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Does baseline total testosterone improve the yielding of prostate cancer screening?

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Abstract Previous studies suggest an association between total testosterone (tT) and prostate cancer, but results are conflicting and it is not clear if accounting for the tT levels improves the yielding of patient selection for prostatic biopsy. We evaluated the potential for tT levels and the tT/total PSA (tPSA) ratio to be used as diagnostic tools for prostate cancer and its relation with cancer aggressiveness.

We measured tT, tPSA and free PSA (fPSA) in fasting blood samples of 1570 subjects consecutively referred for prostate biopsy due to abnormal digital rectal examination and/or elevated tPSA levels. These values were compared between groups defined according to the pathological results of the biopsy.

No significant difference was observed in tT levels when comparing cases with prostate cancer, high grade prostate intraepithelial neoplasia, pathological prostatitis, benign prostatic hyperplasia or no alteration (median: 4.26 versus 4.44 versus 4.31 versus 4.16 pg/mL, respectively; $p = 0.643$). The tT/tPSA ratio had a better area under the curve than tT alone (0.62, 95% CI, 0.59–0.65 versus 0.50, 95% CI, 0.47–0.53), but worse than the f/tPSA ratio (0.70, 95% CI, 0.67–0.73). In multivariate analysis, using the median of distribution as cut-off no significant association was observed between tT or tT/tPSA and prostate cancer (OR = 1.06, 95% CI, 0.84–1.33; OR = 0.94, 95% CI, 0.70–1.27, respectively). The tT levels were not significantly different across Gleason score groups ($p = 0.553$).

In patients suspected of having prostate cancer the tT levels are not useful to improve the yielding of patient selection for prostatic biopsy or to predict cancer aggressiveness.

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1. Introduction

Prostate cancer is the most commonly diagnosed non-dermatologic malignancy and the third leading cause of cancer mortality among men in Europe.[1] Total prostatic specific antigen (tPSA) is widely used for prostate cancer screening, despite its low accuracy across different cut-offs.[2] The need to avoid both unnecessary diagnostic procedures and missed diagnoses has led to the study of several other biomarkers that could further contribute to improve the yielding of patient selection for prostatic biopsy.

Androgens, particularly testosterone, are required for normal growth and functioning of the human prostate.[3] The responsiveness of prostate cancer to androgen withdrawal[4] and the fact that it is very rare among eunuchs[5] supports the hypothesis that prostate cancer is associated with androgens' blood levels. On the other hand, prostate cancer patients may have lower circulating tT concentrations due to increased intra-prostatic accumulation and metabolization.[6,7]

The currently available evidence on the relation serum tT and prostate cancer does not support the use of this biomarker to determine prostate cancer risk, increase specificity of PSA testing, or assess tumour aggressiveness.[3] The results from studies addressing specifically the relation between serum tT and the diagnosis of prostate cancer are conflicting[7–9] with some authors only finding association using the tT/tPSA ratio[10] or among patients with tPSA < 4 ng/mL.[11,12] However, none of the previous reports presented tT levels separately for men with signs of prostatic inflammation or high grade prostate intraepithelial neoplasia (HGPIN) in the biopsy evaluation, and none included more than 600 patients; the median number of participants per study is 197.5. In addition, in some of the previous studies[7,8,13–17] the control group was described as composed by healthy men or subjects with benign prostatic disease, which may contribute to an overestimation of the yielding of the diagnostic test due to the lack of alternative diseases that might produced false-positive results.[18] Furthermore, controls did not perform a prostate biopsy, despite a noteworthy prevalence of prostate cancer may be expected in patients with low PSA levels, even with negative digital rectal examination.[19]

Some studies also found an association between lower levels of total testosterone and higher Gleason score[20,21] but this was not confirmed by others.[22–26] Interestingly, there are some lines of evidence that prostate cancers are stimulated to dedifferentiate in a testosterone-deficient environment, leading to more aggressive tumours.[27]

We aimed to evaluate the potential for serum tT levels and the tT/tPSA ratio to be used as diagnostic tools for prostate cancer, using patients with increased risk of disease and presenting different prostatic pathologies [including benign prostatic hyperplasia (BPH), histological evidence of prostatitis, HGPIN and prostate cancer]. As secondary objectives we aimed to quantify the relation between tT and tT/tPSA ratio and the combined Gleason score in the prostate biopsy.

2. Patients and methods

2.1. Patient selection

Between January 2005 and March 2009 we enrolled 1570 subjects consecutively referred to ultrasound guided trans-rectal prostate biopsy, on the basis of abnormal digital rectal examination and/or elevated tPSA levels ($\geqslant 2.5$ ng/mL), in the Department of Urology of S. João Hospital, a University Hospital in Porto. None of them received previous hormone therapy, radiotherapy or chemotherapy before undergoing prostate biopsy. All participants provided written informed consent to this research study.

2.2. Measurement of biomarkers

Before undergoing prostatic biopsy all participants provided a fasting blood sample, collected between 09.00 and 10.30 AM. The blood was allowed to clot for 30 min before centrifugation and the serum used for assessment of tPSA, free PSA (fPSA) and serum tT. The latter was evaluated by chemo-luminescence with a commercially available Immunoassay kit (ABBOTT Diagnostics Division).

2.3. Outcome evaluation

The result of the prostatic pathology assessment and the combined Gleason score of the prostate cancer cases were defined by biopsy results. The number of biopsy cores ranged from 12 to 14. All prostatic biopsies were reviewed independently by two different pathologists, who were blinded to the patients' tPSA, fPSA and tT values. Patients were grouped in four mutually-exclusive groups as follows (ordered by increasing severity): normal prostate or Benign Prostatic Hyperplasia (N/BPH), prostatitis (if presenting with leucocyte infiltrates), HGPIN and prostate cancer. When the prostate biopsy revealed simultaneously signs of BPH and inflammation (leucocyte infiltrates) the patients were classified as having prostatitis. Eleven patients with inconclusive biopsy results were excluded from analysis.

2.4. Statistical analysis

The Kruskal–Wallis and Chi-square tests were used to compare quantitative and categorical variables across groups, respectively. Spearman correlation coefficients

were computed to quantify the association between serum tT and age, tPSA and fPSA/tPSA ratio. Unconditional logistic regression was used to quantify the association between groups of tT and tT/tPSA levels (cut-off defined using the median of the distributions) and prostatic cancer. Both the crude and age-, tPSA- and fPSA/tPSA-adjusted odds ratios (OR) and respective 95% confidence intervals (95% CI) are presented. The only co-variable that did not present a normal distribution was tPSA and was modelled after log-transformation.

A receiver operating characteristic (ROC) analysis was used to evaluate the potential for tT and tT/tPSA to distinguish between malignant and benign diagnoses across different cut-offs.

The analyses were conducted also among the subgroup of patients with tPSA < 4 ng/mL. Statistical significance was defined as $p < 0.05$, and the reported p-values are two-sided. All analyses were performed using STATA®, version 11.0.

3. Results

The median values for age, tPSA and fPSA/tPSA ratio were 67 years, 7.0 ng/mL and 0.17, respectively. Prostatic biopsies revealed adenocarcinoma in 618 (39.4%) of the participants, HGPIN in 51 (3.2%), prostatitis in 623 (39.7%) and N/BPH in the remaining 278 (17.7%). Among prostate cancer cases the combined Gleason score was 6 or lower in 17.9% patients, 7 in 57.4% and 8 or higher in 24.7%.

The median serum tT level in our sample was 4.25 pg/mL (range: 0.29–15.01 pg/mL; P25–P75: 3.31–5.44 pg/mL). No significant or meaningful correlation was observed between tT and age ($r = -0.002$; $p = 0.943$), tPSA ($r = -0.034$; $p = 0.208$), or fPSA/tPSA ratio ($r = 0.029$; $p = 0.282$). The tT/tPSA ratio was significantly associated with age ($r = -0.169$; $p < 0.001$), tPSA ($r = -0.849$; $p < 0.001$) and fPSA/tPSA ratio ($r = 0.228$; $p < 0.001$).

Compared to subjects with benign histology, prostate cancer patients had significantly higher age (median: 69 versus 67 years; $p < 0.001$) and tPSA (median: 8.1 versus 6.3 ng/mL; $p < 0.001$). The median fPSA/tPSA ratio was significantly lower in patients with malignant disease compared to patients with benign histology (0.13 versus 0.20; $p < 0.001$).

Table 1 summarises the participants' characteristics according to prostatic histology. No statistically significant differences were observed when comparing serum tT levels across different prostatic biopsy diagnoses, or between prostatic cancer and benign pathology patients (4.27 versus 4.26 pg/mL; $p = 0.794$). In men with hipogonadic levels of tT, 56% presented with prostate cancer, compared to 61% in those without analytical criteria of hipogonadism ($p = 0.207$). If only the patients with tPSA lower than 4 ng/mL were considered, serum tT concentrations were slightly higher in patients with benign disease but the difference was not statistically significant (4.43 versus 4.35 pg/mL; $p = 0.308$). The tT/tPSA ratio was significantly lower in prostate cancer patients than in the remaining histological diagnosis. The ratios were 0.51 for malignant prostatic pathology and 0.65 for the benign conditions ($p < 0.001$). Similar differences, though non-statistically significant, were observed when only patients with tPSA < 4 ng/mL were considered (prostate cancer versus benign prostatic disease: 1.45 versus 1.59; $p = 0.083$).

The ROC curves for serum tT, serum tT/tPSA ratio, tPSA and fPSA/tPSA ratio to distinguish between prostate cancer and benign prostatic conditions are presented in Fig. 1 for all patients and in Fig. 2 for those with tPSA < 4 ng/mL. Considering all patients, the area under the curve (AUC) was higher for tT/tPSA ratio than for tT alone (0.62, 95% CI: 0.59–0.65 versus 0.50, 95% CI: 0.47–0.53), but lower than fPSA/tPSA ratio (0.70, 95% CI: 0.67–0.73). All these tests have AUCs ⩽ 70, corresponding to diagnostic tests with poor accuracy (a test that perfectly discriminates between benign from malignant pathologies will have an AUC of 1, while a completely useless test will have an AUC of 0.5).[28] The best diagnostic test for patients with

Table 1
Characteristics of the participants stratified by prostatic histology.

		Prostatic histology				p
		N/BPH ($n = 278$)	Prostatitis ($n = 623$)	HGPIN ($n = 51$)	Prostate cancer ($n = 618$)	
Median age (years)		67.0	66.0	67.0	69.0	<0.001
Median tPSA (ng/mL)		5.8	6.6	6.2	8.1	<0.001
Median fPSA/tPSA ratio		0.21	0.20	0.21	0.13	<0.001
Median tT (pg/mL)		4.16	4.31	4.44	4.26	0.643
tT (pg/mL)[a,b]	⩽2.50	28 (10.1)	44 (7.1)	2 (3.9)	58 (9.4)	0.207
	>2.50	250 (89.9)	579 (92.9)	49 (96.1)	560 (90.6)	
Median tT/tPSA		0.71	0.62	0.71	0.51	<0.001

fPSA: free prostatic specific antigen; HGPIN: high grade prostate intraepithelial neoplasia; N/BPH: normal prostate or benign prostate hyperplasia; tT: total Testosterone; tPSA: total prostatic specific antigen.

[a] Results are presented as n (%).

[b] The cutoff corresponds to the lower limit of the reference interval for serum total testosterone in this population.

tPSA < 4 ng/mL is also fPSA/tPSA (AUC = 0.61, 95% CI: 0.52–0.70).

The diagnostic OR for the distinction between benign and malignant prostatic pathology are presented in Table 2. In general, no significant association was detected for tT levels. Patients with lower tT/tPSA ratio had higher probability of prostate cancer (1st versus 2nd half: OR = 1.81; 95% CI: 1.47–2.23). This association was no longer observed in multivariate analysis (1st half versus 2nd half: OR = 0.94; 95% CI: 0.70–1.27), but age, tPSA and fPSA/tPSA ratio remained independent predictors of malignant pathology.

Considering only patients with total PSA lower than 4 ng/mL (Table 3), adjusted associations between tT and tT/tPSA levels and prostate cancer were weak and non-statistically significant (OR = 1.06; 95% CI: 0.56–2.03 and OR = 1.33; 95% CI: 0.65–2.71, respectively). Age and the fPSA/tPSA ratio remained independent predictors of malignant pathology in this sub-population, but not tPSA.

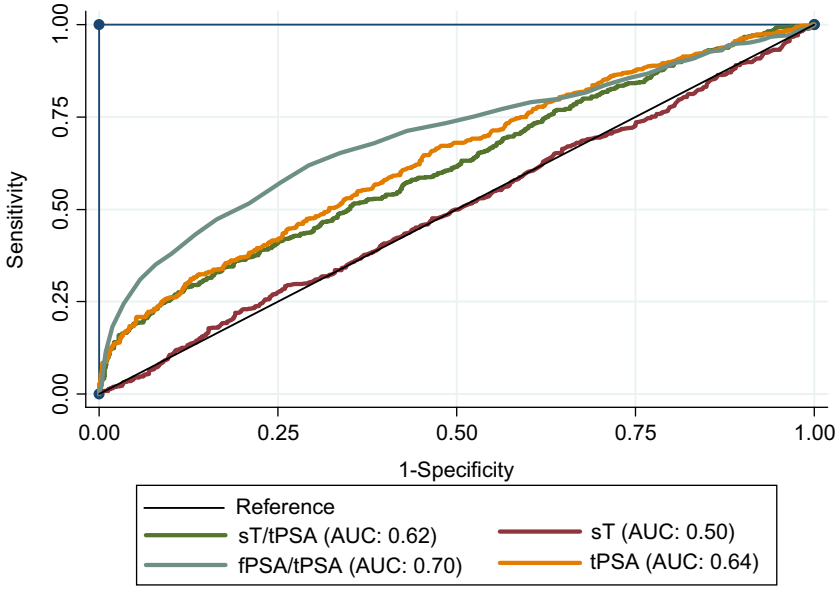

Fig. 1. Receiver operating characteristic (ROC) curves for total testosterone levels as a test for diagnosis prostatic carcinoma using the biopsy results as the gold standard (all participants).

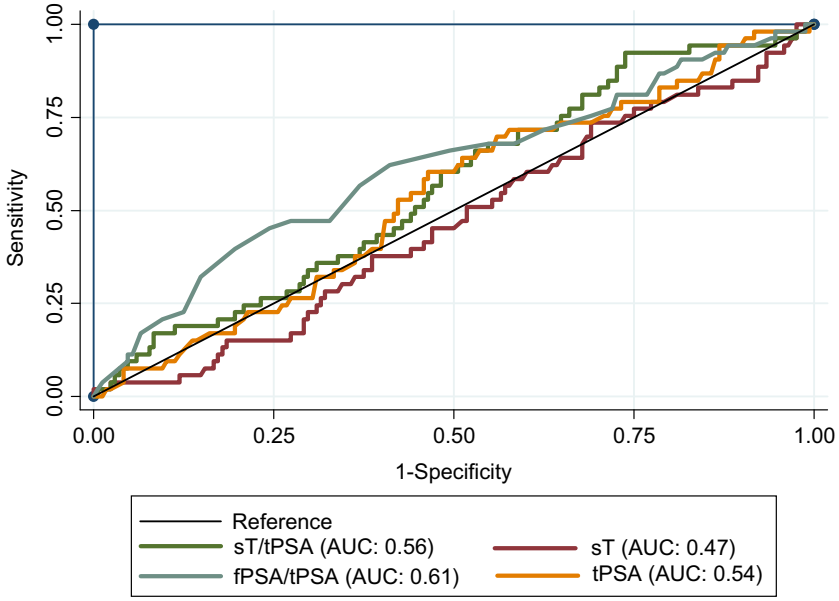

Fig. 2. Receiver operating characteristic (ROC) curves for total testosterone levels as a test for prostatic carcinoma diagnosis using the biopsy results as the gold standard (participants with tPSA < 4 ng/mL).

Table 2
Diagnostic odds ratios for the association between total testosterone and total testosterone/total prostatic specific antigen ratio and prostate cancer.

	Prostate cancer	
	Crude OR (95% CI)	Adjusted OR[d] (95% CI)
tT[a] > 4.25 pg/mL	0.99 (0.94–1.05)	1.06 (0.84–1.33)
Age[b]		1.56 (1.33–1.84)
tPSA[c] 4–10 ng/mL		1.38 (0.97–1.96)
tPSA[c] > 10 ng/mL		2.26 (1.54–3.32)
fPSA/tPSA[c] < 0.15		4.09 (3.22–5.19)
tT/tPSA[a] < 0.601	1.81 (1.47–2.23)	0.94 (0.70–1.27)
Age[b]		1.57 (1.33–1.85)
tPSA[c]4–10 ng/mL		1.41 (0.98–2.04)
tPSA[c] > 10 ng/mL		2.39 (1.49–3.84)
fPSA/tPSA[c] < 0.15		4.09 (3.22–5.19)

CI: confidence interval; tT: total testosterone; tT/tPSA: total testosterone/total prostatic specific antigen ratio; benign histology – includes normal prostate, benign prostate hyperplasia, prostatitis and HGPIN.
[a] Median of the distribution was used to define cut-offs.
[b] This variable was introduced in the model as continuous but divided by a factor of 10; the presented OR corresponds to the independent increased risk of prostate cancer per a 10-year increase in age.
[c] These are classically used cut-offs for these variables, predetermined to be used in this analysis; reference category: PSA < 4 ng/mL and fPSA/tPSA > 0.15.
[d] Adjusted or derived from models including age, tPSA, fPSA/tPSA ratio and tT or tT/tPSA, as applicable.

Table 3
Diagnostic odds ratios for the association between total testosterone and total testosterone/total prostatic specific antigen ratio and prostate cancer, in patients with total prostatic specific antigen lower than 4 ng/mL.

	Prostate cancer	
	Crude OR (95% CI)	Adjusted OR[d] (95% CI)
tT[a] > 4.43 pg/mL	1.23 (0.67–2.25)	1.06 (0.56–2.03)
Age[b]		1.69 (1.07–2.70)
tPSA[c] > 2.5 ng/mL		1.19 (0.57–2.47)
fPSA/tPSA[c] < 0.15		3.63 (1.73–7.63)
tT/tPSA[a] < 1.54	1.43 (0.78–2.63)	1.33 (0.65–2.71)
Age[b]		1.69 (1.07–2.66)
tPSA[c] > 2.5 ng/mL		1.03 (0.46–2.32)
fPSA/tPSA[c] < 0.21		3.62 (1.73–7.58)

CI: confidence intervals; tT: total testosterone; tT/tPSA: total testosterone/total prostatic specific antigen ratio; benign histology – includes normal prostate, benign prostate hyperplasia, prostatitis and HGPIN.
[a] Median of the distribution in patients with tPSA < 4 ng/mL was used to define cut-offs.
[b] This variable was introduced in the model as continuous but divided by a factor of 10; the presented OR corresponds to the independent increased risk of prostate cancer per a 10-year increase in age.
[c] These are classically used cut-offs for these variables, predetermined to be use in this analysis; reference category: PSA < 4 ng/mL and fPSA/tPSA > 0.15.
[d] Adjusted or derived from models including age, tPSA, fPSA/tPSA ratio and tT or tT/tPSA, as applicable.

In prostatic cancer patients the serum tT levels were not significantly different across combined Gleason score groups (Table 4). The median values ranged from 4.59 pg/mL in patients with histological Gleason score 6 or lower to 4.11 pg/mL in those scoring 9 or more ($p = 0.553$). The tT/tPSA ratio was significantly different across Gleason score groups with mean values gradually ranging from 0.67 in patients with the lowest scores to 0.22 in subjects in the highest Gleason score category ($p < 0.001$). Again, this association disappeared after adjusting for tPSA levels. These results follow a similar trend when considering only the population with tPSA lower than 4 ng/mL.

4. Discussion

In our large consecutive series of patients undergoing prostatic biopsy due to abnormal digital rectal examination and/or elevated tPSA levels the serum tT levels were similar between cancer and the different benign prostatic disease diagnoses, including prostatitis and HGPIN. The tT/tPSA ratio was associated with diagnosis of prostate cancer, but not independently of age, tPSA or fPSA/tPSA levels. This is probably because the tT/tPSA ratio was associated with prostate cancer due to the tPSA component and not the relation between tT and tPSA. The conclusions were the same when the analysis was restricted to patients with tPSA concentrations lower than 4 ng/mL.

Some of the previous articles have found lower tT concentrations in prostatic cancer patients,[7,13,16,29] particularly in those with lower tPSA levels,[11,12] while other investigations yielded no differences between subjects with benign prostatic pathology and patients with malignant disease.[8–10,14,15,17,30] On the other hand, Yano et al.[31] reported that higher levels of serum tT were independently associated with prostate cancer in patients with PSA lower than 10 ng/mL, but no association was found in the whole sample. Rhoden et al.[10] reported an association between lower tT/tPSA ratios and prostate cancer in men with symptomatic hypogonadism, but this was not confirmed in a subsequent study.[32]

We did not find a clear significant association between tT levels and combined Gleason score, which is in accordance with most previous reports.[22–26] However we can see a small non-significant trend towards lower levels of tT among higher Gleason scores, pointing in the direction of two studies that found a significant association.[20,21]

Serum tT levels depend on factors such as the subjects' age, general condition and the timing and method of assaying. Age might be a source of confounding as older patients have lower testosterone levels, and in all series, patients with prostate cancer are older than patients with non-malignant disease. Some of the studies[13,16] that reported an association between prostate cancer and tT levels did not provide results adjusted for age and other possible confounders; in others,[12,29] the association was no longer statistically significant after adjustment.

Some diseases can affect tT levels and the fact that this was not taken into account could have contributed

Table 4
Total testosterone and total testosterone/total PSA ratio in relation with Gleason score among subjects with prostate cancer.

	Gleason score	*n* (%)	Median tT (pg/mL)	*p*	Median tT/tPSA	*p*
All participants	⩽6	111 (18.0)	4.59		0.67	
	7	354 (57.4)	4.25	0.553	0.57	<0.001
	8	93 (15.1)	4.22		0.36	
	⩾9	59 (9.6)	4.11		0.22	
Participants with PSA < 4 ng/mL	⩽6	16 (28.6)	4.51		1.60	
	7	35 (62.5)	4.30	0.347	1.46	0.211
	8	2 (3.6)	3.01		0.78	
	⩾9	3 (5.4)	3.98		1.21	

tT: total testosterone; tT/tPSA: total testosterone/total prostatic specific antigen ratio.

for a non-differential bias. However our main objective was to evaluate tT as a diagnostic tool for prostate cancer and therefore we used a representative sample of the patients in this situation. Also, signs and symptoms of late-onset hypogonadism were not specifically collected for in this study, so no conclusion can be drawn for clinical hypogonadism and prostate cancer diagnosis.

The gold-standard for true quantification of serum tT is the measurement by Isotope-Dilution Gas Chromatography–Mass Spectrometry. A previous study reported that the immunoassay test used in our study yields serum tT levels lower by 0.11 pg/mL, on average, with a correlation coefficient (r) of 0.95, when compared to the gold-standard.[33] However, the price of spectrometry would militate against its widespread use as a complement to traditional screening, so testing an immunoassay exam is more useful.

Our study adds to previous research on this topic the evidence from a large contemporary consecutive series of patients undergoing prostate biopsy because of elevated serum tPSA and/or an abnormal DRE, which contributes for an unbiased sample of subjects among which serum tT could be evaluated as a diagnostic tool. Moreover all biopsies were obtained using a modern scheme of ⩾12 cores, making false-negative results less likely. Furthermore, in our study, the sampling time was similar for all fasting patients, always prior to any prostate specific treatment or organ manipulation, and all hormone assays were performed in the same laboratory, thus minimising measurement bias. Due to the facts pointed we believe that our study provides the best evidence available about the potential role of serum tT as a biomarker for prostate cancer diagnosis.

Serum tT is known to be a natural stimulus for prostate cancer growth, probably due to a series of genetic rearrangements, early in carcinogenesis, conjoining oncogenic ETS transcription factors with androgen-responsive elements.[3] There are several potential explanations for serum tT not being elevated in patients with prostate cancer. The most potent androgen is dihydrotestosterone (DHT)[34] which is rapidly and irreversibly converted from testosterone.[6] Prostate cancer cells can lower tT serum levels by acting as an androgen store through an increase in the number of binding sites for testosterone,[7] and also by suppressing the pituitary–gonadal axis, possibly by secretion of inibin.[35]

Prostate cancer can also locally produce androgens from adrenal hormones[36] or get a testosterone enriched blood flow directly from the testicles, due to a damaged venous valvular system of the internal spermatic vein, frequent in the ageing men[37] making prostatic levels very different from those observed in the serum. Additionally, according to a recent saturation model[38,39] the androgens may have a limited ability to further stimulate prostate cancer growth when the serum testosterone concentration is beyond a relatively low threshold, even within the physiologic range.

A recent collaborative analysis with approximately 3900 patients with prostate cancer and 6500 control subjects found no association between serum total testosterone and future risk of prostate cancer diagnosis.[40] This result remained constant independently of the time between blood collection and prostate cancer diagnosis, supporting our own results. This, however, does not exclude other potential applications of testosterone levels in this malignancy. In the future tT may have an important clinical role as a marker of prognosis in the clinical course of the disease or as a marker of treatment response, as has been suggested during androgen-deprivation therapy.[41] In an important series of patient submitted to radical prostatectomy, tT was a significant independent predictor of extraprostatic disease despite not associated with biochemical recurrence.[42] Also free and bioavailable testosterone were not evaluated and despite a negative previous result[43] they can become interesting biomarkers for prostate cancer.

In conclusion, tT levels are similar between all types of prostatic pathology and, among prostate cancer patients, are not associated with combined Gleason score. Its role as an independent predictor of cancer or histological aggressiveness is not supported by the present series.

Conflict of interest statement

None declared.

References

1. Ferlay J, Parkin DM, Steliarova-Foucher E. Estimates of cancer incidence and mortality in Europe in 2008. *Eur J Cancer* 2010;**46**(4):765–81.
2. Brawer MK. Prostate-specific antigen. *Semin Surg Oncol* 2000;**18**(1):3–9.
3. Schulman CC, Irani J, Morote J, et al. Testosterone measurement in patients with prostate cancer. *Eur Urol* 2010;**58**(1):65–74.
4. Crawford ED, Hou AH. The role of LHRH antagonists in the treatment of prostate cancer. *Oncology (Williston Park)* 2009;**23**(7):626–30.
5. Henderson BE, Ross RK, Pike MC, Casagrande JT. Endogenous hormones as a major factor in human cancer. *Cancer Res* 1982;**42**(8): 3232–9.
6. Meikle AW, Smith JA, Stringham JD. Production, clearance, and metabolism of testosterone in men with prostatic cancer. *Prostate* 1987;**10**(1):25–31.
7. Mearini L, Costantini E, Zucchi A, et al. Testosterone levels in benign prostatic hypertrophy and prostate cancer. *Urol Int* 2008;**80**(2):134–40.
8. Schatzl G, Reiter WJ, Thurridl T, et al. Endocrine patterns in patients with benign and malignant prostatic diseases. *Prostate* 2000;**44**(3):219–24.
9. Hong SK, Han BK, Jeong JS, et al. Serum measurements of testosterone, insulin-like growth factor 1, and insulin-like growth factor binding protein-3 in the diagnosis of prostate cancer among Korean men. *Asian J Androl* 2008;**10**(2):207–13.
10. Rhoden EL, Riedner CE, Morgentaler A. The ratio of serum testosterone-to-prostate specific antigen predicts prostate cancer in hypogonadal men. *J Urol* 2008;**179**(5):1741–4.
11. Morgentaler A, Rhoden EL. Prevalence of prostate cancer among hypogonadal men with prostate-specific antigen levels of 4.0 ng/mL or less. *Urology* 2006;**68**(6):1263–7.
12. Kravchick S, Peled R, Dorfman D, et al. Predictive criteria for prostate cancer detection in men with serum PSA concentration of 2.0 to 4.0 ng/mL. *Urology* 2005;**66**(3):542–6.
13. Ghanadian R, Puah CM, O'Donoghue EP. Serum testosterone and dihydrotestosterone in carcinoma of the prostate. *Br J Cancer* 1979;**39**(6):696–9.
14. Andersson SO, Adami HO, Bergstrom R, Wide L. Serum pituitary and sex steroid hormone levels in the etiology of prostatic cancer – a population-based case–control study. *Br J Cancer* 1993;**68**(1) :97–102.
15. Bartsch W, Horst HJ, Becker H, Nehse G. Sex hormone binding globulin binding capacity, testosterone, 5alpha-dihydrotestosterone, oestradiol and prolactin in plasma of patients with prostatic carcinoma under various types of hormonal treatment. *Acta Endocrinol (Copenh)* 1977;**85**(3):650–64.
16. Hill P, Wynder EL, Garbaczewski L, Walker AR. Effect of diet on plasma and urinary hormones in South African black men with prostatic cancer. *Cancer Res* 1982;**42**(9):3864–9.
17. Raivio T, Santti H, Schatzl G, et al. Reduced circulating androgen bioactivity in patients with prostate cancer. *Prostate* 2003;**55**(3): 194–8.
18. Rutjes AW, Reitsma JB, Vandenbroucke JP, Glas AS, Bossuyt PM. Case–control and two-gate designs in diagnostic accuracy studies. *Clin Chem* 2005;**51**(8):1335–41 [Epub 2005 June 16].
19. Thompson IM, Pauler DK, Goodman PJ, et al. Prevalence of prostate cancer among men with a prostate-specific antigen level < or =4.0 ng per milliliter. *N Engl J Med* 2004;**350**(22):2239–46.
20. Schatzl G, Madersbacher S, Thurridl T, et al. High-grade prostate cancer is associated with low serum testosterone levels. *Prostate* 2001;**47**(1):52–8.
21. Zhang PL, Rosen S, Veeramachaneni R, et al. Association between prostate cancer and serum testosterone levels. *Prostate* 2002;**53**(3):179–82.
22. Teloken C, Da Ros CT, Caraver F, et al. Low serum testosterone levels are associated with positive surgical margins in radical retropubic prostatectomy: hypogonadism represents bad prognosis in prostate cancer. *J Urol* 2005;**174**(6):2178–80.
23. Fodstad P, Bjoro T, Torlakovic G, Fossa SD. No association of serum gonadal or pituitary hormones with prognostic parameters in stages T1 to T3 PN0M0 prostate cancer. *J Urol* 2002;**168**(3) :1188–92.
24. Hoffman MA, DeWolf WC, Morgentaler A. Is low serum free testosterone a marker for high grade prostate cancer? *J Urol* 2000;**163**(3):824–7.
25. Imamoto T, Suzuki H, Yano M, et al. Does presence of prostate cancer affect serum testosterone levels in clinically localized prostate cancer patients? *Prostate Cancer Prostatic Dis* 2009;**12**(1):78–82.
26. Sher DJ, Mantzoros C, Jacobus S, et al. Absence of relationship between steroid hormone levels and prostate cancer tumor grade. *Urology* 2009;**73**(2):356–61.
27. Morgentaler A. Testosterone deficiency and prostate cancer: emerging recognition of an important and troubling relationship. *Eur Urol* 2007;**52**(3):623–5.
28. Altman DG, Bland JM. Diagnostic tests 3: receiver operating characteristic plots. *BMJ* 1994;**309**(6948):188.
29. Sofikerim M, Eskicorapci S, Oruc O, Ozen H. Hormonal predictors of prostate cancer. *Urol Int* 2007;**79**(1):13–8.
30. Morote J, Ramirez C, Gomez E, et al. The relationship between total and free serum testosterone and the risk of prostate cancer and tumour aggressiveness. *BJU Int* 2009;**104**(4):486–9.
31. Yano M, Imamoto T, Suzuki H, et al. The clinical potential of pretreatment serum testosterone level to improve the efficiency of prostate cancer screening. *Eur Urol* 2007;**51**(2):375–80.
32. Morote J, Planas J, Ramirez C, et al. Evaluation of the serum testosterone to prostate-specific antigen ratio as a predictor of prostate cancer risk. *BJU Int* 2009;**105**(4):481–4.
33. Taieb J, Mathian B, Millot F, et al. Testosterone measured by 10 immunoassays and by isotope-dilution gas chromatography–mass spectrometry in sera from 116 men, women, and children. *Clin Chem* 2003;**49**(8):1381–95.
34. Marks LS, Mostaghel EA, Nelson PS. Prostate tissue androgens: history and current clinical relevance. *Urology* 2008;**72**(2):247–54.
35. Miller LR, Partin AW, Chan DW, et al. Influence of radical prostatectomy on serum hormone levels. *J Urol* 1998;**160**(2): 449–53.
36. Mizokami A, Koh E, Izumi K, et al. Prostate cancer stromal cells and LNCaP cells coordinately activate the androgen receptor through synthesis of testosterone and dihydrotestosterone from dehydroepiandrosterone. *Endocr Relat Cancer* 2009;**16**(4): 1139–55.
37. Gat Y, Joshua S, Gornish MG. Prostate cancer: a newly discovered route for testosterone to reach the prostate: treatment by super-selective intraprostatic androgen deprivation. *Andrologia* 2009;**41**(5):305–15.
38. Isbarn H, Pinthus JH, Marks LS, et al. Testosterone and prostate cancer: revisiting old paradigms. *Eur Urol* 2009;**56**(1):48–56.
39. Morgentaler A, Traish AM. Shifting the paradigm of testosterone and prostate cancer: the saturation model and the limits of androgen-dependent growth. *Eur Urol* 2009;**55**(2):310–20.
40. Roddam AW, Allen NE, Appleby P, Key TJ. Endogenous sex hormones and prostate cancer: a collaborative analysis of 18 prospective studies. *J Natl Cancer Inst* 2008;**100**(3):170–83.
41. Perachino M, Cavalli V, Bravi F. Testosterone levels in patients with metastatic prostate cancer treated with luteinizing hormone-releasing hormone therapy: prognostic significance? *BJU Int* 2010;**105**(5):648–51.
42. Massengill JC, Sun L, Moul JW, et al. Pretreatment total testosterone level predicts pathological stage in patients with localized prostate cancer treated with radical prostatectomy. *J Urol* 2003;**169**(5):1670–5.
43. Faydaci G, Bilal E, Necmettin P, et al. Baldness, benign prostate hyperplasia, prostate cancer and androgen levels. *Aging Male* 2008;**11**(4):189–92.