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Brief Correspondence

Serum Luteinizing Hormone Testing Can Identify Optimal Medical Castration

Juan Morote^{a,b,c}, Imma Comas^d, Roser Ferrer^d, Lucas Regis^{a,b}, Anna Celma^{a,b}, Anna Santamaría^b, Jacques Planas^{a,b,*}, Enrique Trilla^{a,b,c}

Prostate cancer (PCa) guidelines recommend serum testosterone testing to assess the efficacy of castration and to define castration resistance when biochemical or clinical progression is detected. The currently accepted serum testosterone castrate level is 50 ng/dl, although levels below 20 ng/dl have been associated with longer castration resistance–free survival [1]. This castrate level of serum testosterone was established during the 1980s by the US Food and Drug Administration to evaluate products for castration. It arose from previous studies on PCa patients who had undergone surgical castration and corresponded to the lowest sensitivity level of the radioimmunoassays available [2].

In 2007, on the basis of previous studies [3,4], the American Endocrine Society and the Centers for Disease Control and Prevention recommended only methods based on mass spectrometry to measure testosterone, especially for the low levels detected in women and children [5]. In 2016, we highlighted that chemiluminescence was used in all clinical studies analyzing the prognostic value of serum testosterone. Chemiluminiscent assays are the methods most used in clinical laboratories owing to high sensitivity, automatability, high speed, and low cost; however, their lack of accuracy and reproducibility at low levels make these results uncertain [6]. We have observed discrepancies in serum testosterone results from different chemiluminiscent assays [7] and we have suggested mass spectrometry as the appropriate method for measuring serum testosterone in PCa patients undergoing medical castration [8]. Finally, we have observed that serum luteinizing hormone (SLH) is better associated with medical castration activity than serum testosterone is, even when measured with an appropriate method. We established a castrate level of SLH as 1.10 UI/l [9]. SLH has also been used to monitor the switch between luteinizing hormone-releasing hormone (LH-RH) antagonist and agonist [10]. The aim of the present study was to analyze the

association between SLH and serum testosterone, measured with mass spectrometry and chemiluminescence, in PCa patients undergoing medical castration and to determine if SLH can define optimal castration.

We prospectively measured serum testosterone with mass spectrometry and chemiluminescence in 138 frozen serum samples (–80 °C) selected among PCa patients undergoing continuous medical castration with a 3-month depot LH-RH agonist. We used a Chromsystems reagent kit for the Shimadzu liquid chromatography/mass spectrometry 8050 system (Izasa Scientific) with a sensitivity limit of 0.5 ng/dl and an Atellica Solutions chemiluminiscent assay (Siemens Inc.) with a sensitivity limit of 7.0 ng/dl for measurement of serum testosterone. Selected serum samples had a previous LH measurement within the castration range. LH was measured using the Advia-Centaur XPI chemiluminiscent assay (Siemens Inc.) with a sensitivity limit of 0.12 UI/l. We randomly selected 69 serum samples with LH ≤0.12 UI/l and 69 with SLH between >0.12 and 1.10 UI/l. The decision to use this threshold was based on our previous study, in which approximately 75% of patients on active LH-RH agonist treatment had SLH ≤0.12 UI/l [8]. Optimal castration was defined as serum testosterone <20 ng/dl [6]. The study was approved by our ethics committee (PR/AG 048/2016). Associations were analyzed using the median test between the SLH interval and quantitative serum testosterone measurements, and the McNemar test between the SLH interval and semi-quantitative serum testosterone. SPSS v.20 was used for these analyses.

The median age was 72 yr (interquartile range [IQR] 63–78) for men with SLH ≤0.12 UI/l and 73 yr (IQR 66–80) for those with SLH >0.12 UI/l ($p = 0.136$). For the groups with SLH ≤0.12 versus >12 UI/l, serum testosterone measured with mass spectrometry was 9.0 ng/dl (IQR 3.7–17.5) versus 12.0 ng/dl (IQR 7.9–21.4; $p = 0.028$), and serum testosterone

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Table 1 – Analysis of the association between ST levels and SLH by ST measurement method.

Method	Median ST, ng/dl (interquartile range)			p value
	All patients	SLH ≤ 0.12 UI/l	SLH 0.13–1.10 UI/l	
LC-MSMS	11.1 (5.9–19.8)	9.0 (3.7–17.5)	12.0 (7.9–21.4)	0.028
Chemiluminiscence	17.4 (10.0–32.0)	17.6 (7.0–39.8)	17.2 (13.0–24.1)	0.918

LC-MSMS = liquid chromatography–tandem mass spectrometry; SLH = serum luteinizing hormone; ST = serum testosterone.

Table 2 – Distribution of patients with ST <20 ng/dl by SLH interval and ST measurement method.

ST method	Patients with ST <20 ng/dl, n (%)		p value
	SLH ≤ 0.12 UI/l	SLH 0.13–1.10 UI/l	
LC-MSMS	54 (78.3)	15 (21.7)	<0.001
Chemiluminiscence	37 (53.6)	32 (46.4)	0.295

LC-MSMS = liquid chromatography–tandem mass spectrometry; SLH = serum luteinizing hormone; ST = serum testosterone.

measured with chemiluminiscence was 17.6 ng/dl (IQR 7.0–39.8) versus 17.2 ng/dl (IQR 13.0–24.1; $p = 0.918$; Table 1). The distribution of serum testosterone results measured with both methods is presented by SLH interval in Table 2. For testosterone measured with mass spectrometry, 78.3% of patients with SLH ≤ 0.12 UI/l and 21.7% with SLH > 0.12 UI/l had serum testosterone <20 ng/dl ($p = 0.001$). For testosterone measured with chemiluminiscence, the corresponding rates with testosterone <20 ng/dl were 53.6% and 46.4% ($p = 0.295$). No patients had serum testosterone >50 ng/dl when measured with mass spectrometry, while five patients (3.6%) had serum testosterone >50 ng/dl when measured with chemiluminiscence, of whom three (4.3%) had SLH ≤ 0.12 UI/l and two (2.9%) had SLH > 0.12 UI/l.

We observed a significant association between SLH and serum testosterone only when it was measured with mass spectrometry. Moreover, the proportion of patients with serum testosterone <20 ng/dl was significantly higher in the group with SLH ≤ 0.12 UI/l (78.3%). This proportion is similar to the 75% observed in our previous study, in which we concluded that SLH was more efficient than serum testosterone in identifying patients with active medical castration [9]. These are the reasons why we proposed SLH testing for assessing the efficacy of medical castration and defining castration resistance [11].

The present study is limited by its retrospective design and the lack of randomized selection of frozen serum samples. The SLH threshold of 0.12 UI/l was based on our previous results, according to which 75% of patients on medical castration therapy had SLH below the lowest sensitivity level. The size of the study for statistical power was not calculated owing to the absence of previous data analyzing the association between SLH and serum testosterone measured with mass spectrometry.

In contrast to testosterone measurement, chemiluminiscence is an appropriate method for measuring low

levels of SLH, especially after immunoassay calibration according to the World Health Organization international standard for human pituitary luteinizing hormone (WHO/BS/2014.2240). This difference in immunoassay testing between testosterone and SLH may be due to the specific chemical structure of LH, in contrast to the similar chemical structure of testosterone and many other steroids [12].

We conclude that in patients with PCa undergoing medical castration, SLH is significantly associated with serum testosterone if it is appropriately measured. We found that SLH ≤ 0.12 UI/l was associated with optimal castration, defined as serum testosterone <20 ng/dl. Future studies should verify the hypothesis that SLH may be better at predicting castration resistance–free survival than serum testosterone is.

Author contributions: Jacques Planas had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Morote, Comas, Ferrer.

Acquisition of data: Celma, Planas, Regis, Comas, Ferrer.

Analysis and interpretation of data: Morote, Comas, Ferrer.

Drafting of the manuscript: Morote.

Critical revision of the manuscript for important intellectual content: Trilla, Santamaría.

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^aDepartment of Urology, Vall d'Hebron Hospital, Barcelona, Spain

^bUrology Research Unit, Vall d'Hebron Research Institute, Barcelona, Spain

^cUniversitat Autònoma de Barcelona, Barcelona, Spain

^dDepartment of Biochemistry, Vall d'Hebron Hospital, Barcelona, Spain

*Corresponding author. Department of Urology, Vall d'Hebron Hospital, Paseig Vall d'Hebron 119–129, 08035, Barcelona, Spain.

Tel.: +34 93 2746100; Fax: +34 932746058.

E-mail address: jplanas@vhebron.net (J. Planas).