Asian Journal of Andrology (2020) 22, 441–446
www.asiaandro.com; www.ajandrology.com

Open Access

REVIEW

Prostate Cancer

How do we define "castration" in men on androgen deprivation therapy?

Sarin Itty, Robert H Getzenberg

Androgen deprivation therapy (ADT) is the mainstay for the treatment of advanced prostate cancer. Since the clinical evolution from surgical orchiectomy, we have typically used ADT and orchiectomy to be synonymous terms for castration. The goal of this study is to determine if, in contemporary medical practice, surgical and chemical castration provide for similar levels of diminishment of total and free testosterone. Further, what approaches should be used to most accurately measure testosterone levels in men with advanced prostate cancer and what cutoff values, for example for total testosterone 50 ng dl^{-1} or 20 ng dl^{-1}, should be utilized. Studies available in the literature have been analyzed and compiled to address these questions. Finally, evidence is provided that free testosterone, the biologically active component, should be utilized to provide clinically relevant state of castration.

Asian Journal of Andrology (2020) **22**, 441–446; doi: 10.4103/aja.aja_139_19; published online: 21 January 2020

Keywords: androgen; androgen deprivation therapy; castration; free testosterone; testosterone

INTRODUCTION

Throughout history, castration has been used as a form of criminal punishment, a display of religious devotion, a means toward obtaining a soprano voice, and for eugenic reasons. Currently, castration is performed primarily for medical treatment. Castration, defined as, "to deprive (a male animal or person) of the testes," was originally performed by surgical means (orchiectomy) and although that is less common today, there are two oncological reasons which may warrant orchiectomy, prostate cancer, and testicular cancer. Certain individuals may elect for the surgery for cosmetic purposes as well: for example, sex reassignment surgery (SRS) for transgender women. Cases of testicular torsion or severe testicular injury may also require an orchiectomy, depending on the loss or survival of the testicles. A congenital anomaly called undescended testicles can also require orchiectomy; this is when the testicles are located in the abdominal cavity and need to be surgically moved into the scrotum. If irreparably damaged, a surgeon may remove one testicle or both testicles.

Today, what we term, "castration," can be performed either surgically or chemically. Surgical castration occurs via an orchiectomy, removal of the testes which can be unilateral (removing one testicle) or bilateral (removing both testicles). A study of eunuchs in China revealed that as a result of the castration, in 21 out of 26 eunuchs, the prostate was nearly impalpable; thus, the gland requires the continued presence of androgens throughout life for proper functioning.[1] The disappearance (shrinkage) of the prostate may be a function of time after castration as well as the age at which the castration occurs.[2]

Testosterone, an androgen responsible for male secondary sexual characteristics, is produced primarily in the testes (95%) and also in small amounts by the adrenal glands (5%). Testosterone is the main sex hormone for men, regulating various functions such as libido, bone density, red blood cell count, male characteristics, and male behaviors. Testosterone is also the fundamental hormone in the regulation of prostate cancer growth. Blood levels of total testosterone are composed of bound and free forms of testosterone. Most of the circulating testosterone is bound to either albumin or sex hormone-binding globulin (SHBG). As its name implies, most of the sex hormones in males and females are bound to SHBG, a protein produced by the liver, as they circulate through the body. In addition to protection from degradation, SHBG serves to regulate the amount of free testosterone that is available for biological activity by keeping it bound and therefore inactive. Free testosterone is the metabolically active form of testosterone that carries out the biological functions that are associated with its activity.[3] For the purpose of this manuscript, the terms free testosterone and bioavailable testosterone are synonymous and are used that way throughout.

Blood testosterone levels vary depending on the method of castration. Surgical castration usually involves the removal of the testes, which leads to a decrease in testosterone production. Overall, surgical castration results in lower levels of testosterone than that of hormonal treatments.[4] For men undergoing lutenizing hormone-releasing hormone (LHRH) agonist therapy, the total testosterone has been shown to range from 0.69 ng dl^{-1} to 29.5 ng dl^{-1}, whereas the range for total testosterone in orchiectomized patients was 0.69 ng dl^{-1} to 13.01 ng dl^{-1}.[4] In these assays, the reported 0.69 ng dl^{-1} is the approximated limit of detection of the assay.

A study published in the *Asian Journal of Andrology* described total testosterone and free testosterone levels in orchiectomized patients.[4] The study population comprised 1389 men from 150 sites in the USA and Mexico, 56 of which had undergone orchiectomy. The free testosterone levels were obtained using equilibrium dialysis (EqD) and radioimmunoassay (RIA). In the orchiectomy-alone patients, the mean free testosterone level was 1.14 pg ml^{-1} (0.114 ng dl^{-1}) and the

Dr. Kiran C. Patel College of Allopathic Medicine, Nova Southeastern University, Fort Lauderdale, FL33328, USA.
Correspondence: Dr. RH Getzenberg (rhgetzenberg@gmail.com)
Received: 03 July 2019; Accepted: 25 October 2019

total testosterone level averaged 0.69 nmol l^{-1} (19.9 ng dl^{-1}). In this study, within all the groups which underwent orchiectomy, serum free testosterone averaged 1.9 pg ml^{-1} (0.19 ng dl^{-1}), which was described as the value for the optimal suppression of free testosterone levels and the ultimate goal of medical androgen deprivation therapy.

MATERIALS AND METHODS

One approach to define castration is through the study of clinical and regulatory guidelines and guidance documents. In this article, we reviewed the existing guidelines and guidance documents in order to determine if there is a consensus for what a castrate level of testosterone is. To accomplish this goal, we utilized a comprehensive PubMed search (terms included free testosterone, total testosterone, androgen deprivation therapy, prostate cancer, testosterone measurement, castration threshold, and orchiectomy) combined with searches of the existing clinical and regulatory guidelines. We focused the search on studies examining the association between total and free testosterone levels and men on androgen deprivation therapy (ADT) but included those that contained information only related to total testosterone levels as descriptors of the efficacy of ADT. Although the FDA does not have a guidance definition of castration, various guidance documents illustrate a historical definition of a clinically castrate range as being a total testosterone level below 50 ng dl^{-1}.[5] The European Medical Association's (EMA) Sixth International Consultation on New Developments in Prostate Cancer and Diseases agreed that because total serum testosterone levels below 20 ng dl^{-1} are typical in men that have undergone bilateral orchiectomy, this cutoff should be utilized for chemical castration.[3] As for clinical guidelines, the American Urological Association (AUA) lists 50 ng dl^{-1} as the threshold for chemical castration.[6] The European authorities have not established a clear definition of castration, although the European Association of Urology (EAU) has noted that testosterone levels <20 ng dl^{-1} are associated with improvement in outcomes compared to men within the 20–50 ng dl^{-1} range.[7]

RESULTS

Methods for measuring testosterone

As androgen deprivation therapy (ADT), another term used for clinical "castration," typically results in significant lowering of serum testosterone levels; sensitive assays are required that can detect the blood levels at the lower ends of the scale. Although several studies have validated techniques for the measurement of relatively higher levels of testosterone (above 100 ng dl^{-1}), for ADT measurements, the range of testosterone levels for ADT falls much lower. Ideally speaking, the goal of medically induced ADT would follow the surgical gold standard of <20 ng dl^{-1}.

Obviously, one of the limitations to the standardization of free and total testosterone levels constitutes the assays used to measure them. There may be assay differences related to the ability to detect low levels of the forms of the hormone in the blood. The primary techniques utilized for the measurement of testosterone are liquid chromatography-mass spectrometry (LC/MS), RIA, and chemiluminescence. The assays and associated instrumentation used to measure testosterone are important to consider because of the reliability, accuracy, sensitivity, ability to be standardized, and comparability of the results, when making any conclusions regarding clinically appropriate testosterone levels and cutoffs to define castration.

As a means to evaluate the level of free testosterone within the blood, it is necessary to separate out the free and bound components. The typical approach used for such a separation is EqD which, as its name implies, uses dialysis to separate out the free and bound components. After performing the EqD, the two forms of testosterone found in the blood (free and total testosterone) are then measured through RIA and/or LC/MS. RIA is an immunoassay which uses radioactively labeled compounds (antigens) to bind to antibodies and form immune complexes. From the data collected, a curve is formed and the amount of antigens in the serum can be estimated. Results from various studies support measuring free testosterone through RIA,[8] and it was the standard used for many years.[9] The advantages of RIA include that it is widely used and therefore represents a preponderance of the testosterone measurement data, it is less expensive than LC/MS, and it has high specificity and sensitivity. The disadvantages of RIA include the lack of accuracy, overestimates of values, difficulty in creating appropriate ligands or receptors, potential analogs, and the hazards associated with using radioactive substances. There is also potential for cross-reactivity with hormones other than testosterone. In addition to this, a study by Wang *et al*.[9] has illustrated that RIA tends to underestimate free testosterone levels by 20%–60%.

Termed the gold standard by the Endocrine Society, LC/MS is a technique from analytical chemistry with huge potential for clinical use.[8] There are validated LC/MS methods that measure free and total testosterone.[10] The advantages of using LC/MS include higher sensitivity, higher precision, analytical specificity, performance, and excellent diagnostic ability.[11] Disadvantages include that it is more expensive than RIA, there is a lack of standardization and generalizability, and that noise may influence the machinery's extreme sensitivity. In order to obtain clinical proficiency as measured by the US Centers for Disease Control and Prevention (CDC) certification for standardization, laboratories have to demonstrate a mean bias of 6.4%.[12]

On comparing RIA and LC/MS, it is evident that LC/MS has a higher analytical sensitivity and better diagnostic ability than RIA.[11] Other studies have found a concordance between LC/MS and RIA in the measurement of total testosterone.[13] In a study on 56 men (aged 26–77 years) comparing RIA and EqD, a robust correlation ($r = 0.966$) in results was observed,[14] but as this study was performed with samples from normal, healthy men, the measurements were not at the lower end of the concentration spectrum where assay differences, if they exist, may be expected to occur.

Currently, it seems that immunoassays remain the assay form most typically used in the measurement of testosterone levels for patients in the normal testosterone ranges. In most clinical and laboratory settings, testosterone immunoassays assays are performed on automated platforms with nonradioactive methods.[15] The automated immunoassays now use chemiluminescence detection instead of radioactive ligands to quantify testosterone. While RIA was more commonly used, the accuracy and reliability of the immunoassay is questionable considering that it often uses testosterone analogs as standards and propriety reagents.[15] A study found the variability of immunoassays to be 23%, which is really high, especially for the diagnosis of disorders that rely on smaller measurements of testosterone.[9] However, when measuring smaller amounts through LC/MS, the machine was able to measure concentrations as low as 20 ng dl^{-1} accurately due to its high precision and accuracy.

In measuring lower levels of testosterone, the difference in accuracy and sensitivity is more applicable, especially in cases where testosterone levels below 20 ng dl^{-1} need to be measured. Although LC/MS is a more expensive and not currently as clinically available approach, it is more accurate and should be used for the diagnostic measurements of testosterone. Its high sensitivity and reliability is essential when working on this smaller end of the scale.

Use of hormonal therapy for prostate cancer treatment

In 1966, Charles Huggins was awarded the Nobel Prize in Physiology for his use of hormonal therapy in prostate cancer (PCa) treatment.[16] The studies reported by him and his colleagues illustrated that ADT in men with metastatic PCa triggered an anticancer response and "ameliorated cancer-associated disruptions in bone metabolism".[17] Huggins developed the concept of chemical castration through ADT as a means to treat advanced PCa.

With these disease-altering findings, castration became a mainstay in the treatment of advanced PCa. At that point in time, orchiectomy (surgical castration) was principally used for the treatment of advanced PCa as it was/is a less expensive and yet effective means of castration. The surgical removal of the testes ensures a decrease in serum testosterone typically resulting in a decrease in tumor burden as well as the associated androgen responsive markers. However, orchiectomy is no longer the mainstay as a treatment for PCa principally as a result of the public perception that it is a barbaric as it results in an alteration in physical appearance. Although appearance can be modified by the use of prosthetics, the act also carries a psychological impact.[18] It is also irreversible[3] unlike ADT which can be discontinued depending on the morbidity associated with the observed side effects as well as disease progression.

The goal of medical ADT is to reduce testosterone levels to below "castrate" levels, which historically have been considered to be ≤50 ng dl^{-1}. Surgical castration (orchiectomy) usually leaves the patient with testosterone levels <20 ng dl^{-1}.[7] The threshold of ≤20 ng dl^{-1} has not yet been widely incorporated into clinical practice due to the paucity of literature to support this cutoff, the lack of accuracy and sensitivity of the assays/instruments, and the self-fulfilling prophecy based on the current regulatory guidelines. Decreasing the threshold which defines castration and reforming the goal of medical ADT to that equivalent for what is found in orchiectomized men (≤20 ng dl^{-1}) would seem to result in a more complete castration and therefore may lead to better oncological outcomes. In many ways, with an incomplete castration, we may be continuing to feed the PCa and at the same time provide for an environment in which resistant clones can evolve. A similar analogy could be microbes developing resistance due to improper antibiotic use; not using sufficient levels of antibiotics may appear to reduce the infection but, in reality, it provides enough of the population remaining that resistance can be selected for.

Total testosterone

As described above, the measurement of total testosterone consists of bound (bound to SHBG or albumin) and unbound (free) testosterone. The normal range of total testosterone levels for a male is 280–1100 ng dl^{-1}. The FDA defines testosterone as a hormone essential for the growth and development of the male sex organs and maintenance of secondary male characteristics.[19] There are no FDA guidelines on what a "castrate" level of total testosterone is, although the historically accepted definition, within the USA, has been ≤50 ng dl^{-1}. The current National Comprehensive Cancer Network (NCCN) guidelines also define castration as a serum total testosterone of ≤50 ng dl^{-1}.[20]

In addition to the complexity of the assays used to measure testosterone levels and the differences between free and total testosterone, the AUA has written that the diagnosis of low testosterone should only be made after two separate testosterone measurements supporting the importance of repeated measurements. They also describe that the measurements be conducted in the early morning as a result of the known diurnal variation in testosterone levels.[21]

Androgen deprivation therapy

The historical goal of castration is to reduce the level of serum testosterone as androgens are known to stimulate the growth of most prostate cancers. Thus, ADT can be used to treat advanced PCa patients. ADT can be given for various periods of time either alone or in combination with external beam radiation therapy or brachytherapy (interstitial radiation). The course(s) of ADT are typically predicated on the disease path and the morbidity of the side effects that are known to accompany castrate levels of testosterone and therefore estrogens in these men. The American Cancer Society defines ADT as orchiectomy, LHRH agonists, and antagonists, and Cyp17 inhibitors.[22] Using antiandrogens with LHRH agents is called a combined androgen blockade (CAB). CAB with finasteride or dutasteride is triple androgen blockade and is considered to be the most effective medical form of ADT.

There are several different types of ADT, consisting of both surgical and chemical means. The three main types of chemical castration are brought about through the action of pharmacological agents. The first type are the LHRH agonists or antagonists; LHRH agonists can sometimes result in "testosterone flares" that can be avoided with the antagonists. Examples of LHRH agonists include leuprolide, goserelin, triptorelin, and histreline, whereas examples of LHRH antagonists include degarelix and the CYP17 inhibitors, such as abiraterone. Abiraterone is a second-generation androgen receptor (AR) pathway inhibitor that lowers the production of androgens but may also lead to less inhibition of LH and LHRH. The second group are classified as anti-androgens, and are involved in blocking the effects of androgens directly on the cells usually through a blockade of androgen receptor itself. Examples of this group include flutamide, bicalutamide, nilutamide, enzalutamide, apalutamide, and darolutamide. In the USA, the anti-androgens are typically used in combination with either an LHRH agonist or antagonist. The third group is administration of estrogens/female hormones. Estrogens work through a feedback mechanism by turning off androgen production and therefore reducing testosterone levels. Although they were commonly used in the 1960s and 1970s, as a result of some of the serious potential side effects, they are rarely used today. The major side effect associated with estrogen administration is that its high potency (due to the presence of estrogen receptors on platelets) can lead to serious or even fatal, venous thrombolic events (VTEs). The major advantages of ADT are that the resulting lowering of testosterone levels is rapid and effective, is nonsurgical, is reversible, and has the potential for oral or subcutaneous administration rather than intravenous nature of current chemotherapies. The side effects that result from ADT are significant and can often result in patients discontinuing their use or using them intermittently. Among the major limitations are hot flashes, a loss of libido, fatigue, osteoporosis, erectile dysfunction, and more.[23] Perhaps, the biggest limitation to the use of ADT is that it is invariably followed by the recurrence of the disease and the development of castration-resistant prostate cancer (CRPC).[20]

In addition to the direct hormonal approaches to ADT, treatment modalities can be combined to create more effective approaches and treatment plans leading to therapeutic synergism. For example, docetaxel is a chemotherapy that is used in different PCa disease settings. As a taxane, docetaxel interferes with microtubules, impacting chromosomal segregation in cell division and can therefore impart cell death in actively dividing cells. In addition to its direct effects on inhibiting the growth of these actively growing cells by disrupting the microtubules, taxanes can also inhibit the transport of the AR into the nucleus.

Free testosterone

Perhaps, one of the most important yet understudied questions in helping to define the clinically desirable castration of men with advanced PCa is what form of testosterone should be measured. As described above, total testosterone is what is principally used today, but free testosterone is the biologically active form and therefore is also termed bioavailable testosterone. Perhaps, free testosterone would be a more clinically and biologically relevant measure of castration than total testosterone. Free testosterone has been demonstrated to be an important biomarker for cancer-specific survival in PCa; cancer-specific survival is longer for patients with a free testosterone level below the cutoff, almost 26 months longer than the group with free testosterone above the cutoff.[24] Bioavailable testosterone (including free testosterone) may correlate better with symptoms than total testosterone.

Only 2% of all testosterone in the body is unbound and free. According to the free hormone hypothesis, only the 2% of testosterone which is free can diffuse into cells and bind to the AR.[25] As free testosterone is the biologically active form of testosterone, it should be given more attention in the treatment of PCa.

A cross-sectional study evaluating assays which measure free testosterone, the goal of which was to determine the concordance of the various assays to one another, was conducted on fifty males.[26] This study demonstrated that there was a significant correlation among the free testosterone index, free testosterone by EqD, and bioavailable testosterone. The free testosterone value was calculated by the Vermeulen method, a formula relying on the total testosterone and SHBG values obtained from immunoassay.[27] This approach is a practical measure of bioavailable testosterone as a result of its simplicity and efficiency in detecting hypogonadism.[26]

Patients with advanced PCa that achieve lower free testosterone levels have been shown to have overall better survival rates.[5] Despite these findings, more studies need to be conducted examining the levels of serum free testosterone and determining any correlations with PCa disease progression. A study published in *Oncology Letters* examining a group of 34 patients illustrated that the mean free testosterone was a significant prognostic factor of cancer-specific survival; also, cancer-specific survival (43.6 *vs* 17.3 months, $P = 0.0063$) was statistically significantly longer in patients with free testosterone levels below the cutoff level than patients above it.[24] Thus, suppressing biologically active testosterone (free testosterone) would appear to be a primary goal of ADT.

There is sparsity of data or even mention of free testosterone and desired castrate levels in regulatory or federal guidelines. Thus, more research needs to be conducted, perhaps comparing total and free testosterone levels within a population undergoing ADT in which clinical outcomes are determined. In the next section, a few such studies are described.

Compiling clinical data regarding the measurement of testosterone levels

Although it is not often used as regularly as it should be, measurement of serum testosterone levels is a critical element of the administration of ADT. Testosterone measurements are utilized in the diagnosis and management of several disorders, including polycystic ovary syndrome (PCOS), testosterone deficiency, and testosterone excess. In ADT, free and total testosterone are viewed as markers measuring ADT effectiveness and reflecting the prognosis of PCa.

A comprehensive literature search was conducted to identify and analyze all studies measuring free and total testosterone. Information collected includes the type of ADT used, the number of patients, the timing of the measurement, the method/assay of measurement, patient demographics, and length of time on ADT, if available. Unfortunately, from our review of the literature, only four published studies[4,28–30] directly addressed these important points, the summaries of which are included here.

The effects of different forms of ADT on hormone levels within the body were studied.[4] Using RIA and EqD, the effects of various forms of ADT and orchiectomy on circulating free testosterone (FT) and total testosterone (TT) were measured. While the group with LHRH agonists (1191 patients) had an average FT value of 1.01 pg ml^{-1} (0.101 ng dl^{-1}) and an average TT value of 0.69 nmol l^{-1} (19.1 ng dl^{-1}), the orchiectomy group (56 patients) had an average FT value of 1.14 pg ml^{-1} (0.114 ng dl^{-1}) and an average TT value of 0.69 nmol l^{-1} (19.1 ng dl^{-1}).[4] In addition to these groups, they also had a group which utilized a combined therapy (27 patients) of both orchiectomy and androgen receptor blockade, which had an average FT value of 1.25 pg ml^{-1} (0.125 ng dl^{-1}) and an average TT value 0.69 nmol l^{-1} (19.1 ng dl^{-1}).[4] The most effective group (10 patients) underwent orchiectomy combined with an LHRH agonist and had an average FT value of 0.35 pg ml^{-1} (0.035 ng dl^{-1}) and an average TT value of 0.75 nmol l^{-1} (21.6 ng dl^{-1}).[4] Unlike the other published work, this study asserted that there was no significant difference in hormone levels between surgically and medically castrated individuals.

A 2016 study evaluated free and total testosterone in 29 patients who were administered LHRH agonists alone as their form of ADT.[28] Within this study, several groups with cutoff levels of free testosterone and total testosterone were established. The total testosterone cutoffs were at 50, 32, and 20 ng dl^{-1} and free testosterone cutoffs were set at 1.7, 1.1, and 0.7 pg ml^{-1} (or 0.17, 0.11, and 0.07 ng dl^{-1}, respectively).[28] The main purpose of this study was to compare serum free and total testosterone levels in an effort to predict castration-resistant survival.[28] The lowest threshold of free testosterone which illustrated significant differences was 1.7 pg ml^{-1} (0.17 ng dl^{-1}).[28] Free testosterone was discovered to be a better option than total testosterone in predicting castration resistance within nonmetastatic PCa patients.[28]

The third study examined the free testosterone levels of PCa patients undergoing LHRH agonist treatment.[29] The cutoffs established in this study for castrate levels were below 50 ng dl^{-1} for total testosterone and below 1.7 pg ml^{-1} (0.17 ng dl^{-1}) for free testosterone. While 116 out of 135 patients met the cutoff for total testosterone after treatment, 128 patients met the cutoff for free testosterone patients; including FT measurement within patient evaluation reduces the failure rate from 14.1% to 5.2%.[29] The majority of participants within their study had free testosterone levels that were reduced below 1.7 pg ml^{-1} or 0.17 ng dl^{-1}. This work revealed that although free and total testosterone levels were correlated, they provide complementary information about the status of the current ADT.[29]

Finally, a study was performed which examined the relationship of testosterone, SHBG, and calculated free testosterone in PCa patients undergoing orchiectomy or estrogen administration as a means for ADT.[30] In the orchiectomy-treated group ($n = 33$ patients), one-third of the group with the lowest free testosterone or total testosterone had a better survival over 2 years than the two-thirds that had higher levels.[30] Despite these findings, there was no evidence of a rise in free testosterone which accompanied clinical progression in these patients.[30] In addition, free testosterone was lower in estrogen-treated patients than the orchiectomized patients.[30] Within **Table 1**, the three groups (one orchiectomy group and two different estrogen groups) are listed with their prospective values.

Table 1: Analysis of studies examining serum free testosterone and total testosterone levels

Study	*ADT utilized*	*Patients (n)*	*Method of measurement*	*Timing*	*Mean age (year)*	*Length of ADT (month)*	*FT*	*TT*
Schweizer *et al.*[4] 2018	LHRH agonist	1191	TT: RIA FT: RIA and EqD		76		1.01 pg ml^{-1} (0.101 ng dl^{-1})	0.69 nmol l^{-1} (19.9 ng dl^{-1})
Schweizer *et al.*[4] 2018	Orchiectomy	56	TT: RIA FT: RIA and EqD		76		1.14 pg ml^{-1} (0.114 ng dl)$^{-1}$	0.69 nmol l^{-1} (19.9 ng dl^{-1})
Schweizer *et al.*[4] 2018	Orchiectomy and androgen receptor blockage	27	TT: RIA FT: RIA and EqD		74		1.25 pg ml^{-1} (0.125 ng dl^{-1})	0.69 nmol l^{-1} (19.9 ng dl^{-1})
Schweizer *et al.*[4] 2018	LHRH agonist and orchiectomy	10	TT: RIA FT: RIA and EqD		75		1.25 pg ml^{-1} (0.125 ng dl^{-1})	0.75 nmol l^{-1} (21.6 ng dl^{-1})
Regis *et al.*[28] 2017	LHRH agonist	126	TT: Chemiluminescent enzyme immunoassay FT: RIA	Morning (8 a.m.–10 a.m.)	71.9	6	Recommended below 1.7 pg ml^{-1} (0.17 ng dl^{-1})	Recommended below 20 ng dl^{-1}
Morote *et al.*[29] 2005	LHRH agonist	135	RIA		73.0	42	128 patients below 1.7 pg ml^{-1} (0.17 ng dl^{-1})	116 patients below 50 ng dl^{-1}
Levell *et al.*[30] 1987	Orchiectomy	44	Calculated FT		71.1	6	Mean±s.d.: 23±1.4 pmol l^{-1} (0.66±0.04 ng dl^{-1})	
Levell *et al.*[30] 1987	Estrogen (stilbestrol/ Estracyt)	28	Calculated FT		71.1	6	Mean±s.d.: 5.9±0.9 pmol l^{-1} (0.18±0.03 ng dl^{-1}) dl^{-1}	

ADT: androgen deprivation therapy; LHRH: luteinizing hormone-releasing hormone; TT: total testosterone; FT: free testosterone; RIA: radioimmunoassay; EqD: equilibrium dialysis; s.d.: standard deviation

Testosterone and clinical responses in men on second-generation inhibitors of the androgen axis

Several recent studies have explored the use of testosterone as a predictor or indicator of response. In a review of the available literature together with a meta-analysis, Claps *et al.*[31] examined the ability of total testosterone to serve as a prognostic indicator in men with PCa. In a study of Japanese patients with metastatic PCa treated with second-generation agents along with taxane-based therapies, the authors found that progression-free survival was greater among men with higher serum total testosterone levels.[32] In men with CRPC treated at a single institution, a higher pre-treatment total serum testosterone level (≥13 ng dl^{-1}) was associated with a better prognosis in men treated with enzalutamide or abiraterone.[33] Similarly, in CRPC patients again treated with enzalutamide or abiraterone, men with very low testosterone levels (<5 ng dl^{-1}) seemed to not benefit significantly from these AR-targeted therapies.[34] These findings agree with the theory that if testosterone levels are suppressed significantly with CRPC, the potential benefit from androgen axis-targeted agents may be limited. Accordingly, men with metastatic CRPC (mCRPC) with AR copy variations and low basal serum total testosterone levels treated with abiraterone had poorer progression-free survival than those without AR gain and with higher testosterone levels.[35]

DISCUSSION

As described, there appears to be support for increasing the use of the measurement of free testosterone as a tool to determine the level of "castration" in the treatment of PCa. PCa progression may be related to incomplete castration; thus, more studies should examine the extent of castration and the related impact on disease progression and survival. The more common practice regarding castration levels is to measure total testosterone. However, free testosterone should be taken into consideration as well, when determining the cutoff threshold for castration. Based on the existing data, it would appear that the goal for ADT should be a total testosterone level <20 ng dl^{-1} and a free testosterone level of <0.12 ng dl^{-1}.

AUTHOR CONTRIBUTIONS

SI co-developed the concept for this work, performed the analysis, and drafted the manuscript. RHG co-developed the concept for this work, provided feedback during the execution of the study, and edited the manuscript. Both authors read and approved the final manuscript and agree with the order of presentation of the authors.

COMPETING INTERESTS

Both authors declare no competing interests.

REFERENCES

1 Wu CP, Gu FL. The prostate in eunuchs. *Prog Clin Biol Res* 1991; 370: 249–55.
2 Wilson JD, Roehrborn C. Long-term consequences of castration in men: lessons from the Skoptzy and the eunuchs of the Chinese and Ottoman courts. *J Clin Endocrinol Metab* 1999; 84: 4324–31.
3 Oefelein MG, Resnick MI. Effective testosterone suppression for patients with prostate cancer: is there a best castration? *Urology* 2003; 62: 207–13.
4 Schweizer MT, Hancock ML, Getzenberg RH, Yu EY. Hormone levels following surgical and medical castration: defining optimal androgen suppression. *Asian J Androl* 2018; 20: 405–6.
5 Rove KO, Crawford ED, Perachino M, Morote J, Klotz L, *et al*. Maximal testosterone suppression in prostate cancer-free vs. total testosterone. *Urology* 2014; 83: 1217–22.
6 Lowrance WT, Murad MH, Oh WK, Jarrard DF, Resnick MJ, *et al*. Castration-resistant prostate cancer: AUA guideline amendment 2018. *J Urology* 2018; 200: 1264–72.
7 Cornford P, Bellmunt J, Bolla M, Briers E, De Santis M, *et al*. EAU-ESTRO-SIOG guidelines on prostate cancer. Part II: treatment of relapsing, metastatic, and castration-resistant prostate cancer. *Eur Urol* 2017; 71: 630–42.
8 Sartorius G, Ly LP, Sikaris K, McLachlan R, Handelsman DJ. Predictive accuracy and sources of variability in calculated free testosterone estimates. *Ann Clin Biochem* 2009; 46: 137–43.
9 Wang C, Cattlin DH, Demers LM, Starcevic B, Swerdloff RS. Measurement of total serum testosterone in adult men: comparison of current laboratory methods versus liquid chromatography-tandem mass spectrometry. *J Clin Endocrinol Metab* 2004; 89: 534–43.
10 Rhea JM, French D, Molinaro RJ. Direct total and free testosterone measurement by liquid chromatography tandem mass spectrometry across two different platforms. *Clin Biochem* 2013; 46: 656–64.
11 Helden J, Weiskirchen R. Cross-method comparison of serum androstenedione measurement with respect to the validation of a new fully automated chemiluminescence immunoassay. *Clin Biochem* 2018; 62: 32–8.
12 Vesper HW, Botelho JC. Testosterone. An overview of CDC's standardization initiative. *Clin Lab News* 2012. Available from: https://www.aacc.org/publications/cln/articles/2012/june/testosterone.

13 Tran TS, Center JR, Seibel MJ, Eisman JA, Kushnir MM, *et al*. Relationship between serum testosterone and fracture risk in men: a comparison of RIA and LC-MS/MS. *Clin Chem* 2015; 61: 1182–90.
14 Kacker R, Hornstein A, Morgentaler A. Free testosterone by direct and calculated measurement versus equilibrium dialysis in a clinical population. *Aging Male* 2013; 16: 164–8.
15 Matsumoto AM, Bremner WJ. Serum testosterone assays-accuracy matters. *J Clin Endocrinol Metab* 2004; 89: 520–4.
16 Hansson N, Moll F, Schultheiss D, Krischel M. Remembering Charles B. Huggins' Nobel Prize for hormonal treatment of prostatic cancer at its 50th anniversary. *Eur Urol* 2016; 69: 971–2.
17 Nelson WG. Commentary on Huggins and Hodges: studies on prostatic cancer. *Cancer Res* 2016; 76: 186–7.
18 Wiechno PJ, Kowalska M, Kucharz J, Sadowska M, Michalski W, *et al*. Dynamics of hormonal disorders following unilateral orchiectomy for a testicular tumor. *Med Oncol* 2017; 34: 84.
19 Seftel AD. Re: testosterone products: drug safety communication – FDA cautions about using testosterone products for low testosterone due to aging; requires labeling change to inform of possible increased risk of heart attack and stroke. *J Urol* 2015; 194: 759–60.
20 Mohler JL, Antonarakis ES, Armstrong AJ, D'Amico AV, Davis BJ, *et al*. Prostate cancer, version 2.2019, NCCN clinical practice guidelines in oncology. *J Natl Compr Canc Netw* 2019; 17: 479–505.
21 Mulhall JP, Trost LW, Brannigan RE, Kurtz EG, Redmon JB, *et al*. Evaluation and management of testosterone deficiency: AUA guideline. *J Urol* 2018; 200: 423–32.
22 American Cancer Society. Hormone Therapy for Prostate Cancer. Available from: https://staging.cancer.org/cancer/prostate-cancer/treating/hormone-therapy.html. [Last assessed on 2019 Feb 08].
23 Miyamoto H, Messing EM, Chang C. Androgen deprivation therapy for prostate cancer: current status and future prospects. *Prostate* 2004; 61: 332–53.
24 von Klot CA, Kuczyk MA, Boeker A, Reuter C, Imkamp F, *et al*. Role of free testosterone levels in patients with metastatic castration-resistant prostate cancer receiving second-line therapy. *Oncol Lett* 2017; 13: 22–8.
25 Mendel CM. The free hormone hypothesis: a physiologically based mathematical model. *Endocr Rev* 1989; 10: 232–74.
26 Morley JE, Patrick P, Perry HM. Evaluation of assays available to measure free testosterone. *Metabolism* 2002; 51: 554–9.
27 Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. *J Clin Endocrinol Metab* 1999; 84: 3666–72.
28 Regis L, Planas J, Carles J, Maldonado X, Comas I, *et al*. Free testosterone during androgen deprivation therapy predicts castration-resistant progression better than total testosterone. *Prostate* 2017; 77: 114–20.
29 Morote J, Esquena S, Abascal JM, Trilla E, Cecchini L, *et al*. Behavior of free testosterone in patients with prostate cancer on androgen deprivation therapy. *Int J Biol Markers* 2005; 20: 119–22.
30 Levell MJ, Siddall JK, Rowe E, Glashan RW, Robinson MR, *et al*. Relationship of testosterone, sex hormone binding globulin, and calculated free testosterone to subsequent clinical progress in patients with carcinoma of the prostate treated with bilateral orchidectomy or estrogens. *Prostate* 1987; 11: 17–21.
31 Claps M, Petrelli F, Caffo O, Amoroso V, Roca E, *et al*. Testosterone levels and prostate cancer prognosis: systematic review and meta-analysis. *Clin Genitourin Cancer* 2018; 16: 165–75.
32 Shiota M, Kashiwagi E, Murakami T, Takeuchi A, Imada K, *et al*. Serum testosterone level as possible predictive marker in androgen receptor axis-targeting agents and taxane chemotherapies for castration-resistant prostate cancer. *Urol Oncol* 2019; 37: 180 e19–24.
33 Sakamoto S, Maimaiti M, Xu M, Kamada S, Yamaga Y, *et al*. Higher serum testosterone levels associated with favorable prognosis in enzalutamide- and abiraterone-treated castration-resistant prostate cancer. *J Clin Med* 2019; 8. pii: E489.
34 Hashimoto K, Tabata H, Shindo T, Tanaka T, Hashimoto J, *et al*. Serum testosterone level is a useful biomarker for determining the optimal treatment for castration-resistant prostate cancer. *Urol Oncol* 2019; 37: 485–91.
35 Lolli C, De Lisi D, Conteduca V, Gurioll G, Scarpi E, *et al*. Testosterone levels and androgen receptor copy number variations in castration-resistant prostate cancer treated with abiraterone or enzalutamide. *Prostate* 2019; 79: 1211–20.