Original Study

Serum Testosterone Levels in Prostate Cancer Patients Undergoing Luteinizing Hormone-Releasing Hormone Agonist Therapy

Juan Morote,[1] Inma Comas,[2] Jacques Planas,[1] Xavier Maldonado,[3] Ana Celma,[3] José Placer,[3] Roser Ferrer,[2] Joan Carles,[4] Lucas Regis[1]

Abstract

Prostate cancer guidelines have recommended serum testosterone measurement during androgen deprivation therapy to assess its efficacy and diagnose castration resistance. The present study compared the testosterone levels from a widely used chemiluminescent assay (CLIA) and liquid chromatography tandem mass spectrometry method, which is the recommended method, in prostate cancer patients undergoing luteinizing hormone-releasing hormone agonist therapy. The CLIA overestimated the testosterone levels and suggested, incorrectly, the presence of inadequate castration in ≤ 15% of patients.

Background: Serum testosterone measurement is recommended to assess the efficacy of androgen deprivation therapy (ADT) and to diagnose castration resistance in patients with prostate cancer (PCa). Currently, the accepted castrate level of serum testosterone is 50 ng/dL. Liquid chromatography and tandem mass spectrometry (LC MSMS) is the appropriate method to measure testosterone, especially at low levels. However, worldwide, chemiluminescent assays (CLIAs) are used in clinical laboratories, despite their lack of accuracy and reproducibility, because they are automatable, fast, sensitive, and inexpensive. **Materials and Methods:** We compared serum testosterone levels measured using LC MSMS and CLIAs in 126 patients with PCa undergoing luteinizing hormone-releasing hormone (LHRH) agonist therapy. **Results:** The median serum testosterone level was 14.0 ng/dL (range, 2.0-67.0 ng/dL) with LC MSMS and 31.9 ng/dL (range, 10.0-91.6 ng/dL) with CLIA ($P < .001$). The serum testosterone levels, measured using LC MSMS, were < 20 ng/dL in 83 patients (65.9%), 20 to 50 ng/dL in 40 (31.7%), and > 50 ng/dL in 3 patients (2.4%). These ranges were found in 34 (27%), 72 (57.1%), and 20 (15.9%) patients when testosterone was measured using CLIA ($P < .001$). The castrate level of serum testosterone using LC MSMS and CLIA was 39.8 ng/dL (95% confidence interval [CI], 37.1-43.4 ng/dL) and 66.5 ng/dL (95% CI, 62.3-71.2 ng/dL), respectively. **Conclusion:** We found that CLIA overestimated the testosterone levels in PCa patients undergoing LHRH agonist therapy. Thus, the castration level was incorrectly considered inadequate with CLIA in almost 15% of patients. The true castration level of serum testosterone using an appropriate method is < 50 ng/dL.

Clinical Genitourinary Cancer, Vol. ■, No. ■, ■-■

Keywords: Androgen deprivation therapy, Chemiluminescence, LHRH agonist treatment, Mass spectrometry, Testosterone

Introduction

Prostate cancer (PCa) guidelines have recommended serum testosterone measurement during androgen deprivation therapy (ADT) to assess its efficacy and define castration resistance. Although different studies have highlighted better outcomes for patients with lower testosterone levels, regulatory authorities have considered the castration level of serum testosterone to be ≤ 50 ng/dL.[1]

During the 1980s, the Food and Drug Administration established the castration level of serum testosterone as ≤ 50 ng/dL. However, testosterone testing was limited at that time. This definition arose from previous studies of PCa patients who had

[1]Department of Urology
[2]Department of Biochemistry
[3]Department of Radiation Oncology
[4]Department of Medical Oncology, Vall d'Hebron Hospital, Universitat Autònoma de Barcelona, Barcelona, Spain

Submitted: Aug 7, 2017; Revised: Oct 18, 2017; Accepted: Oct 30, 2017

Address for correspondence: Lucas Regis, PhD, Department of Urology, Vall d'Hebron Hospital, Universitat Autònoma de Barcelona, PO Box Vall d'Hebron 119-129, Barcelona 08035, Spain
E-mail contact: lregis@vhebron.net

1558-7673/$ - see frontmatter
https://doi.org/10.1016/j.clgc.2017.10.025

undergone surgical castration. Also, this threshold corresponded to the lowest limit of quantification using the radioimmunoassays available at that time.[2] In 2000, using a recently introduced chemiluminescent assay (CLIA), Oefelein et al[3] redefined the castration level of serum testosterone to 20 ng/dL from a group of 35 PCa patients who had undergone surgical castration. The serum testosterone levels in those patients ranged from 5 to 30 ng/dL (median, 15 ng/dL). However, the castration level they selected corresponded to the 75th percentile of the distribution.[3] In 2007, Morote et al,[4] also using a CLIA, reported 32 ng/dL as the castration level of testosterone with clinical impact. It represented the lowest level of serum testosterone capable of significantly discriminating survival free of castration resistance in a group of 73 nonmetastatic PCa patients who had undergone luteinizing hormone-releasing hormone (LHRH) agonist therapy.[4]

Currently, CLIAs are widely used in clinical practice to measure serum testosterone given that they are automatable, sensitive, fast, and inexpensive.[5,6] However, CLIAs have a disturbing lack of accuracy and reproducibility, especially at low testosterone levels.[5,6] This was the reason that in 2007, the American Endocrine Society and the Centers for Disease Control and Prevention recommended LC MSMS as the only appropriate method for serum testosterone testing, mainly for children and women.[7,8] This method extracts testosterone in the first step, avoiding its overdetection caused by contamination with other steroids. In the second step, MSMS ensures an accurate and reproducible measurement of testosterone by identification of its chemical structure.[8]

The method used to measure testosterone is crucial when assessing the efficacy of ADT to identify which patients have achieved adequate castration levels when biochemical or clinical progression has been detected. Owing to the lack of studies analyzing the influence of the method used on the serum testosterone levels in PCa patients undergoing LHRH agonist therapy, we decided to perform the present study. Our objectives were (1) to compare and correlate the serum testosterone levels measured with a CLIA in widespread use and LC MSMS in our reference laboratory in patients with PCa receiving LHRH agonist therapy; (2) to analyze the number of patients with a serum testosterone level < 50 ng/dL, stratified by the measurement method used; and (3) to estimate the true castration level of serum testosterone using the LC MSMS measurement.

Materials and Methods

Our institutional ethical committee approved the present study in January 2016 (approval no. PR046/2016). The study included 126 consecutive patients with histologically confirmed PCa who were undergoing continuous LHRH agonist therapy as monotherapy or as neoadjuvant treatment before radiation therapy. All the patients had received the LHRH agonist for > 3 months, and all had received bicalutamide for 2 weeks before and after the first LHRH agonist administration. Serum testosterone measurements were requested as a part of the routine follow-up protocol from February 1 to June 30, 2016, during the LHRH agonist treatment period of 4 to 48 months. The clinical characteristics of the study cohort are listed in Table 1.

Blood was extracted between 8:00 and 10:00 AM, and 2 aliquots of 1 mL of serum were prepared to measure serum testosterone using CLIA and LC MSMS. The CLIA measurement was performed in our reference laboratory using an automated platform (Advia Centaur XPi; Siemens Inc, New York, NY). The measurement range is 10 to 1500 ng/dL. The intra-assay coefficient of variation ranges from 2.3% to 6.2%, and the interassay coefficient of variation ranges from 1.4% to 4.4% (manufacturer information: 10629910_ES Rev. U, 2014-08, 1-18). The second aliquot of serum was stored at $-80°C$, after solid phase extraction, and LC MSMS was performed in an external and qualified laboratory during the week of July 16. This method uses ultra-high-pressure liquid chromatography with the 1290 Infinity Binary LC System (Agilent Technologies, Santa Clara, CA). The system is connected in parallel to tandem mass spectrometry using the 6430 Series Tripe Quadruple LC/MS System (Agilent Technologies). The measurement ranged from 2 to 1500 ng/dL. The intra-assay coefficient of variation ranges from 4% to 5%, and the interassay coefficient of variation ranges from 7% to 8% for testosterone levels ≤ 50 ng/dL.[9]

Table 1 Patient Characteristics

Characteristic	n (%)
Patients	126 (100.0)
Age (y)	
Median	73
Range	48-86
Indication for LHRH agonist	
Metastatic disease	45 (35.7)
Neoadjuvant to radiation therapy	59 (46.8)
PSA failure after primary treatment	22 (17.5)
Duration of LHRH agonist (mo)	
Median	18
Range	4-48

Abbreviations: LHRH = luteinizing hormone-releasing hormone; PSA = prostate-specific antigen.

Statistical Analysis

We began the statistical analysis by assessing the type of serum testosterone distribution using the Kolmogorov-Smirnov test. We used the Mann-Whitney *U* test to compare the testosterone levels measured using the CLIA and LC MSMS. Thereafter, the testosterone distribution was analyzed according to the frequently used intervals[1-4] of < 20 ng/dL, 20 to 50 ng/dL, and < 50 ng/dL. These distributions were compared using the Kruskal-Wallis test. Finally, owing to absence of a kurtosis distribution for both testosterone measurements, the castration levels were assessed from the 95th percentile of the distributions after logarithmic transformation.[10] SPSS, version 20, was used to perform the statistical analysis.

Results

Neither distribution of the serum testosterone levels, measured using CLIA and LC MSMS, followed the normal curve ($P < .001$; Figure 1). Boxplots of the testosterone levels are presented in Figure 2. The mean value of testosterone measured using CLIA and LC MSMS was 34.2 ng/dL (95% confidence interval [CI], 31.1-37.2 ng/dL) and 17.9 ng/dL (95% CI, 15.8-20.1 ng/dL),