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Determined Free Serum Testosterone is Better than Total Testosterone as a Predictor of Prostate Cancer Risk

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Abstract: *Background: Circulating testosterone remains the main source of androgens, however only 1%-2% circulates as free testosterone (fT), hormone active form. To compare the performance of serum fT and total testosterone (tT) as predictors of prostate cancer (PCa) detection and aggressiveness.*

Methods: Serum fT (RIA, pg/mL) and tT (LC/MS, ng/dL) were prospectively determined in 3364 consecutive men with PCa suspicion scheduled to TRUS guided biopsy. Tumor aggressiveness was assessed by Gleason grade (8 to 10) and D´Amico risk (cT3a+ or PSA>20 ng/mL or Gleason 8 to 10). Recodification of fT and tT in four groups according to percentile (ptile) distribution were done. tT/1 (min to 10 ptile): 62-291 ng/dl, tT/2

(ptile 10.1 to 50): 291,1 to 451 ng/dl, tT/3 (ptile 50.1 to 90): 451,1 to 676 ng/dl, and tT/4 (ptile 90.1 to max): 676.1 to 1440 ng/dl. fT/1: 0,1 to 0,85, fT/2: 0,86 to 5.20, fT/3: 5,21 to 9.65, and fT/4: 9.66 to 18 pg/ml.

Results: PCa detection rates were for tT/1: 43.8%, tT/2: 40.6%, tT/3: 35.6%, and tT/4: 31.7%, p=0.0001. For fT/1: 48.0%, fT/2: 41.0%, fT/3: 34.8%, and fT/4: 28.6%, p=0.0001. Logistic regression analysis shown fT and tT as independent predictors of PCa detection, respectively OR= 0.857 (95%CI: 0.769-0.956), p= 0.006, and OR= 0.792 (95%CI: 0.709-0.883), p=0.0001.

Conclusions: Serum fT and tT were independent predictors of PCa detection. fT has better performance than tT to predict the PCa risk, but none of them were related to tumor aggressiveness.

Impact: Free testosterone could compose a nomogram to improve PCa detection.

Keywords: *prostate cancer, serum testosterone, free testosterone, prediction, diagnosis.*

1. INTRODUCTION

Prostate cancer (PCa) is the most common cancer in elderly males in Europe. The incidence is highest in Northern and Western Europe (> 200 per 100,000), while rates in Eastern and Southern Europe have showed a continuous increase [1] . For more than seventy years, it has been widely accepted that PCa growth is dependent on serum testosterone concentrations, based on experiments by Huggins et al. that castration caused PCa regression, whereas testosterone administration caused more rapid PCa growth [2]. Although from an epidemiological point of view, this relationship had been questioned in view of the fact that serum testosterone level decreases with age [3], while the prevalence of PCa increases substantially. Moreover, an association between circulating androgens and PCa has not been clearly confirmed in epidemiologic studies [4, 5].

Circulating testosterone remains the main source of androgens for PCa cells. Up to 95% of testosterone is produced by the Leydig cells in the testes in response to luteinizing hormone (LH) release from the anterior pituitary while the remainder of testosterone is derived from the adrenal glands [6]. However, these values largely reflect the concentration of testosterone bound to plasma proteins, including sex hormone binding globulin (SHBG) and albumin. About 30%-44% of total testosterone (tT) is bound to SHGB and it is considered to be unavailable for androgenic signaling. Only about 1%-2% circulates as free testosterone (fT), constituting the active form of the hormone able to diffuse into cells and bind to androgen receptors. This theory has been largely accepted as the free hormone hypothesis [7]. The remaining testosterone is loosely bound to albumin in a reversible fashion and may be biologically available in some tissues [8]. During the last few years some studies have reported an association of low serum testosterone levels with an increase in the risk of prostate cancer, more aggressive tumors and worse survival rates [9-14]. Most of the articles that failed to find a significant association between testosterone and risk of PCa and/or its aggressiveness assumed serum tT value as the main representation of the androgenic activity [15-17]. Alternatively, other authors have published the relationship of the active form of the hormone and PCa, as calculated fT [18-20], bioavailable testosterone (bT) [21] and a few of them using immunoassay determined fT [22]. We believe that "determined fT reflects better than tT the concentration of intraprostatic T" is a plausible hypothesis. The aim of this study was to compare the performance of serum determined fT and tT as predictors of PCa detection risk and tumor aggressiveness in a large cohort of patients eligible for transrectal ultrasound (TRUS) prostate biopsy.

2. PATIENTS AND METHODS

2.1 Patients

We prospectively determined serum tT and fT levels in 3364 consecutive men scheduled for a TRUS-guided biopsy of the prostate performed by an experienced urologist between January 2007 and June 2014. Biopsies were taken using an end-fire ultrasound transducer (Falcon 2101, B-K Medical Inc., Denmark) and an automatic 18-G needle. The criteria for prostate biopsy were an abnormal digital rectal examination (DRE) and/or a serum PSA level of > 4.0 ng/mL. Biopsy scheme included 10 cores plus 2 to 8 additional cores in the first 2518 procedures according to age and prostatic volume, and 12 cores thereafter. All biopsy cores were analyzed by an experienced uropathologist. Patients who were receiving LH-releasing hormone analogs, 5-ARI, testosterone replacement therapy or had previous prostate biopsy were excluded from the analysis. We prospectively recorded age, body mass index (BMI), total and free PSA, findings at DRE, prostate volume (PV), biopsy findings, Gleason scores,

and clinical stage (TNM 2002). The study was carried out in accordance with the ethical standards of the Helsinki Declaration II and approved by our institutional review board. Written informed consent was obtained from each patient before any study investigation was carried out.

2.2 Hormonal assays

Despite a trend toward aged-related reduction in peak serum testosterone concentration at dawn, all patients underwent systematic blood sampling between 7 AM and 10 AM one week before the day of the biopsy to assess tT as well as fT levels according to the Endocrine Society's guidelines [23]. Serum tT (241-827 ng/dL) was measured using a solid-phase, competitive chemiluminescent enzyme immunoassay (LC/MS), using the Immulite 2500 automated analyzer (DPC Inc., Los Angeles, CA, USA). Serum fT (8.9-42.5 pg/mL) was measured using an analogue ligand radioimmunoassay (RIA) (DPC, Inc., Los Angeles, CA, USA).

2.3 Study end points

The primary end points of this study were the PCa detection rate and tumor aggressiveness. Tumor aggressiveness was defined as Gleason score [24] of the biopsy (HG: high grade PCa, GS 8 to 10) and D´Amico risk classification (HR: high risk PCa, clinical stage cT3a+ or PSA>20 ng/ml or GS 8 to 10). We defined advanced PCa as local advanced PCa (T3-4), lymph node dissemination (N1-2) or metastatic dissemination (M1).

2.4 Statistical analyses

Variables such as age, BMI, total PSA and prostate volume were analyzed as continuous variables. DRE was analyzed as a categorical variable (normal versus suspicious). To define whether a hormonal pattern was related to PCa, recodification of fT and tT in four groups according to percentile (ptile) distribution were done. tT/1 (min to 10 ptile): 62-291 ng/dl, tT/2 (ptile 10.1 to 50): 291.1 to 451 ng/dl, tT/3 (ptile 50.1 to 90): 451.1 to 676 ng/dl, and tT/4 (ptile 90.1 to max): 676.1 to 1440 ng/dl. fT/1: 0.1 to 0.85, fT/2: 0.86 to 5.20, fT/3: 5.21 to 9.65, and fT/4: 9.66 to 18.0 pg/ml. The chi-squared test and the nonparametric Mann-Whitney U-test were used to relate qualitative and quantitative variables respectively. In the population study, first a univariate analysis of the clinical, biochemical, and hormonal variables related to the presence of PCa was performed. Those variables that achieved a statistical significance in the univariate analysis ($p < 0.05$) were selected for further multivariate analysis (binary logistic regression and forward stepwise conditional methods).

3. RESULTS

The median age of the 3364 patients was 68 years (range, 46-86), the rate of abnormal DRE was 20.3% (684), and the median PSA was 7.0 ng/ml (range, 0.6 to 1754). The overall PCa detection rate was 38.0% (1280). The median of serum tT and ft concentration was 451 ng/dL and 5.2 pg/dL respectively. 86.7% of PCa diagnosed patients were organ confined ate diagnosis (T1-2 N0 M0) while 10.6% were locally advanced stage (T3-4 N0-2 M0). Only 2.7% of the cohort had metastasis at diagnosis. High-grade disease (Gleason score > 7; n = 331) was found in 25.9% of patients with positive biopsy. In addition, 33.2 % of patients with PCa was classified as high risk disease (adapted from D´Amico risk: cT3a+ or PSA>20 ng/mL or Gleason score 8 to 10; n = 426). Clinical, pathological, biochemical and hormonal data of the entire cohort are presented in table 1. In the univariate analysis, variables such as age, total PSA and finding of DRE were positively correlated with the presence of PCa while prostate volume, tT and fT had an inverse relation (table 2). BMI was not significantly related with PCa ($p > 0.05$).

Graphic representation of the distribution of tT and fT levels and the percentiles distribution of the population sample are shown in fig. 1.

Binary logistic regression analysis showed that both tT and fT were independent predictors of PCa detection. Low serum levels of tT (OR 0.857, 95%CI 0.769-0.956, $p < 0.001$) and fT (OR 0.792, 95% CI 0.709-0.883), $p < 0.001$) were significantly associated with PCa risk, as shown in table 3. Furthermore, total serum PSA and DRE were significant independent predictors of cancer.

The PCa detection rate according to tT and fT percentiles distribution is summarized in fig. 2. The ROC analysis for serum tT and fT to distinguish between PCa and benign prostatic conditions are presented in fig. 3. Considering all patients, the area under the curve (AUC) was higher for fT than for tT (0.571 versus 0.541, respectively).

The second aim of our study was to analyze the relationship between PCa aggressiveness and hormonal pattern. As reported in table 4, serum tT and fT were not statistically significant predictors neither of HGS (OR 1.153, 95%CI 0.966-1.376, p = 0.115; OR 0.903, CI95% 0.756-1.080, p = 0.263, respectively) nor HR (OR 1.058, 95%CI 0.871-1.284, p = 0.572; OR 1.184, CI95% 0.960-1.459, p = 0.114, respectively). Age, DRE and PSA were significant predictors of HR disease while age and PSA were also predictors of HGS ($p < 0.05$).

4. DISCUSSION

The aim of this study was to define a useful hormonal variable to help in detecting patients at risk of PCa. The authors conducted a retrospective study analyzing prostate biopsies from a third level health-care center in which a hormonal profile had been determined as part of a clinical protocol. The main finds of this study are that tT and fT were correlated with PCa. Those hormone parameters appeared to be helpful clinical markers to detect PCa, although this hormonal pattern achieved inaccurate results (fig. 3). A meta-analysis that incorporated 18 prospective studies did not find a relationship between testosterone, calculated free testosterone, dihydrotestosterone, dyhidroepiandrosterone sulfate, androstenedione, androstanediol glucoronide, oestradiol and calculated free oestradiol [25]. However, Roddam et al. found an inverse association between SHBG and PCa detection and, as reported in other published articles [21], it might suggest a role of the free or bioavailable hormone portion in increasing PCa risk [25] .

Our group has previously analyzed the relationship between hormonal pattern and PCa risk [26-28]. To our knowledge, this is the largest prospective, consecutive single center study that has tried to identify a hormonal clinical marker, which contributes to PCa detection and prediction of its level of aggression. Previously, many studies had reported an association of low serum testosterone levels with PCa aggressiveness [9] [10-14]. Most of them failed to find a significant association between testosterone and risk of PCa and/or its aggressiveness and assumed serum tT value as the main representation of the androgenic activity [15-17, 29-31]. Daniels et al. analyzed a total of 1927 patients for a mean period of five years. These authors did not find any association between the diagnosis of PCa and tT serum levels [16]. As we know, it has been largely accepted that the biological activity of a given hormone is affected by its unbound (free) rather than protein-bound concentration plasma. Mendel et al. proposed this theory, called The Free Hormonal hypothesis, after mathematical and physiological hormonal model tests [7, 8]. Consequently, our group believes that determined fT is a better reflector/indicator of the concentration of intraprostatic testosterone than tT. Therefore, to study the real relationship between the hormonal pattern of testosterone and PCa, the free portion of the hormone must be determined.

Some authors have published the relationship of the active form of testosterone and PCa [18, 21, 32]. Grosman et al. found a positive correlation between the free androgen index (FAI), fT and bT and PCa in a cohort of 150 men scheduled for prostate biopsy [18]. Similarly, García-Cruz et al. studied 1000 men and found that calculated fT (using Vermeulen's formula) and bT were significantly associated with PCa detection [21]. To provide further support, Leon et al recently published that estimated fT and bT were significantly linked with high-grade PCa [20]. In contrast, there are published studies that have failed to achieve an association between estimated fT or bT and PCa [33-35]. In this present study, in order to verify the real relationship with PCa, determined fT (RIA) was incorporated into the clinical protocol evaluation, which is a more accurate method to represent the free androgen profile. It is well known that calculated fT is highly dependent on the accuracy and sensitivity of the tT and SHBG assays and that the FAI is no longer recommended due to poor correlation with reference methods in men [36]. Our results demonstrated that patients with low levels of determined fT and tT had an increased PCa risk as both were independent predictors of PCa detection. Furthermore, fT seems to be a better PCa detection marker than tT due to the fact that it has achieved a better performance with a more powerful trend: more PCa detection, less fT levels and it demonstrated more intense PCa rates changes inter percentiles groups (fig. 3, table 3). Rivera et al. published the results of a biopsies sample analysis showing that the average values of total and determined free testosterone were significantly smaller in patients with PCa [37], which supports the findings of our present study. Although, fT and tT serum profile probably have a limited clinical application, both tests achieved AUCs < 0.70 corresponding to diagnostic tests with poor accuracy (a test that discriminates benign from malignant pathologies will have an AUC of 1, while a unreliable test will have an AUC of 0.5) [38].

The second aim of our study was to analyze the relationship of PCa aggressiveness and hormonal pattern. It has been hypothesized that PCa in a low testosterone environment may dedifferentiate and subsequently influence more undifferentiated and aggressive tumors given the fact that prostate cells grow in an environment lacking one of their most important activators, testosterone, which is reflected in higher Gleason score tumors [39]. Our data failed in achieve any association of testosterone, total or free portion, and high risk or high-grade tumors. Thus our study contrasts with some of those published within the last decade in which low testosterone levels seem to be linked to some aspects related to tumor aggressiveness: several studies claim that low testosterone levels might be related to poor clinical and pathological outcomes: high risk tumors [40, 41], high grade cancers [22, 42-44], advanced stage diseases [9, 45], positive surgical margins [10], worse prognosis and treatment response [46].

Published data are sometimes paradoxical. On one hand, testosterone deficiency is related to a poor prognosis in PCa while on the other hand, testosterone supplementation does not increase PCa risk. Testosterone is converted to the more androgenic DHT (by the action of 5alfa-reductase) within the prostate but the proportion of prostate-produced DHT that reaches the serum has not been determined and the correlation between tT and fT serum levels and DHT has not been established. Hence, the relationship between the concentration of different androgen isoforms in serum and in the prostate is unclear and could explain the lack of an association between serum levels and prostate cancer aggressiveness. Our study is a daily practice experience and no inclusion or exclusion criteria were considered. Regarding blood collection and preservation, our laboratory followed ISA-ISSAM-EAU Guideline recommendations. Part of the contradictory results might be explained by these methodological differences.

Our study has some limitations. This pooled analysis relied on the measurement of serum hormone levels in only one sample at only one time. These single measurements provide an imperfect estimate of a man's usual hormonal status and can be influenced both by within-person errors and analytical errors. Both types of errors are likely to lead to attenuation of the relationship between hormone concentration and risk [47]. In addition, prostate cancers develop with aging, so the timing is highly relevant. Our results represent a single point biopsy and do not take into consideration the time factor in relation to the development of PCa. Another possible limitation of our study is that one biopsy outcome was used as the definitive diagnosis of the patient. However, there is still a small chance of missing a PCa in this procedure.

5. FIGURES AND TABLES

5.1 Figures

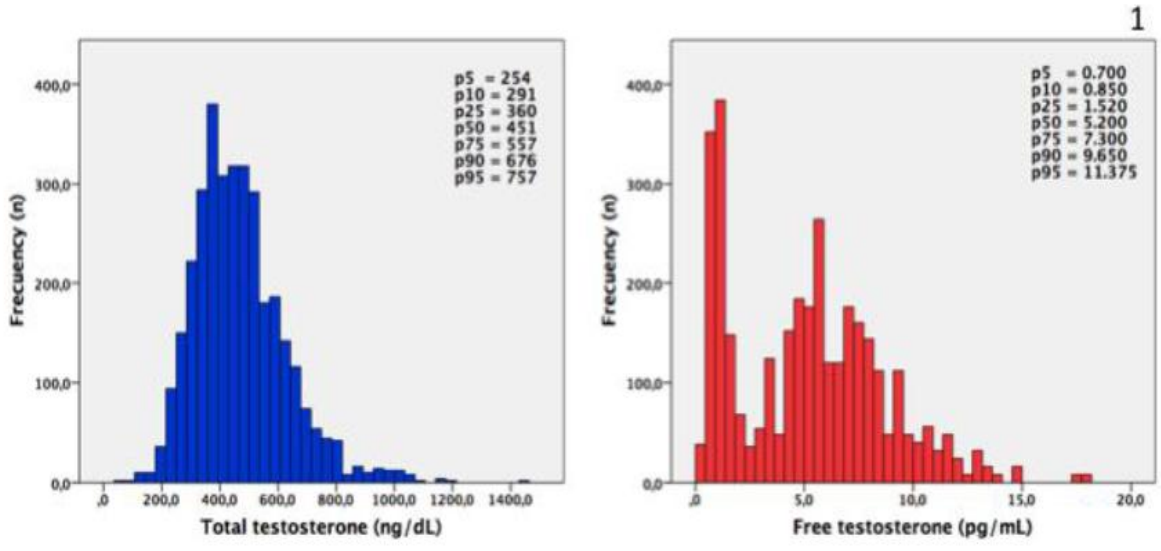

Figure1. *A. Total testosterone graphic and percentile distribution at the population sample. B. Free testosterone graphic and percentile distribution at the population sample.*

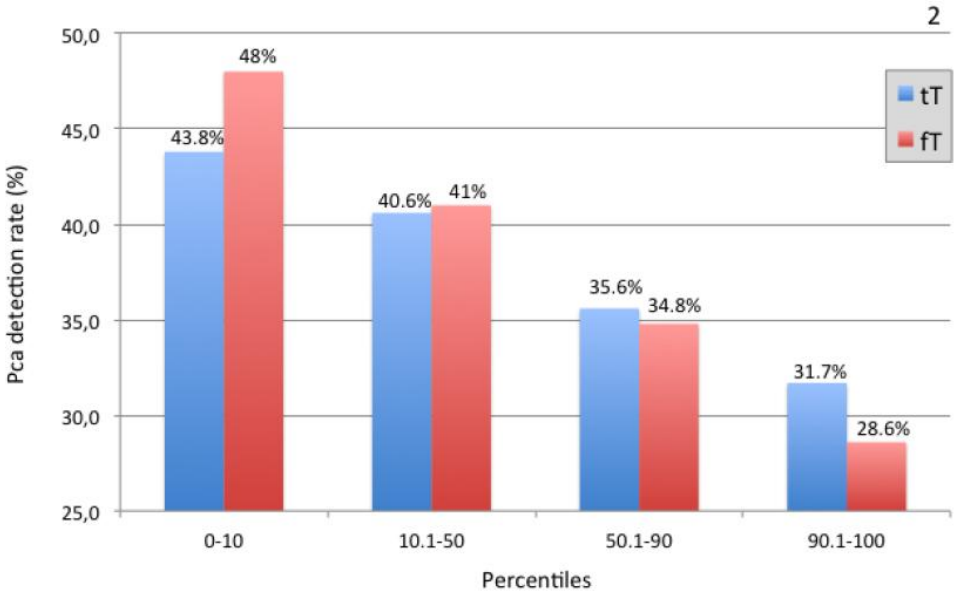

Figure2. *PCa detection rate according to total and free testosterone percentiles distribution.*

tT (ng/dL): p 0 - 10 = 0 to 291; p 10.1 – 50 = 292 to 451; p 50.1 – 90 = 452 to 676; p 90.1 – 100 = 677 to 1500. fT (pg/dL): p 0 - 10 = 0 to 0.850; p 10.1 – 50 = 0.851 to 5.200; p 50.1 – 90 = 5.201 to 9.650; p 90.1 – 100 = 9.651 to 20.

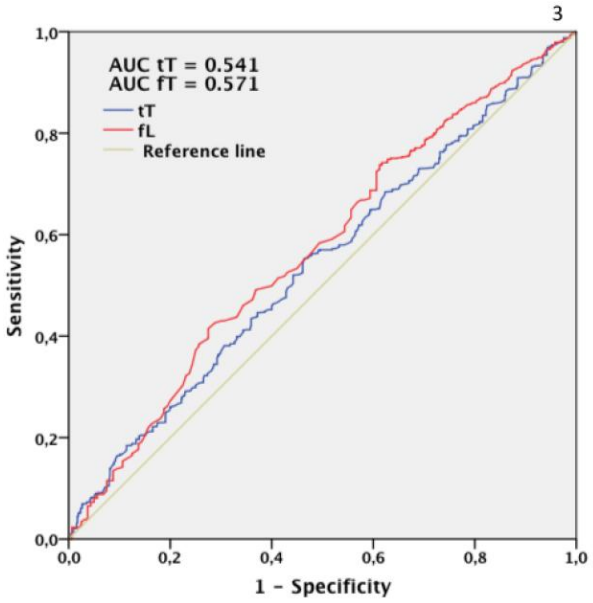

Figure3. *Receiver operating characteristics (ROC) curve of total testosterone and free testosterone in relation of PCa detection. AUC: area under the curve; tT: total testosterone; fT: free testosterone*

5.2 Tables

Table1. *Demographic and clinical data of the population sample.*

Men, no	3364
Age*, years	68 (46-86)
PSA*, ng/mL	7 (0.7-1724)
DRE, positive (%)	684 (20.3)
PV*, cm3	43.2 (7-159)
tT*, ng/dL	451 (62-1440)
fT*, pg/mL	5.2 (0.1-18)
PCa detection rate, no (%)	1280 (38%)
Clinical stage, no (%)	
T1-2 N0 M0	1110 (86.7)
T3-4 N0-2 M0	136 (10.6)

T1-4 N0-1 M1	34 (2.7)
Biopsy Gleason score, no (%)	
6	380 (11.3)
7	569 (16.9)
8-10	331 (9.8)
PCa risk**, no (%)	
Low	272 (21.3)
Intermedium	582 (45.5)
High	426 (33.2)

DRE: digital rectal examination; PV: prostate volume; tT: total testosterone; fT: free testosterone; Pca: prostate cancer.

**quantitative variables are expressed as median (range);*

*** PCa risk according to D'Amico classification*

Table2. *Demographic and clinical data according to PCa diagnosis*

data	PCa	No PCa	p Value
Age*, years	69 (48-86)	68 (46-86)	< 0.001
PSA*, ng/mL	7.4 (1.6-1724)	6.9 (0.7-48.6)	< 0.001
DRE, positive (%)	602(17.9)	82 (2.4)	< 0.001
PV*, cm3	37.7 (10-136)	48.7 (7-159)	< 0.001
tT*, ng/dL	434(62-1167)	459.5 (107-1440)	< 0.001
fT*, pg/mL	4.8 (0.1-18)	5.5 (0.1-17.4)	< 0.001

DRE: digital rectal examination; PV: prostate volume; tT: total testosterone; fT: free testosterone; PCa: prostate cancer.

**quantitative variables are expressed as median (range)*

Table3. *Binary logistic regression analysis of the PCa risk with percentile distribution of total and free testosterone*

variable	OR	95%CI	p Value
DRE	19.335	15.002-24.919	< 0.001
Age, years	1.036	1.024-1.049	< 0.001
PSA, ng/mL	1.027	1.015-1.039	< 0.001
fT1/4	0.792	0.709-0.883	< 0.001
tT1/4	0.857	0.769-0.956	< 0.005

DRE: digital rectal examination; tT1/4: total testosterone percentiles 0-10/10.1-50/50.1-90/90.1-100; fT1/4: free testosterone percentiles 0-10/10.1-50/50.1-90/90.1-100

OR: odds ratio. CI: confidence interval.

Table4. *Binary logistic regression analysis of the PCa aggressiveness with percentile distribution of total and free testosterone*

	High	Grade	High	Risk*
variable	OR (95%CI)	p value	OR (95%CI)	p value
DRE	1.085 (0.818-1.439)	p = 0.569	1.528 (1.129-2.066)	p < 0.006
Age, years	1.073 (1.050-1.096)	p < 0.001	1.090 (1.064-1.116)	p < 0.001
PSA, ng/mL	1.022 (1.016-1.029)	p < 0.001	1.181 (1.146-1.217)	p < 0.001
fT1/4	0.903 (0.756-1.080)	p = 0.263	1.184 (0.960-1.459)	p = 0.114
tT1/4	1.153 (0.966-1.376)	p = 0.115	1.058 (0.871-1.284)	p = 0.572

DRE: digital rectal examination; tT1/4: total testosterone percentiles 0-10/10.1-50/50.1-90/90.1-100; fT1/4: free testosterone percentiles 0-10/10.1-50/50.1-90/90.1-100

** Adaptation of D'Amico risk classification that includes local advanced disease*

OR: odds ratio. CI: confidence interval.

6. CONCLUSION

In summary, in the present study we confirm the hypothesis that low testosterone levels increase the risk of prostate cancer. Moreover, our data suggest that fT is better correlated with PCa detection rather than tT. Although both parameters probably have a limited clinical application due to poor accuracy achieved in further analysis. We also failed to confirm that low testosterone levels increase tumor aggressiveness. The convenience of serum tT or fT assessment before the decision of PCa biopsy remains unclear.

REFERENCES

[1] Heidenreich A., Bastian P. J., Bellmunt J., Bolla M., Joniau S., van der Kwast T. et al.: EAU guidelines on prostate cancer. part 1: screening, diagnosis, and local treatment with curative intent-update 2013. Eur Urol 65(1), 124-137 (2014).

[2] Huggins C., Clark P. J.: Quantitative studies of prostatic secretion: II. The effect of castration and of estrogen injection on the normal and the hyperplastic prostate glands of dogs. J Exp Med 72(6), 747-762 (1940).

[3] Kaufman J. M., Vermeulen A.: The decline of androgen levels in elderly men and its clinical and therapeutic implications. Endocr Rev 26(6), 833-876 (2005).

[4] Isbarn H., Pinthus J. H., Marks L. S., Montorsi F., Morales A., Morgentaler A. et al.: Testosterone and prostate cancer: revisiting old paradigms. Eur Urol 56(1), 48-56 (2009).

[5] Botelho F., Pina F., Figueiredo L., Cruz F., Lunet N.: Does baseline total testosterone improve the yielding of prostate cancer screening? Eur J Cancer 48(11), 1657-1663 (2012).

[6] Nishiyama T., Hashimoto Y., Takahashi K.: The influence of androgen deprivation therapy on dihydrotestosterone levels in the prostatic tissue of patients with prostate cancer. Clin Cancer Res 10(21), 7121-7126 (2004).

[7] Mendel C. M.: The free hormone hypothesis: a physiologically based mathematical model. Endocr Rev 10(3), 232-274 (1989).

[8] Matsumoto A. M., Bremner W. J.: Serum testosterone assays--accuracy matters. J Clin Endocrinol Metab 89(2), 520-524 (2004).

[9] Massengill J. C., Sun L., Moul J. W., Wu H., McLeod D. G., Amling C. et al.: Pretreatment total testosterone level predicts pathological stage in patients with localized prostate cancer treated with radical prostatectomy. J Urol 169(5), 1670-1675 (2003).

[10] Teloken C., Da Ros C. T., Caraver F., Weber F. A., Cavalheiro A. P., Graziottin T. M.: Low serum testosterone levels are associated with positive surgical margins in radical retropubic prostatectomy: hypogonadism represents bad prognosis in prostate cancer. J Urol 174(6), 2178-2180 (2005).

[11] Morgentaler A.: Testosterone and prostate cancer: an historical perspective on a modern myth. Eur Urol 50(5), 935-939 (2006).

[12] Yano M., Imamoto T., Suzuki H., Fukasawa S., Kojima S., Komiya A. et al.: The clinical potential of pretreatment serum testosterone level to improve the efficiency of prostate cancer screening. Eur Urol 51(2), 375-380 (2007).

[13] Muller R. L., Gerber L., Moreira D. M., Andriole G., Castro-Santamaria R., Freedland S. J.: Serum testosterone and dihydrotestosterone and prostate cancer risk in the placebo arm of the Reduction by Dutasteride of Prostate Cancer Events trial. Eur Urol 62(5), 757-764 (2012).

[14] Mearini L., Zucchi A., Nunzi E., Villirillo T., Bini V., Porena M.: Low serum testosterone levels are predictive of prostate cancer. World J Urol 31(2), 247-252 (2013).

[15] Garcia-Cruz E., Castaneda-Argaiz R., Carrion A., Alcover J., Sallent A., Leibar-Tamayo A. et al.: Preoperative hormonal pattern in patients undergoing radical prostatectomy due to prostate cancer. Actas Urol Esp 37(5), 280-285 (2013).

[16] Daniels N. A., Nielson C. M., Hoffman A. R., Bauer D. C.: Sex hormones and the risk of incident prostate cancer. Urology 76(5), 1034-1040 (2010).

[17] Rhoden E. L., Riedner C. E., Morgentaler A.: The ratio of serum testosterone-to-prostate specific antigen predicts prostate cancer in hypogonadal men. J Urol 179(5), 1741-1744 (2008).

[18] Grosman H., Fabre B., Mesch V., Lopez M. A., Schreier L., Mazza O. et al.: Lipoproteins, sex hormones and inflammatory markers in association with prostate cancer. Aging Male 13(2), 87-92 (2010).

[19] Schnoeller T., Jentzmik F., Rinnab L., Cronauer M. V., Damjanoski I., Zengerling F. et al.: Circulating free testosterone is an independent predictor of advanced disease in patients with clinically localized prostate cancer. World J Urol 31(2), 253-259 (2013).

[20] Leon P., Seisen T., Cussenot O., Drouin S. J., Cattarino S., Comperat E. et al.: Low circulating free and bioavailable testosterone levels as predictors of high-grade tumors in patients

undergoing radical prostatectomy for localized prostate cancer. Urol Oncol (2015).

[21] Garcia-Cruz E., Huguet J., Piqueras M., Marquez M. P., Peri L., Izquierdo L. et al.: Low testosterone bioavailability is related to prostate cancer diagnose in patients submitted to prostate biopsy. World J Urol 30(3), 361-365 (2012).

[22] Albisinni S., De Nunzio C., Tubaro A., Barry W. T., Banez L. L., Freedland S. J.: Greater percent-free testosterone is associated with high-grade prostate cancer in men undergoing prostate biopsy. Urology 80(1), 162-167 (2012).

[23] Rosner W., Vesper H.: Toward excellence in testosterone testing: a consensus statement. J Clin Endocrinol Metab 95(10), 4542-4548 (2010).

[24] Epstein J. I., Allsbrook W. C. J., Amin M. B., Egevad L. L.: The 2005 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma. Am J Surg Pathol 29(9), 1228-1242 (2005).

[25] Roddam A. W., Allen N. E., Appleby P., Key T. J.: Endogenous sex hormones and prostate cancer: a collaborative analysis of 18 prospective studies. J Natl Cancer Inst 100(3), 170-183 (2008).

[26] Morote J., Ramirez C., Gomez E., Planas J., Raventos C. X., de Torres I. M. et al.: The relationship between total and free serum testosterone and the risk of prostate cancer and tumour aggressiveness. BJU Int 104(4), 486-489 (2009).

[27] Morote J., Planas J., Ramirez C., Gomez E., Raventos C. X., Placer J. et al.: Evaluation of the serum testosterone to prostate-specific antigen ratio as a predictor of prostate cancer risk. BJU Int 105(4), 481-484 (2010).

[28] Regis L., Planas J., Celma A., de Torres I. M., Ferrer R., Morote J.: Behavior of total and free serum testosterone as a predictor for the risk of prostate cancer and its aggressiveness. Actas Urol Esp (2015).

[29] Weiss J. M., Huang W. Y., Rinaldi S., Fears T. R., Chatterjee N., Hsing A. W. et al.: Endogenous sex hormones and the risk of prostate cancer: a prospective study. Int J Cancer 122(10), 2345-2350 (2008).

[30] Gill J. K., Wilkens L. R., Pollak M. N., Stanczyk F. Z., Kolonel L. N.: Androgens, growth factors, and risk of prostate cancer: the Multiethnic Cohort. Prostate 70(8), 906-915 (2010).

[31] Koo J. M., Shim B. S.: Significance of Serum Testosterone for Prostate-Specific Antigen (PSA) Elevation and Prediction of Prostate Cancer in Patients with PSA Above 10 ng/ml. Korean J Urol 51(12), 831-835 (2010).

[32] Hyde Z., Flicker L., McCaul K. A., Almeida O. P., Hankey G. J., Chubb S. A. et al.: Associations between testosterone levels and incident prostate, lung, and colorectal cancer. A population-based study. Cancer Epidemiol Biomarkers Prev 21(8), 1319-1329 (2012).

[33] Kaaks R., Lukanova A., Rinaldi S., Biessy C., Soderberg S., Olsson T. et al.: Interrelationships between plasma testosterone, SHBG, IGF-I, insulin and leptin in prostate cancer cases and controls. Eur J Cancer Prev 12(4), 309-315 (2003).

[34] Wiren S., Stocks T., Rinaldi S., Hallmans G., Bergh A., Stenman U. H. et al.: Androgens and prostate cancer risk: a prospective study. Prostate 67(11), 1230-1237 (2007).

[35] Sawada N., Iwasaki M., Inoue M., Sasazuki S., Yamaji T., Shimazu T. et al.: Plasma testosterone and sex hormone-binding globulin concentrations and the risk of prostate cancer among Japanese men: a nested case-control study. Cancer Sci 101(12), 2652-2657 (2010).

[36] Shea J. L., Wongt P. Y., Chen Y.: Free testosterone: clinical utility and important analytical aspects of measurement. Adv Clin Chem 63, 59-84 (2014).

[37] Rivera P., Tagle R., Mir S., Gonzalez R.: [Relationship between serum testosterone levels and prostatic cancer]. Actas Urol Esp 27(10), 788-792 (2003).

[38] Altman D. G., Bland J. M.: Diagnostic tests 3: receiver operating characteristic plots. BMJ 309(6948), 188 (1994).

[39] Morgentaler A.: Testosterone deficiency and prostate cancer: emerging recognition of an important and troubling relationship. Eur Urol 52(3), 623-625 (2007).

[40] Salonia A., Gallina A., Briganti A., Abdollah F., Suardi N., Capitanio U. et al.: Preoperative

hypogonadism is not an independent predictor of high-risk disease in patients undergoing radical prostatectomy. Cancer 117(17), 3953-3962 (2011).

[41] Salonia A., Abdollah F., Capitanio U., Suardi N., Briganti A., Gallina A. et al.: Serum sex steroids depict a nonlinear u-shaped association with high-risk prostate cancer at radical prostatectomy. Clin Cancer Res 18(13), 3648-3657 (2012).

[42] Hoffman M. A., DeWolf W. C., Morgentaler A.: Is low serum free testosterone a marker for high grade prostate cancer? J Urol 163(3), 824-827 (2000).

[43] Zhang P. L., Rosen S., Veeramachaneni R., Kao J., DeWolf W. C., Bubley G.: Association between prostate cancer and serum testosterone levels. Prostate 53(3), 179-182 (2002).

[44] Platz E. A., Leitzmann M. F., Rifai N., Kantoff P. W., Chen Y. C., Stampfer M. J. et al.: Sex steroid hormones and the androgen receptor gene CAG repeat and subsequent risk of prostate cancer in the prostate-specific antigen era. Cancer Epidemiol Biomarkers Prev 14(5), 1262-1269 (2005).

[45] Xylinas E., Ploussard G., Durand X., Fabre A., Salomon L., Allory Y. et al.: Low pretreatment total testosterone (< 3 ng/mL) predicts extraprostatic disease in prostatectomy specimens from patients with preoperative localized prostate cancer. BJU Int 107(9), 1400-1403 (2011).

[46] Yamamoto S., Yonese J., Kawakami S., Ohkubo Y., Tatokoro M., Komai Y. et al.: Preoperative serum testosterone level as an independent predictor of treatment failure following radical prostatectomy. Eur Urol 52(3), 696-701 (2007).

[47] McShane L. M., Midthune D. N., Dorgan J. F., Freedman L. S., Carroll R. J.: Covariate measurement error adjustment for matched case-control studies. Biometrics 57(1), 62-73 (2001).

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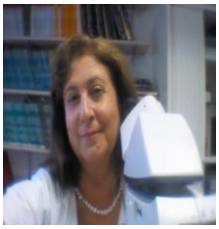

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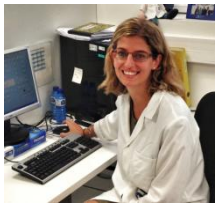

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