Role of Testosterone in Managing Advanced Prostate Cancer


Androgen deprivation therapy is frequently used to treat patients with advanced prostate cancer. New therapies for metastatic castration-resistant prostate cancer have drawn increased attention to serum and intratumoral testosterone levels. The present review examines the role of testosterone in prostate cancer progression, discusses the nuances and potential pitfalls in measuring serum testosterone using available assays, and summarizes current data relevant to the arguments for and against achieving and maintaining the lowest possible testosterone levels during androgen deprivation therapy, including the adverse effects of such treatment. Incorporating this information, we have made recommendations incorporating testosterone evaluation and its effect on the clinical decision-making process.

The use of androgen deprivation therapy (ADT) to treat prostate cancer (PCa) and slow disease progression has increased significantly in all PCa risk groups in the United States.1,2 The clinical significance of the failure to lower or maintain the serum testosterone level at less than the castrate level of ≤50 ng/dL, a definition determined more by measurement methods than evidence, remains unclear. Current guidelines (American Urological Association (AUA), National Comprehensive Cancer Network [NCCN], European Association of Urology (EAU)) regarding ADT are vague with respect to the specific monitoring of serum testosterone, primarily owing to a lack of evidence from well-designed clinical trials. No evidence is available to suggest that serum testosterone is responsible for the development of PCa; however, PCa, itself, is dependent on intratumoral androgens for survival, growth, and proliferation, as postulated and proved by Huggins and Hodges3 in 1941. This is particularly evident for men undergoing ADT, in which the disease state is largely reflected by both the serum testosterone and the serum prostate-specific antigen (PSA) levels. In addition, the arrival of new therapies targeting androgen synthesis and androgen receptor (AR) activity has also focused renewed interest on serum testosterone.

ROLE OF TESTOSTERONE IN PCA

The critical role of androgens in the development and growth of prostatic tissue, including its influence on benign prostatic hyperplasia, has been well characterized.3 Within the target tissues, testosterone is converted to the more potent androgen dihydrotestosterone (DHT) by 5α-reductase. DHT has 10 times greater affinity to AR than testosterone and is about 10 times more concentrated than testosterone within the prostate.4 In men genetically deficient of 5α-reductase, DHT levels are markedly lower despite normal serum testosterone levels, and there is no observed incidence of benign prostatic hyperplasia or PCa. However, the exact role of androgens in the development of PCa remains unknown.5 Numerous epidemiologic studies have shown no association between the serum testosterone levels and the risk of PCa development; however, lower serum testosterone levels at diagnosis have been associated with more aggressive tumors.6,7 A recent study examined the association be-
between the serum testosterone level in ADT-naive men immediately before undergoing radical prostatectomy and the occurrence of high-risk or high-grade PCa. Men presenting with low testosterone (<300 ng/dL) were more likely to be diagnosed with high-risk or high-grade PCa on univariate analysis, although this correlation lost significance on multivariate analysis. Furthermore, severe hypogonadism (serum testosterone <100 ng/dL) was significantly associated with the diagnosis of high-risk or high-grade PCa when both univariate and multivariate logistic regression analysis.8 This apparent contradiction to the overall hypothesis that androgens remain important for prostate growth and development is intriguing and underscores the important distinction between sustaining the growth of tissues and the underlying pathogenesis of PCa.

When bound to androgen, AR homodimerizes, translocates into the nucleus, and associates with AR response elements in the promoters of androgen-regulated genes, altering their transcription. The normal expression of these genes is necessary to maintain the balance between proliferative and apoptotic signaling and promote healthy prostatic tissue and for the production of PSA and other prostate-specific proteins.4,9 Multiple pharmacologic agents have been developed to disrupt this androgen axis, including 5α-reductase inhibitors, luteinizing hormone-releasing hormone (LHRH) agonists, LHRH antagonists, antiandrogens, androgen biosynthesis inhibitors, and other compounds.

Approximately 94%-97% of circulating testosterone is synthesized by the testis in response to luteinizing hormone.10,11 Successful disruption of the androgen axis effectively cuts off essential fuel for tumor growth, causing involution of the prostatic tissue and PCa. There are, however, persistent levels of testosterone (20%) and DHT (30%) within the prostate after LHRH agonist therapy, derived from the intraprostatic conversion of adrenal steroids.10 The clinical effect of these residual intraprostatic androgens on PCa progression remains unclear.

Although the direct connections among testosterone, DHT, and PSA are understood, using 1 as a surrogate for the other is not as straightforward. No correlation has been observed between PSA and serum androgen levels in ADT-naive patients with localized PCa7 or in healthy eugonadal men.12 In addition, assessment of serum testosterone and DHT at PCa diagnosis demonstrated no relationship with intraprostatic DHT levels.11 However, after 6 months of ADT, mean serum testosterone levels decline and become positively correlated with intraprostatic DHT levels (P = .033),11 suggesting that serum testosterone levels after ADT reflect additional mechanisms of testosterone synthesis in the prostate. Similarly, PSA levels correlate strongly with serum androgen levels after 6 months of ADT (P < .01 for all androgens tested), suggesting that the PSA levels also reflect the androgen milieu in localized PCa treated with hormonal therapies.7,13

The critical role of serum testosterone in hormone-sensitive PCa growth and progression is clearly demonstrated by the clinical benefits of ADT seen in most patients. However, in the vast majority of patients receiving ADT, the disease progresses to castrate-resistant PCa (CRPC). Furthermore, ADT has been associated with an increased risk of osteoporosis and skeletal fracture, metabolic disruptions, incident diabetes, cardiovascular disease and mortality, reduced cognitive function, and sexual side effects.13-15

CRPC likely develops through a number of compensatory pathways, including the development of dysfunctional AR—by increased AR expression and the resulting AR-dependent gene expression at very low androgen levels, by AR gene mutations that increase or broaden its function (increased ligand promiscuity), or by activation of ARs by other signaling pathways independent of androgens; mutations in AR coactivators or corepressors, including microRNAs; intratumoral androgen synthesis; and the development of proliferative pathways that circumvent AR.9,16-18

CRPC intratumoral androgens are primarily derived from the uptake and metabolism of adrenal androgens, along with the acquired ability of tumor cells for de novo steroidogenesis.9,17 During progression to CRPC, the enzymes involved in the steroidogenic pathways are upregulated, along with other androgen-responsive genes.19,20 These genes are normally downregulated in ADT-naive patients, suggesting that the progression to CRPC occurs through continued stimulation of the AR and its increased ability to activate transcription versus bypassing AR in alternate pathways.20 AR is further implicated in CRPC because many patients respond to secondary hormonal manipulation.

MEASURING TESTOSTERONE LEVELS

Serum testosterone measurements are readily available to the clinician and provide a window into androgen activity. For patients with PCa receiving ADT, routine testosterone measurements can aid in monitoring treatment efficacy and verify progression when the PSA level increases during treatment. Although various assays are available for research and clinical use, considerable inter- and intra-assay variation exists, including varying levels of accuracy at low testosterone levels. No universal, accuracy-based standards are available against which different assays can be compared. Furthermore, the reference intervals for serum testosterone vary widely, depending on the specific assay, the laboratory in which it is performed, and even the population used to establish the intervals.21 Other steroidal compounds in serum can potentially interfere with the assay. Finally, the serum testosterone levels in men are influenced by diurnal variation and age.22 These factors make it difficult to translate the data from the research laboratory to clinical practice.21,22 Currently, collaborative initiatives are underway to develop universally standardized assays.21,22
Endocrine Society recommends that serum testosterone be measured in 3 samples taken on different days between 8:00 AM and 10:00 AM to account for diurnal variation and variations that can occur on even smaller time scales.22

Rapid automated radioimmunoassays (RIAs) and chemiluminescent (CL) immunoassays are the most commonly used assays in the clinical measurements of serum testosterone.22,23 Liquid chromatography-tandem mass spectrometry (LC/MS-MS) assays are more accurate at the lower androgen levels found in women, children, and hypogonadal men.22,23 Because of the greater sensitivity and decreased variability compared with RIA and CL assays (coefficient of variability [CV] upward of 40%), LC/MS-MS is advocated as the standard assay for men on ADT—large commercial laboratories have now adopted LC/MS-MS assays as the preferred test for hypogonadal men and men on ADT.9,22,24-26 Despite the reported increased sensitivity of LC/MS-MS assays, precision remains an issue, because the CV has been reported from 2.7% to 25.6% at a serum testosterone level of 8.5 ng/dL.27 The variability is influenced by several factors, including different assay tolerances, limitations in reference standards, and varied sample preparation techniques. The generally recognized range for serum testosterone levels in eugonadal men is 300-1000 ng/dL;23 however, the reference ranges vary by laboratory and the type of assay used. Thus, the serum testosterone assay results should be assessed within the context of the individual laboratories’ reference ranges.

DEFINING CASTRATE TESTOSTERONE LEVELS

Standardization of testosterone assays is needed to accurately characterize castrate testosterone levels. Historically defined by levels achieved with bilateral orchiectomy, the reference standard against which all ADTs are assessed, the threshold of 50 ng/dL was determined by older assays using a double-isotope dilution technique.28 However, when the serum testosterone levels in surgically castrated PCa patients were measured using the CL assay, a median testosterone level of 15 ng/dL (range <10-30 ng/dL) was determined.29 Assessments of serum testosterone levels in random samples sent to a national reference laboratory were concordant with those determined by the investigators. From this assessment, <20 ng/dL was suggested as the new definition for castrate levels.29

Subsequently, a review of published data reporting serum testosterone levels in surgically castrated patients revealed that median testosterone levels, when measured using RIA or CL immunoassays, ranged from 15 to 42 ng/dL.28 From these data, a 2005 expert panel issued the following consensus statement, “Using orchiectomy as the benchmark, achieving testosterone levels below/equal to 20 ng/dL after LHRH agonist therapy would be desirable.”30 Despite a continued regulatory definition of castration testosterone level as ≤50 ng/dL, some studies assessing ADT efficacy have set castration levels at ≤20 ng/dL.31,32 A review of the reported mean testosterone levels achieved with LHRH agonists, when measured using RIA or CL immunoassays, found a range of 5.0 to 29.5 ng/dL.28 In a randomized trial comparing the LHRH antagonist degarelix and the LHRH agonist leuprolide, the median serum testosterone levels during the last 11 months of the 12-month trial, as measured using LC/MS-MS, were 8 ng/dL in all treatment arms.33

ACHIEVING AND MAINTAINING CASTRATE TESTOSTERONE LEVELS WITH ADT

The ability of available LHRH agonists, the current mainstay of ADT,34 to achieve and maintain castrate testosterone levels, whether defined as ≤50 or ≤20 ng/dL, varies. A comprehensive review of the achievement of castrate testosterone levels after any ADT was beyond the scope of our report. Other reviews have stated that ≤17% of patients receiving LHRH agonists do not reach a testosterone level of ≤50 ng/dL, and 38% fail to achieve a serum testosterone level of ≤20 ng/dL.28,35 An analysis of the baseline characteristics of men with advanced PCa enrolled in 2 clinical trials with an inclusion criterion of serum testosterone <50 ng/dL found that 18.3% had serum testosterone levels >20 ng/dL.36 No significant differences between ADT types (including orchiectomy) and testosterone levels were observed. In a separate study of 138 patients undergoing ADT, the failure rate to achieve or maintain a serum testosterone level of <50 or <20 ng/dL was 11% and 46%, respectively.37

On initiation of LHRH agonist therapy, testosterone surges can occur from continuous pituitary stimulation and a resulting increase in LH levels, which will lead to an initial increase in testicular testosterone production.33,38 Testosterone surges can lead to clinical manifestations, or flares, such as bladder outlet obstruction or, in the presence of bone metastasis, bone pain.38 Testosterone surges can be managed with coadministration of nonsteroidal antiandrogens during the first 2 weeks of LHRH agonist therapy. These surges do not occur using LHRH antagonists or after bilateral orchiectomy.33,34 Acute-on-chronic responses reflect a testosterone surge after readministration of LHRH agonist due to an LH surge and have been noted in ≤10% of patients receiving standard LHRH agonist therapy.36 The effect of testosterone surges on clinical outcomes is not known.

Testosterone escapes or breakthroughs, defined as ≥1 testosterone value over castrate levels, can occur at any time and have been documented in ≤13% of patients receiving LHRH agonists at a testosterone threshold of 50 ng/dL.35,38 A recent database analysis reviewed 2290 patients treated with curative radiotherapy who received ≥3 months of LHRH agonist therapy and regular serum testosterone monitoring. The risk of testosterone break-
throughs >50, >32, or >20 ng/dL was 3.3%, 6.6%, and 26.8%, respectively. Morote et al followed 73 patients with advanced PCa receiving 3-month depot LHRH agonists for ≤48 months. Serum testosterone was measured prospectively using CL immunoassays every 6 months. The probability of future breakthroughs after the first 3 serum testosterone measurements is illustrated in Figure 1. Based on the achievement of a serum testosterone level <20 ng/dL, the probability of future breakthroughs decreased as the number of serum testosterone determinations at <20 ng/dL increased.

**CURRENT SOCIETY GUIDELINES AND RECOMMENDATIONS**

The current society guidelines regarding achieving and monitoring target serum testosterone levels in patients undergoing ADT are unclear. The 2012 NCCN guidelines define “adequate suppression” of serum testosterone as <50 ng/dL. Although the clinical benefits are not clear, the NCCN guidelines suggest that patients who do not achieve adequate suppression can be considered for additional hormonal manipulation. To date, the AUA has no guidelines regarding target testosterone levels or specific recommendations regarding serum testosterone monitoring in hormonally suppressed patients. Although the EAU does not provide a definitive castrate testosterone level, it recommends a serum testosterone measurement 3 months after initiating LHRH therapy and then at 6 months to ensure treatment efficacy and maintenance of castrate levels. An increase in PSA levels or the indication of clinical progression should trigger a testosterone level measurement in all cases to confirm CRPC.

**SHOULD WE STRIVE FOR ACHIEVING AND MAINTAINING THE LOWEST TESTOSTERONE LEVEL POSSIBLE?**

The lack of comprehensive guidelines and recommendations regarding the serum testosterone levels in patients with androgen-suppressed PCa reflects a lack of level I evidence to (a) define castrate serum testosterone levels and (b) demonstrate that achieving and maintaining testosterone levels below a set goal positively affects clinical outcomes. The variability between testosterone assays makes comparing the existing data difficult. Nonetheless, there is continued interest in establishing whether testosterone levels lower than those achieved by orchiectomy improve clinical outcomes. Original randomized studies comparing the effectiveness of bilateral orchiectomy and ADT demonstrated no overall survival benefit. New prospective studies assessing the correlation between testosterone levels during or after ADT and clinical outcomes are needed, because only a few studies have specifically addressed this question. When Morote et al prospectively followed 73 patients with nonmetastatic advanced PCa treated with continuous LHRH agonist therapy, they found that a serum testosterone breakthrough >50 ng/dL was an independent predictor of PSA progression (P = .008; hazard ratio 2.8), based on the first three 6-month testosterone measurements. Patients with testosterone breakthroughs >20 and between 20 and 50 ng/dL also had reduced survival free of progression. The lowest serum testosterone threshold able to significantly affect progression-free survival was 32 ng/dL (P = .0258). Patients with all 3 determinations of serum testosterone <32 ng/dL had a mean survival free of progression of 137 versus 88 months for those with any
ADT increased the risk of death (hazard ratio 1.33, months. A greater testosterone level at 6 months after were treated with a continuous LHRH agonist. Serum with newly diagnosed bone-only metastatic PCa who were treated with a continuous LHRH agonist. Serum testosterone and PSA levels were measured every 3 months. A greater testosterone level at 6 months after ADT increased the risk of death (hazard ratio 1.33, P < .05).44 A continuous relationship was found between the testosterone levels and cancer-specific survival. For similar pretreatment Gleason scores and 6-month PSA levels, the lower the 6-month testosterone level, the longer the survival. Despite the various limitations of these studies, their results suggest that achieving and maintaining testosterone at castrate levels or lower might improve clinical outcomes.

The reports by Morote et al13 and Perachino et al44 are not without their criticisms. Some have questioned the reliability of the conclusions drawn from the associations made with serum testosterone measurements, because the assay used, at least in the study by Morote et al,43 has a reported CV of 24.3% at 27.1 ng/dL (Immulite 2500 platform).40 Although the assay CV was concluded to not to be solely responsible for subsequent serum testosterone breakthroughs in men on ADT, the CV might still confound the results.

Greater androgen suppression can be achieved with complete androgen blockade (CAB). However, the individual trial results examining overall survival with CAB compared with LHRH agonist monotherapy have been conflicting.35 Any modest survival benefits offered by CAB are often offset by increased rates of adverse events and reduced quality of life.34 As such, the recommendations regarding the use of CAB have also been conflicting—the American Society of Clinical Oncology states that CAB should be considered for the initial hormonal management of metastatic, recurrent, or progressive PCa45 and the NCCN guidelines state that CAB provides no proven benefit over castration alone.41

Support for additional androgen suppression comes from the examination of CRPC tissue samples. Compared with noncancerous control tissues or primary PCa samples from eugonadal patients, the testosterone concentrations in metastatic CRPC tumors from castrate patients were up to fourfold greater (P < .0001), well within the range known to stimulate the AR and support PCa cell proliferation.46 Newer therapies that directly target androgen synthesis, such as abiraterone,47 might impede cell proliferation in PCa cells still responsive to androgens.

Perhaps the most interesting data regarding the benefits, or lack thereof, of maintaining continuous optimal androgen suppression have come from intermittent ADT (IADT) trials, in which patients’ testosterone levels were cycled between on-treatment and off-treatment periods. IADT was introduced on the basis that castration leads to the development of, and selection for, androgen-independent tumor cells and that intermittently halting ADT before the change to an androgen-independent phenotype occurs might restore the apoptotic potential and maintain androgen dependence.48 A systematic review of published trials found that IADT generally demonstrated comparable efficacy to continuous ADT with respect to a variety of outcomes, including biochemical progression, progression-free survival, and overall survival but with associated improved patient tolerability.38 Subsequent studies have found no differences in overall survival or adverse event rates (except for hot flashes) between IADT and continuous ADT39 and no difference in the interval to CRPC or death according to the achievement of castrate testosterone levels (<50 ng/dL).50 Although IADT may be non-inferior to continuous ADT for overall survival, the distribution of the causes of death differ significantly. Patients receiving IADT are more likely to die of PCa, and patients receiving continuous ADT are more likely to die of cardiovascular disease and other causes.49,51 Furthermore, the proportion of patients recovering normal testosterone levels in the IADT studies decreased with each new cycle.48 The significance of this phenomenon remains unclear.

Although continuous ADT and IADT might have comparable outcomes, in a comparison of 3 different dosing regimens (Fig. 2), Blumberg et al52 found that testosterone-based dosing was associated with a significantly lower risk of PSA progression (hazard ratio 0.65; P = .02) compared with continuous dosing. PSA-based dosing (traditional IADT) showed a trend toward a lower treatment failure risk but did not reach statistical significance (hazard ratio 0.80; P = .3).52 These data suggest that continuously maintaining the lowest testosterone level possible might not be effective in all patients; however, cycling between very low levels and at just castrate levels might optimize the apoptotic effects of androgen suppression without creating an intratumoral environment that facilitates the development of androgen-independent stem cells.

Finally, continuous optimal androgen suppression might increase the risk of development of dyslipidemia, bone loss and fractures, sexual dysfunction, depression, metabolic syndrome, type 2 diabetes, and cardiovascular disease and mortality induced by the hypogonadal state.13-15 Increased rates of adverse events have been noted for CAB versus monotherapy,34 and IADT might relieve certain hypogonadal-related side effects.48 Additional studies are still needed to correlate the rate of treatment-related adverse events with incremental levels of serum testosterone below castrate levels. To date, no study has prospectively measured the objective response by computerized tomography for patients receiving ADT.
CONSENSUS STATEMENT REGARDING MEASURING TESTOSTERONE LEVELS IN PATIENTS UNDERGOING ADT

Although some data have examined lower testosterone levels and clinical outcomes, no level I evidence is available to support the premise that lower serum testosterone levels correlate with improved clinical outcomes in patients receiving ADT. However, ample evidence has suggested that the standard surgical and medical forms of ADT routinely achieve testosterone levels far below the current bar of 50 ng/dL, a value derived from older assays proved unreliable at castrate testosterone levels. We suggest that 20 ng/dL be set as the bar to indicate successful testosterone suppression. From the evidence presented, a significant number of patients exists who exhibit testosterone breakthroughs during ADT to varying degrees, depending on the level at which one defines the castrate state.36,39,40 Men who exhibit breakthroughs might be more likely to have future breakthroughs and subsequent treatment failure.37 Our consensus surrounding serum testosterone measurements is as follows (Fig. 3):

1. Serum testosterone should be measured
   a. Before initiation of ADT
   b. Along with the first PSA measurement 2 months after ADT initiation
   c. Every 6 months or with each readministration of LHRH therapy, whichever is more frequent
   d. Whenever the PSA level increases
   e. When switching therapies

2. During ADT, serum testosterone measurements should be made using LC/MS-MS assays in accordance with the recommendations by the Endocrine Society22

3. Within an individual clinical practice, the serum testosterone assays should be consistently performed by the same clinical laboratory

4. Failure to achieve serum testosterone levels <50 ng/dL during ADT are clinically relevant and might affect the treatment outcome; consequently, clinical decisions to change treatment regimens should be made when the serum testosterone levels fails to decrease to the castrate range before reclassification of the disease as CRPC

FUTURE DIRECTIONS

The lack of level I evidence needed to correlate serum testosterone levels after ADT with clinical outcomes highlights the need for additional, preferably prospective, studies. Until testosterone assays outside of research settings can be harmonized, comparisons across studies and even the validity of data within studies will remain difficult to interpret. A lower definition for castrate serum testosterone levels is of little value if the variability in patients’ laboratory results reduces the accuracy and subsequent clinician interpretability.

Data emerging from clinical trials of abiraterone acetate and MDV3100 should provide additional information regarding the clinical consequences of ultralow serum testosterone levels. Abiraterone acetate, a potent inhibitor of CYP17, a key enzyme in androgen synthesis,47,53 suppresses serum testosterone to below-detectable levels,54 and in patients with metastatic CRPC after chemotherapy, provided significantly greater overall survival than placebo.47,53 MDV3100 is a second-generation antiandrogen that demonstrates greater binding affinity for AR than bicalutamide without demonstrating any
agonistic AR activity. Recent Phase III data have revealed that MDV3100 reduced the risk of death by 37% relative to placebo in patients with metastatic CRPC previously treated with docetaxel. Currently, both drugs are under evaluation in the setting of prechemotherapy metastatic CRPC. With new classes of drugs inhibiting testosterone biosynthesis, inhibiting conversion of testosterone to DHT, and selectively targeting AR function forthcoming, the ideal goal remains the selective lowering of intraprostatic and intratumoral androgen levels and inactivating subsequent AR signaling.

CONCLUSIONS
Testosterone remains an important clinical target in the treatment of advanced PCa, and its monitoring during ADT can modestly inform the clinician about the disease state or lack of therapeutic efficacy. However, key issues remain unresolved—the standardization of laboratory assays, universally accepted definition of castrate testosterone levels, deleterious side effects of prolonged ADT, and the lack of understanding around the relationships of testosterone, AR, and progression to CRPC. Serum testosterone remains an important biomarker in the castrate disease state; however, research continues to elucidate its mechanistic relationships to disease progression and possible prognostic value.

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References


