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lower testosterone levels with LHRH agonist therapy than with surgical castration: new insights attained by mass spectrometry

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ABSTRACT

PURPOSE. Androgen deprivation therapy by bilateral orchiectomy (surgical castration) or luteinizing hormone-releasing hormone (LHRH) agonist therapy (medical castration) is recommended for advanced or metastatic prostate cancer. Both methods aim at reducing serum testosterone concentrations to a castrate level which is currently defined as less than 50 ng/dL. The results of previous studies are based on testosterone immunoassays that have insufficient accuracy in the low range. In this study we reevaluated serum testosterone concentrations in men on androgen deprivation therapy using isotope dilution-liquid chromatography-tandem mass spectrometry (ID-LC-MS/MS), an accurate method of measuring testosterone in the castrate range.

MATERIALS AND METHODS. Subjects underwent surgical castration (34) or received a LHRH agonist (32). Serum samples were obtained more than 3 months after surgery or initiation of LHRH agonist therapy. Testosterone levels were determined using ID-LC-MS/MS. Dihydroepiandrosterone sulfate, androstenedione, sex hormone-binding globulin and inhibin B levels were determined.

RESULTS. All subjects had serum testosterone values less than 50 ng/dL and 97% had testosterone concentrations less than 20 ng/dL. Medically castrated men had significantly lower testosterone levels (median 4.0 ng/dL, range less than 2.9 to 20.2) than those surgically castrated (median 9.2 ng/dL, range less than 2.9 to 28.8, $p < 0.001$). No difference was found in dehydroepiandrosterone sulfate, androstenedione, and sex hormone-binding globulin levels between the groups, whereas inhibin B levels were significantly higher in the LHRH agonist treated group.

CONCLUSION. Using an accurate technique for testosterone measurement, subjects on LHRH agonist therapy had significantly lower testosterone concentrations than men who underwent surgical castration. The clinical relevance of these findings remains to be determined.

INTRODUCTION

The hormone dependency of prostate cancer was first demonstrated by Huggins and Hodges in 1941 [1]. The authors demonstrated that testosterone is the driving force for prostate cancer, and that surgical castration or administration of oral estrogens such as diethylstilbestrol causes a major decrease in prostatic tumor activity. After this discovery the standard treatment for advanced and metastasized prostate cancer was androgen deprivation therapy (ADT) by bilateral orchiectomy (surgical castration) or administration of estrogens (medical castration). Surgical castration was performed by total bilateral orchiectomy or a subcapsular technique as described by Riba [2]. Based on the interpretation of trials that have been conducted since the 1980s, it is usually assumed that luteinizing hormone-releasing hormone (LHRH) agonist therapy is equivalent to estrogen administration or surgical castration in patients with advanced

prostate cancer [3–6]. LHRH agonists are preferred to estrogens because of the less severe side effects [7]. In recent clinical guidelines, medical castration using a LHRH agonist is recommended in advanced or metastatic prostate cancer [8], whereas surgical castration is optional and assumed to be equally effective.

Monitoring of serum testosterone is warranted to evaluate treatment efficacy in men on ADT for advanced or metastatic prostate cancer. Generally, fully automated immunoassays are used for testosterone determination in serum. Although this technique has been proven to be reasonably accurate to estimate serum testosterone levels at male physiological ranges, it is notoriously inaccurate at castrate concentrations [9]. Ironically, Herold and Fitzgerald characterized the performance of automated immunoassays for testosterone measurement in the castrate ranges as an educated guess [10]. This and other findings led to the statement from the Endocrine Society and endorsing organizations that direct automated immunoassays jeopardize the health of patients whose medical care depends on accurate measurement of testosterone levels [11]. We recently reported on a method of serum testosterone determination using isotope dilution-liquid chromatography-tandem mass spectrometry (ID-LC-MS/MS) in female patients after bilateral oophorectomy [12]. In these women, with a low testosterone level, the measurements were proven to be precise. Therefore, ID-LC-MS/MS is preferred over commonly used automated immunoassays in low testosterone ranges because of improved accuracy [13,14]. In this study, we describe the results of serum testosterone measurement in men with advanced or metastatic prostate cancer on ADT and who are expected to have castrate levels of testosterone. We reevaluated testosterone levels in castrated men using a highly sensitive and specific ID-LC-MS/MS method and studied whether differences exist between the study groups.

EXPERIMENTAL SECTION

Study population. In this retrospective study, 66 subjects were included. In total, 34 subjects underwent surgical castration, 24 of whom because of advanced or metastatic prostate cancer. Ten patients with a gender identity disorder underwent radical orchiectomy as part of the gender transition. There were 32 men who received LHRH agonist by goserelin ($n = 21$), leuprorelin ($n = 10$) or buserelin ($n = 1$) (figure 1). Eight medically castrated and 5 surgically castrated patients received concomitant docetaxel based chemotherapy. None of the subjects were administered other hormonal therapies or any other medication that could interfere with the gonadal axis. Patients who participated in prostate cancer related drug trials were excluded from study. The patients receiving LHRH agonists were treated for at least 3 months before

entering this study. Samples from castrated subjects were drawn at least 3 months after surgery.

Serum testosterone determination. A single venous blood sample was collected randomly during the day from each subject. Serum was aliquoted and stored at -200C until assayed. Measurements were performed at the Endocrine Laboratory, VU University Medical Center (CCKL accredited). Serum total testosterone was determined by the Coat-a-Count® total testosterone radioimmunoassay (Siemens). The assay has a limit of quantification (LOQ) of 28.8 ng/dL. The intra-assay coefficient of variation (CV) at 57.6 ng/dL is 8%, and the inter-assay CVs at 17.3 and 57.6 ng/dL are 20% and 10%, respectively. In addition, serum total testosterone was measured by ID-LC-MS/MS. Sample preparations were performed as described by Bui et al.[12] A stable deuterated internal standard (testosterone-2,2,4,6,6-D₅; obtained from CDN Isotopes, Pointe-Claire, Quebec, Canada) was added to every specimen. Testosterone was extracted (hexane/ether) and derivatized with methoxylamine hydrochloride. Samples were cleaned up and injected on LC-MS/MS (Waters Quattro Premier™ XE) (LOQ 2.9 ng/dL, intra-assay and inter-assay CV at levels less than 28.8 ng/dL were less than 5% and less than 13%, respectively). The ID-LC-MS/MS method was compared to the reference method isotope dilution-gas chromatography-mass spectrometry [14] using 40 serum samples ranging from 5.7 to 703 ng/dL. Deming regression analysis showed a slope of 1.00, intercept of -0.05 nmol/L and R² of 1.000.

Other parameters. Androstenedione was measured by a radioimmunoassay (RIA)(DSL, Webster, Texas) which featured a LOQ of 0.5 nmol/L. Intra-assay and inter-assay CV for levels greater than 6 nmol/L were 6% and 9%, respectively, and for levels less than 6 nmol/L were 8% and 12%, respectively. Radioimmunoassay was also used for dehydroepiandrosterone sulfate (DHEAS). The LOQ was 0.2 μmol/L. Intra-assay and inter-assay variation at 3 μmol/L was 6% and 10%, respectively, and at 10 μmol/L was 4% and 9%, respectively. Inhibin B was measured by an immunometric assay (Serotec Limited, Oxford, United Kingdom). LOQ was 15 ng/L; intra-assay CV at 3 levels (15, 50, and 100 ng/L) was 30%, 11% and 5%, respectively; and inter-assay CV at 3 levels (35, 65, and 165 ng/L) was 15%, 11% and 9%, respectively. An immunometric assay on an Immulite® 2500 was used to determine the sex hormone-binding globulin (SHBG) concentration. The LOQ for SHBG was 2 nmol/L, the intra-assay and inter-assay CV for the whole range was less than 3% and 4%, respectively.

Statistics. Statistical analysis was done using SPSS® 15.0. Statistical analysis of groups was performed using the Mann-Whitney U test. The median and 95% confidence intervals for testosterone, other steroid hormones, and SHBG in this study population were calculated.

RESULTS

Patient characteristics are listed in table 1. There were no significant differences between the groups in terms of clinical and tumor characteristics, nor were there differences between the groups in prostate specific antigen (PSA) level or hormonal status of the tumor. For all evaluated patients, testosterone values determined using radioimmunoassay (RIA) were less than 50 ng/dL. Using ID-LC-MS/MS, patients treated with a LHRH agonist showed a median serum testosterone concentration of 4.0 ng/dL (range less than 2.9 to 20.2) vs 9.2 ng/dL (range less than 2.9 to 28.8) for those surgically castrated ($p < 0.001$, figure 2). All surgically and medically castrated men had serum testosterone levels less than 50 ng/dL, and 31 (97%) and 33 (97%) had levels less than 20 ng/dL, respectively. There were no significant differences between the groups in SHBG, DHEAS and androstenedione levels. Patients who underwent surgical castration had inhibin B levels below the limit of quantification compared to normal levels in the subjects who underwent medical castration (table 1).

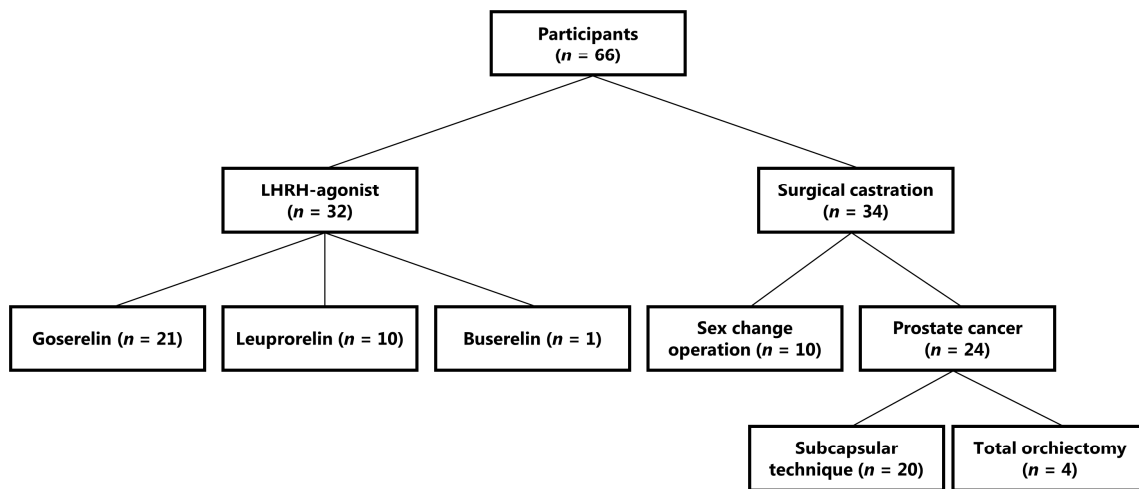


FIGURE 1: Patient treatment modality.

DISCUSSION

ADT by bilateral orchiectomy or LHRH agonist therapy aims at decreasing serum testosterone to a castrate level to halt prostate cancer growth and progression, reduce signs and symptoms of advanced or disseminated disease, and lengthen the lives of those affected. Although all forms of ADT decrease serum androgen levels, the

mechanism of action differs substantially. Bilateral orchiectomy surgically eliminates the primary testosterone producing organs (the testes), whereas LHRH agonist therapy acts by suppressing production of gonadotropins such as luteinizing hormone (LH), resulting in hypogonadotropic hypogonadism. Thus, the testosterone producing (Leydig) cells within the testes are deprived of their stimulating hormones.

TABLE 1: Patient characteristics and serum hormone levels.

	Bilateral orchiectomy (n = 34)	LHRH-agonist (n = 32)	p-value *
Mean age (range)	71.6 (58.0–86.8)	73.0 (57.3–89.6)	not significant
Hormone naive cancer (%)*	10/24 (41.7)	16/32 (50.0)	not significant
Castration resistant prostate cancer (%)*	14/24 (58.3)	16/32 (50.0)	not significant
Metastatic disease (%)*	4/24 (16.7)	7/32 (21.9)	not significant
Median PSA (ng/mL) (range)	6.4 (0.6–67)	24 (0.1–2802)	not significant
Concomitant use of docetaxel (%)*	5/24 (20.8)	8/32 (25.0)	not significant
Serum testosterone by RIA (ng/dL)	< 28.8	< 28.8	not significant
Median serum testosterone by ID-LC-MS/MS (ng/dL) (range)	9.2 (< 2.9–28.8)	4.0 (< 2.9–20.2)	< 0.001
Median SHBG (nmol/L) (range)	43.2 (20.0–126.0)	43.6 (15.0–75.0)	not significant
Median androstenedione (nmol/L) (range)	2.4 (0.8–8.3)	2.3 (< 0.5–7.4)	not significant
Median DHEAS (μmol/L) (range)	1.4 (0.2–7.1)	1.5 (< 0.2–4.9)	not significant
Median Inhibin B (ng/L) (range)	< 15 (< 15)	22 (< 15–193)	< 0.001

* Cancer specific characteristics in the bilateral orchiectomy group only apply to 24 subjects who underwent castration because of prostate cancer.

In the present study, we assessed testosterone levels in men on ADT by bilateral orchiectomy or LHRH agonist therapy. Our data show that all men in both groups had castrate levels of testosterone using a cutoff of 50 ng/dL as measured by immunoassay. These observations corroborate previous studies [15–17]. When measuring testosterone using ID-LC-MS/MS, testosterone levels were even less than 20 ng/dL in 97% of cases. Remarkably, testosterone levels were significantly lower in men on LHRH agonist therapy than in those treated with bilateral orchiectomy. Analysis after the exclusion of transgender patients and/or subcapsular orchiectomy still demonstrated a significant difference ($p < 0.004$, data not shown). This difference could not be detected using the immunoassay because of the known inadequate sensitivity and specificity in this range. For testosterone measurements in patients undergoing ADT, the ID-LC-MS/MS method is superior to analysis by immunoassays, and this finding (including our recommendations) has also been extensively reviewed by our research group [14].

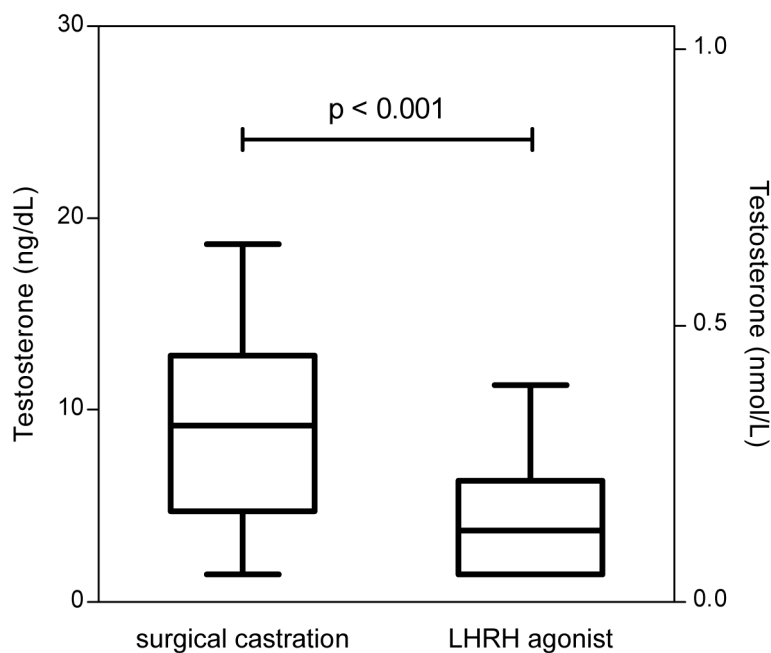


FIGURE 2: Box plot showing serum testosterone levels in patients after orchiectomy ($n = 34$) and after LHRH agonist therapy ($n = 32$) using ID-LC-MS/MS. Upper and lower quartiles are represented by rectangle, and maximum and minimum observed values are represented by whiskers. Median value for surgical castration was 9.2 ng/dL and median value for LHRH agonist was 4.0 ng/dL ($p < 0.001$).

There are several possible explanations for the higher testosterone concentrations found in patients treated by bilateral orchiectomy. First, testosterone may be produced by remaining Leydig cells in the residual testes after subcapsular orchiectomy. Indeed, in 1959, McDonald and Calams cast doubt on the efficacy of subcapsular orchiectomy to remove all testicular parenchyma [18]. Using an immunoassay, they showed that testosterone concentrations in subjects after radical orchiectomy were equal to the levels in men after subcapsular orchiectomy [18]. However, the finding in our study that the inhibin B level, as a marker of functional testicular tissue, was below the limit

of quantification in all surgically treated patients confirms the improbability of remaining Leydig cells. Second, it is unlikely that LHRH agonist therapy or surgical castration influences the hypothalamic-pituitary-adrenal axis and, thus, influences the adrenal production of testosterone or its precursors. Levels of androstenedione and DHEAS were not significantly different between the treatment groups in our study. This finding also indicates that the difference in testosterone levels is not explained by extratesticular conversion of adrenal androgens into testosterone. Finally, in the surgical castration group it is expected that the concentration of LH is increased due to the feedback mechanisms in the pituitary-testicular axis. One may argue that prostate cancer tissue might be stimulated by circulating LH. LH and LHRH receptors have indeed been identified in prostate cancer cells, and it has been reported that LH mediated activation of the LH receptor significantly up regulates the expression of genes and enzymes required for steroidogenesis and increases steroid production [19,20]. Thus, this process might result in higher testosterone levels in surgically castrated men.

It remains to be established whether the reported difference in testosterone concentration between those surgically vs medically castrated is clinically relevant, and if it could translate into a biochemical and clinical benefit. Previous studies comparing the outcomes among the various forms of ADT reported no survival advantage when surgical castration and LHRH agonist therapy were compared [3,4]. However, the outcome of these comparative studies should be interpreted with caution as the British and United States prostate study groups were statistically underpowered to show a relatively small difference in survival. Furthermore, our study on testosterone levels in the castrate testosterone ranges raises questions regarding the equality and interchangeability of different forms of ADT [21]. It might be assumed that differences in testosterone levels in the castrate range exist among different LHRH agonists, or among the different monthly depots of LHRH agonists. These questions will be addressed in further research efforts from our group.

Despite the importance of the gonadal axis in the natural history and the treatment of patients with prostate cancer, current European and United States guidelines provide few recommendations on the indications for and the timing of testosterone measurement [8]. In a survey of practices of oncologists and urologists, only 71% indicated that they monitor the testosterone level in their patients with prostate cancer [22]. This is striking because decreasing serum testosterone is the main goal of ADT, whereas serum PSA and alkaline phosphatase only act as surrogates for treatment efficacy [23]. It has been suggested that the testosterone level achieved with ADT is directly related to the risk of death in men with metastatic prostate cancer [24].

Recently, an expert panel refocused on the testosterone issue and formulated practical recommendations for testosterone measurement during ADT. Testosterone should be

monitored every 3 months in patients starting on ADT to verify the efficacy of treatment. Preferably the determination should be timed just before an injection of a LHRH agonist [25]. After the target level of testosterone has been reached on 3 consecutive measurements, the frequency of testosterone monitoring may be ceased or reduced. In addition, if PSA increases while on ADT, castration levels of testosterone must be demonstrated before castration resistant disease can be assumed. Finally, future prospective studies should further evaluate the potential relevance of testosterone measurement as an independent assessment of prognosis and treatment decision in different stages of the disease.

Despite these recommendations, the target castrate testosterone level in patients on ADT remains to be properly defined. In fact, different definitions of the castrate level of testosterone have been proposed based on detection limits of available assays [26–29]. Whereas previously established testosterone castrate levels were based on commonly used assays of testosterone measurement (less than 50 ng/dL), it has recently been suggested to decrease the castration level to less than 20 ng/dL [29]. The recommendation to decrease the castrate testosterone level to less than 20 ng/dL was prompted by the availability of more accurate assays of serum testosterone determination and the fact that a substantial percentage of men on ADT appeared to have testosterone levels greater than 20 ng/dL [30]. Despite the advantages in methodology, the recommended target level during ADT has not been adopted in the current guidelines of the European Association of Urology and the American Urological Association [8].

CONCLUSION

We observed that all men on ADT had castration levels of serum testosterone (less than 50 ng/dL), and concentrations reached less than 20 ng/dL in 97% of all cases. Therefore, the target testosterone value in patients on ADT could be adjusted to less than 20 ng/dL. However, the interpretation of testosterone levels should be approached with caution if less accurate methods are used. Using ID-LC-MS/MS a difference in testosterone concentrations between surgically and medically castrated patients could be detected which would have gone unnoticed by immunoassay. Patients on LHRH agonist therapy had significantly lower testosterone concentrations than men who underwent surgical castration ($p < 0.001$). Differences could not be explained by residual testicular tissue in surgically treated patients or by differences in the levels of circulating adrenal androgens.

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