered as a physiological phenomenon associated with aging or a common clinical condition that can be corrected by testosterone supplementation.

Serum total T, BAT and free T in adult men normally exhibit diurnal variations with peak concentrations occurring at 06.00-08.00 h and trough concentrations at 18.00-20.00 h; differences in these measurements between peak and trough levels are generally in the order of 40%.[19] Serum SHBG concentrations also show a diurnal variation with peak and trough concentrations at around 16.00 h and 04.00 h respectively. Because of these diurnal variations, it is recommended that in the investigation of testosterone status in men blood samples are taken during the early morning when testosterone concentrations are at their maximum.

3. TESTOSTERONE AND ASSOCIATED LABORATORY TESTS

3.1 Total testosterone

Serum total T is the laboratory test of choice for the initial investigation of male gonadal status.[3,20,21] Multiple types of assays are currently available for the measurement of total T. Isotope dilution gas chromatography – mass spectrometry is regarded as the reference method for total T but few laboratories have the expertise to perform this. In routine clinical diagnostic laboratories total T is usually measured using commercially available, automated immunoassays. Although, the performance of immunoassay methods is in general satisfactory at serum total T concentrations found within the reference range for healthy males, accuracy of these assays at the lower concentrations found in hypogonadal males may be problematic. There are method-related differences in terms of accuracy and precision for all immunoassays and the currently available immunoassays for testosterone are no exception.[22] Liquid chromatography - tandem mass spectrometry (LC-MS) is becoming more widely available in diagnostic laboratories and is regarded by many as a reliable and accurate method of measuring serum total T. It is, however, noteworthy that the accuracy of LC-MS methods depends on the reliability of the sample extraction procedure and calibration.[22]

3.2 Free testosterone

Multiple clinical guidelines on late-onset hypogonadism (LOH) have recommended the use of free testosterone concentrations for the diagnosis of LOH in men with equivocal total T.[3,20,23] However, the measurement of the rather small free T fraction in serum is technically challenging.[24] The reference method for measuring free T is widely regarded as equilibrium dialysis or ultrafiltration.[25,26] Both methods involve separation of the free T fraction by the use of a semi-permeable membrane which only allows passage of the unbound (free T) fraction. Routine measurement of free T by any reference method is impractical in a busy diagnostic laboratory because the assays involved are technically demanding and laborious. Alternatives to direct measurement of free T are discussed below. The measurement of testosterone in saliva has also been suggested as a means of estimating free T in serum and studies have reported encouraging results.[27,28] At present, salivary testosterone assays are not widely used in clinical practice and further evaluation is required before their use can be recommended.

3.3 Direct analogue assays for free testosterone

Direct analogue assays of free T are commercially available but are not recommended for diagnostic purposes.[3,20,23] In terms of methodology, direct analogue assays use a tracer which is testosterone modified in such a way that the analogue binds to

the antibody in the assay but not to serum binding proteins. Theoretically, if the analogue is chosen carefully and the assay optimized appropriately, the amount of analogue that competitively binds to the antibody is inversely proportional to the free T concentration in the sample.[24] However, results generated by such assays are in general inaccurate and show poor correlation with free T concentrations determined by equilibrium dialysis.[29-31] Instead, results from direct analogue assays of free T tend to correlate better with total T.[24] Due to the lack of accurate and practicable assays for the routine laboratory to measure free T, many alternative methods for the estimation of free T have been reported in the literature and are summarised below.

3.4 Free androgen index (FAI)

Free androgen index (FAI), also known as free testosterone index (FTI), was first described by Carter *et al*. (1983) for the investigation of women presenting with hirsutism[32] and is calculated by the formula: 100 x [total T (nmol/L)]/[SHBG (nmol/L)]. Although mathematically easy to calculate, FAI is a dimensionless parameter with little indication of the concentration of free circulating testosterone. FAI is frequently used in clinical practice to estimate free testosterone status in females but it should not be used in estimating free T in men because some of the assumptions inherent in the FAI formula are not applicable to males.[33-35] Moreover, at relatively low concentrations of SHBG commonly found in males, FAI has a tendency to overestimate free T.[35]

3.5 Calculated free testosterone (CFT)

In the literature, there are at least six different published equations for the calculation of serum free T in males. They can be grouped into two main categories. Firstly, equations based on the law of mass action with the assumption that one ligand (testosterone) binds to two proteins (SHBG and albumin).[29,36] Secondly, equations derived from empirical measurements of free T.[26,37,38] All of these CFT equations involve the measurement of total T and SHBG; in addition, the two equations based on the law of mass action also require albumin concentration. Among the published equations, the Vermeulen version appears to be the most widely used in the literature and CFT values derived using this equation were shown to correlate well with free T measured by equilibrium dialysis in the original study.[29] It is important to note that given the same total T, SHBG and albumin concentrations, the various equations can result in CFT values up to approximately two times different from one another.[26,35,38-40] Therefore, CFT values generated by different laboratories may not be comparable if different CFT equations have been employed. To date, which of the above equations gives CFT values closest to serum free T concentrations is unknown.

3.6 Bioavailable testosterone (BAT)

Serum bioavailable testosterone (BAT), also known as non-SHBG-bound testosterone, is the fraction of circulating testosterone which is not bound to SHBG. In practice, BAT is usually considered equivalent to the total concentration of free T and albumin-bound testosterone. BAT can be either assayed or calculated. It is typically assayed by the precipitation of SHBG and SHBG-bound steroids using ammonium sulphate, followed by direct measurement of total T in the supernatant. An alternative method for BAT measurement involves pre-incubation of serum with ^{3}H- testosterone before ammonium sulphate precipitation; percentage of the radiolabelled tracer remaining in the supernatant is then multiplied by the total T concentration to yield BAT.[19] One main disadvantage of BAT measurement is its dependence on adequate SHBG precipitation, temperature and concentration of ammonium sulphate solution used. Also, BAT assays are not easily automated and thus very few clinical laboratories routinely measure BAT. Several equations for the calculation of BAT have been proposed.[29,36,41,42] There is no consensus on whether BAT is better than free T as a biochemical marker of androgenicity.

3.7 Sex hormone binding globulin (SHBG)

Many commercially available immunoassay methods are routinely used for the measurement of serum SHBG concentrations. However, marked method-related differences in bias can occur between these SHBG assays.[40] It is thus extremely important that method-related reference ranges are used for not only serum SHBG concentrations but also CFT and BAT calculated based on such measured SHBG concentrations.

4. CLINICAL INDICATIONS AND INTERPRETATION OF TEST RESULTS

4.1 Late-onset hypogonadism (LOH)

What is LOH?

LOH, also known as male menopause or androgen decline in the aging male (ADAM) in the literature, is a clinical and biochemical syndrome associated with advancing age and characterised by testosterone deficiency and symptoms or signs typical of testosterone deficiency (Table 1).[20] There is no universally accepted definition of male hypogonadism. In most cases, a combination of symptoms, signs and laboratory investigations is used to reach a diagnosis (Table 2).[3,20] Whereas low libido is one of the commonest symptoms experienced by hypogonadal males,[43,44] lethargy and erectile dysfunction are also common.

What is the prevalence of LOH?

Estimates of the prevalence of LOH depend on the diagnostic criteria used and thus vary between published studies. In a cross-sectional study of 1845 men (aged 47.3 ±12.5 years, mean ± SD), 24% and 11% of subjects had total T < 10.4 nmol/L and CFT < 173 pmol/L respectively, whereas only 5.6% of men in this study met the criteria for symptomatic androgen deficiency.[45] In comparison, men were considered to have androgen deficiency in the Massachusetts Male Aging Study (MMAS) if they had at least three signs or symptoms associated with hypogonadism, and either (a) total T < 6.9 nmol/L or (b) total T = 6.9-13.9 nmol/L and CFT < 309 pmol/L; the prevalence of androgen deficiency in the MMAS was reported to increase from 6.0% at baseline (aged 40-70) to 12.3% at follow-up (aged 48-79).[46] The prevalence of LOH is also age-dependent; for example, in the European Male Aging Study (EMAS) study (LOH defined as at least three sexual symptoms in the presence of total T <11 nmol/L and CFT <220 pmol/L) it increased from 0.1% in men aged 40-49 to 5.1% in those aged 70-79 (overall prevalence 2.1%).[44]

How should men with probable LOH be investigated?

Questionnaires for symptoms associated with LOH such as the Androgen Deficiency in Aging Male (ADAM)[47] and the Aging Male Symptom Score (AMS) have been developed to assist the diagnosis of LOH.[48] The use of these questionnaires is not widespread in clinical practice and is not recommended by clinical guidelines for the diagnosis of LOH because of low specificity.[20,49] In an adult male presenting with symptoms or signs of hypogonadism (Table 1), serum total T is usually the initial laboratory investigation for the assessment of androgen status. The measurement of specific fractions of serum testosterone such as free T or BAT is increasingly used by clinicians to assess androgen status in men. Free T or BAT is thought to be preferable to total T alone for the diagnosis of LOH in adult men when serum total T is equivocal.

The Endocrine Society (USA) Clinical Practice Guideline (2010) recommends that a diagnosis of androgen deficiency should only

TABLE 1: Common symptoms and signs associated with male hypogonadism.

Physical	Decreased muscle mass or strength Decreased bone mineral density or osteoporosis Gynaecomastia Reduced body hair including beard Hot flush
Psychological	Depression Lack of energy Fatigue Poor concentration
Sexual	Low libido Decreased sexual thoughts Erectile dysfunction Infertility

be made in men with consistent symptoms or signs, and unequivocally low serum testosterone levels.[3] It is not uncommon for aging males to experience some symptoms associated with LOH; for example, a large proportion (up to 35%) of eugonadal men with testosterone >10.5 nmol/L in the European Male Aging Study (EMAS) reported symptoms that are usually associated with hypogonadism.[50] Men who have low serum testosterone concentrations may also be asymptomatic.[45] To date, published studies disagree on which cut-off values of total T, CFT or BAT should be used for the purpose of the clinical diagnosis of LOH; rather, the prevalence of symptoms and signs of LOH increases with decreasing testosterone concentrations.[44,51] Moreover, there is no current consensus on whether age-related reference ranges of testosterone concentrations should be used in the diagnosis of LOH. Without considering the differences in assay-related performance, various lower limits of reference range for morning total T, ranging from 9.4 nmol/L to 12 nmol/L, have been used in the literature.[20,35,44,52] For practical purpose, total T of 12 nmol/L or more is usually considered not deficient.[20,21] It is widely accepted that total T be measured on at least two occasions before a diagnosis of LOH is made. When total T is borderline low (e.g. 8-12 nmol/L) or an abnormal SHBG concentration is suspected, CFT or BAT may be useful in the diagnosis of LOH.[3,20] There are, however, no universally accepted threshold levels of CFT or BAT for the diagnosis of LOH. Reference ranges derived from established assays and method/ equation for CFT/BAT based on a well-defined normal population should be used.[35] There is little evidence to indicate that CFT is better than BAT, or vice versa, as an estimate of free testosterone concentration.

Table 2 summarises the role for other laboratory tests in the diagnosis of LOH. Measurement of FSH and LH can help differentiate between primary (testicular) and secondary (hypothalamic-pituitary) hypogonadism[3,20] and should be included as part of the laboratory diagnostic profile in men with persistently low testosterone. In men with secondary hypogonadism, investigation of other pituitary functions e.g. serum prolactin concentration is usually indicated.

TABLE 2: Diagnostic criteria and investigations for late-onset male hypogonadism (LOH). Reproduced from Ho & Beckett (2011)[40] with permission from BMJ Publishing Group.

Symptoms and signs	• Persistent symptoms or signs (at least one) associated with male hypogonadism (see Table 1) • No symptom or sign is specific to LOH
Total testosterone (total T)	• Initial investigation of choice • Samples should be taken in early morning (8–11 am) • Total T should be checked at least twice before a diagnosis of LOH is made • No universally accepted cut-off to classify total T as low • Total T at 12 nmol/L or above is usually considered not deficient and does not require replacement therapy
SHBG	• Not routinely required for the diagnosis of LOH • Indicated if total T is borderline low or abnormal SHBG concentration is suspected on clinical grounds
Free and bioavailable testosterone	• May be useful if total T is borderline low • No universally agreed cut-off limit
LH & FSH	• May help differentiate between primary and secondary causes of hypogonadism
Prolactin	• Indicated in confirmed LOH to rule out hyperprolactinaemia especially in secondary hypogonadism

Other considerations

An inverse relationship between body weight and total T was first reported in the 1970s.[53] Subsequent studies have confirmed that total T decreases with increasing body mass index (BMI)[14,16,54] and is positively correlated with serum SHBG concentrations.[55] It is believed that the negative association between total T and BMI is to a large extent due to reduced SHBG synthesis in liver and serum SHBG concentrations in obese men. Because of a reduction in serum SHBG concentrations often seen in obese subjects, the reduction of free testosterone with increasing BMI may be less marked than total T. Therefore, measurement or estimation of free T may be useful in obese men with borderline low total T.

Type 2 diabetes (T2DM) is a recognized risk factor for LOH.[20] Multiple studies have shown that T2DM is associated with lower total T in men compared with controls even after adjustment for age and BMI.[56-59] Serum free T concentrations are also lower in T2DM patients than controls.[59] T2DM patients tend to be overweight and decreased SHBG concentrations can at least partly explain the lower total T in these patients. Studies have demonstrated that testosterone replacement therapy improved glycaemic control or insulin sensitivity but more long-term studies are awaited to determine the benefits of testosterone replacement in T2DM patients.[3,59]

In patients on testosterone replacement therapy, it has been recommended that total T should be increased to approximately mid-normal levels found in healthy, young men.[3,20] The recent joint UK societies' guidelines recommend a minimum total T of 15 nmol/L for the improvement of symptoms,[21] whereas a target total T of 13.9-24.3 nmol/L one week after testosterone enanthate or cypionate injection has been suggested by the Endocrine Society (USA).[3] The Association of British Clinical Diabetologists recommends monitoring of testosterone therapy at 3, 6 and 12 months and then annually in patients with diabetes;[49] in comparison, the Endocrine Society (USA) guidelines suggest monitoring of testosterone 3 to 6 months after initiation of replacement therapy.[3] There is not enough evidence to indicate that free T, CFT or BAT is useful in the monitoring of patients on testosterone replacement therapy.

4.2 Male factor infertility

The definition of infertility is not universally agreed in the literature.[60] The World Health organization has defined infertility as the inability of a sexually active couple to achieve pregnancy despite regular unprotected intercourse for 12 months.[61] Likewise, infertility is considered by the European Society of Human Reproduction and Embryology (ESHRE) as the inability of a couple to conceive after 1 year of sexual intercourse without contraception.[62] In comparison, the UK National Institute for Health and Clinical Excellence (NICE) recommends that infertility should be defined as failure to conceive after regular unprotected sexual intercourse for two years in the absence of known reproductive pathology; NICE also recommends that people who have not conceived after one year of regular unprotected sexual intercourse should also be offered clinical investigations.[63]

The male is the only cause of infertility in approximately 30% of cases, and a combination of male and female factors can be found in another 20% of infertile couples.[64] Male factor infertility can be caused by many clinical conditions, ranging from varicocoele, chromosomal abnormalities, obstructive azoospermia to endocrine aetiologies. In a study on more than 10,000 patients with male factor infertility, no identifiable causes were found in 30-40% of cases (idiopathic male infertility).[65] The objectives of the clinical evaluation of infertile men are to exclude treatable conditions such as gonadotrophin deficiency, obstructive azoospermia, and coital disorders, and to identify those who are candidates for assisted reproductive technologies.[66] A detailed history and physical examination (general and genital) is essential and can identify some causes of infertility in such men.

For the diagnostic work-up of the male partner, semen analysis is considered the cornerstone of initial laboratory investigations.[64] The results of semen analysis should be compared to reference values published by the World Health Organization. Initial laboratory assessment of the infertile man should also include serum concentrations of total T, FSH and LH. Total T should be measured in a morning sample as described above and persistently low total T results suggest that hypogonadism may be a cause of the male factor infertility. After hypogonadism is confirmed, testing of serum FSH and LH levels may be able to differentiate between hypogonadotrophic hypogonadism (low FSH and LH levels) and hypergonadotrophic hypogonadism (elevated FSH and LH levels).

Table 3 summarises the major causes of the two types of hypogonadism. In men with hypogonadotrophic hypogonadism, it is advisable that pituitary dysfunction be screened by measuring hormones such as thyroid stimulating hormone (TSH), free thyroxine, prolactin and cortisol. On average, 15-20% of infertile men are azoospermic [67] and serum FSH level may be helpful in differentiating between obstructive and non-obstructive causes of azoospermia.[64] An elevated FSH in the presence of azoospermia is suggestive, though not diagnostic, of abnormal sperm production.[64] Normal serum FSH levels, however, can also be found in men with primary sperm production problem.[64] Measurement of serum concentrations of inhibin B (produced in Sertoli cells) may be useful as a marker of normal spermatogenesis.[68]

Androgen insensitivity syndrome is a clinical condition which results from the inability for testosterone and dihydrotestosterone to virilise male embryos, and is mainly attributable to molecular defects in the androgen receptor gene.[69] Patients with androgen insensitivity can be classified as having either complete androgen insensitivity syndrome (CAIS) or partial androgen insensitivity syndrome (PAIS). Androgen receptor gene mutations that severely impair the amount, structure or function of the receptor lead to CAIS (commonly known as testicular feminization syndrome), evidenced by the complete feminisation of 46 XY individuals at birth.[70] Mutations of the androgen receptor gene that do not completely disrupt androgen receptor function can cause PAIS; these patients have variable degrees of gynaecomastia and ambiguous genitalia, including partial labial-scrotal fusion, hypospadias and bifid scrotum.[70] In adults with AIS, serum testosterone concentrations are typically within or above the male reference range, whereas LH levels are usually elevated due to the lack of negative feedback regulation.

4.3 Other clinical indications for testosterone measurement

Testosterone testing in adult men is mainly indicated in those who are clinically suspected to have low androgen status. Occasionally, the clinical diagnostic laboratory also receives requests for testosterone measurement in prostate cancer patients who have received medical castration therapy by, for example, gonadotrophin-releasing hormone (GnRH) analogues, though the clinical value of testosterone measurement in such patients is unclear. Anti-androgens such as flutamide, bicalutamide and cyproterone acetate are also used in patients with prostate cancer and sometimes in male-to-female transgender

TABLE 3: Causes of male hypogonadism. Adapted from Dohle *et al* (2010).[65]

Primary (hypergonadotrophic) hypogonadism	Maldescended testis Varicocoele Chromosomal abnormalities e.g. Klinefelter's syndrome, Y chromosome microdeletions Anorchia Trauma, testicular torsion, orchitis Iatrogenic e.g. surgery, medications, irradiation Systemic diseases Testicular tumour Idiopathic
Secondary (hypogonadotrophic) hypogonadism	Hyperprolactinaemia Medications e.g. anabolic steroids Congenital e.g. Kallmann syndrome Hypothalamic or pituitary tumour Fracture of skull base Granulomatous disease Ischaemic or haemorrhagic lesions of hypothalamus
Target organ resistance to androgens	Androgen insensitivity syndromes

individuals; these anti-androgens bind to the androgen receptor and do not tend to markedly alter serum testosterone concentrations.

Hirsutism in men is uncommon and is not easily recognized due to the wide variability of hair growth in healthy adult men. The main condition that needs to be excluded in a hirsute man is androgen-secreting tumour (in the testis or adrenal gland), which is rare in both men and women. Male patients suspected of androgen-secreting tumours should have their serum dehydroepiandrosterone sulphate (major adrenal androgen) and testosterone measured.

5. FINAL REMARKS

For serum samples with total T concentrations which are within or slightly below the reference range applicable to adult males, the technical performance of most commercially available total T assays in terms of accuracy and precision does not usually cause major concern. Estimation of testosterone fractions such as CFT and BAT may be useful when total T concentration is borderline low and in situations where free testosterone and total T concentrations may not correlate well with each other. Importantly, the clinical interpretation of total T and testosterone fractions requires an understanding of the limitations of these assays and calculations. It is likely that clinicians will increasingly use tests for testosterone fractions in the clinical management of adult men but the usefulness of such parameters in the long term management of conditions such as LOH awaits further evaluation.

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