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Total testosterone density predicts high tumor load and disease reclassification of prostate cancer: results in 144 low-risk patients who underwent radical prostatectomy

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Abstract

Objectives The aim of this study is to evaluate the association between total testosterone density (TTD), defined as the ratio of serum TT to prostate volume (PV), and high tumor load (HTL) in low-risk prostate cancer (PCA) patients who underwent radical prostatectomy.

Materials and methods Tumor load was defined as the percentage of prostate volume invaded by cancer (PPI-PCA) in the surgical specimen. Pathologic features including tumor upgrading, upstaging or positive surgical margins in the specimen defined unfavorable disease (UD). PSA, TT, PSA density (PSAD), TTD, percentage of biopsy positive cores (BPC), PV and body mass index (BMI). The association of factors with the risk UD and HTL was evaluated by statistical methods.

Results The cohort included 144 consecutive low-risk PCA patients. Overall, 104 patients (72.2%) had at least one feature indicating UD. TTD was associated with BMI, TT, PSA, PV and PPI-PCA ≥ 20% defined as HTL. A higher PPI-PCA was associated with an increased risk of UD with a fair discriminant power (area under the curve, AUC = 0.775; $p < 0.0001$). Patients with PPI-PCA > 20% were considered the study group versus patients with a PPI-PCA < 20% (control group). BPC, PSAD and TTD were independently associated with the risk of HTL (PPI-PCA ≥ 20%) with receiver-operating characteristics (ROC) curves indicating the same discriminant power for BPC (AUC = 0.628; $p = 0.013$), PSAD (AUC = 0.611; $p = 0.032$) and TTD (AUC = 0.610; $p = 0.032$).

Conclusions Among low-risk PCA patients, TTD is associated with the risk of HTL, which is an independent predictor of UD and should be evaluated in the management of these patients.

Keywords Prostate cancer · Low-risk prostate cancer · Total testosterone · Prostate volume · Testosterone density

Abbreviations

AS	Active surveillances
AUC	Area under the curve
BMI	Body mass index
BPC	Biopsy positive cores
EAU	European association of urology
ePLND	Extended pelvic lymph node dissection HTL—high tumor load
ISUP	International society of urologic pathology
LNI	Lymph node invasion
PW	Prostate weight
PCA	Prostate cancer
PPI-PCA	Percentage of prostate volume invaded by prostate cancer
PSA	Prostate-specific antigen
PSAD	PSA density
PV	Prostate volume
RARP	Robot-assisted radical prostatectomy
ROC	Receiver-operating characteristics
RP	Radical prostatectomy

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RRP	Retropubic radical prostatectomy
TT	Total testosterone
TTD	Total testosterone density
UD	Unfavorable disease
WW	Watchful waiting

Introduction

In clinical practice, Prostate Cancer (PCA) is classified into low-, intermediate- and high risk according to the probability of recurrence after radical treatment by considering PSA serum level, biopsy Gleason score, and clinical TNM stage [1]. The European Urological Association (EAU) has established guidelines on localized and locally advanced PCA. They have stratified the former into low, intermediate and high-risk classes based on PSA, clinical TNM stage and International Society of Urologic Pathology (ISUP) groups [2] which have prognostic potential [3–5].

Patients with low-risk disease are defined by the presence of PSA < 10 ng/ml and ISUP grade 1, and cT1-2a [2]. However, a critical drawback of this system is the discrepancy between clinical and pathological findings. Indeed, low-risk patients who undergo radical prostatectomy (RP) are at risk of unfavorable disease (UD), which includes tumor upgrading, tumor upstaging and positive surgical margins [2]. In this context, we have previously demonstrated that clinical factors including PSA, and the number of positive cores are able to predict tumor reclassification in low-risk PCA patients [6–9]. Interestingly, we also found that prostate volume (PV) and total testosterone levels can stratify low-risk PCA patients and predict the risk of upgrading in this cohort [10, 11]. Although, smaller PV has been proven to be related to more aggressive PCA [12, 13], the relationship between preoperative testosterone serum levels and prostate cancer stage and grade is debated. Indeed, some evidences suggested a linear correlation between preoperative androgen levels and aggressiveness of PCA [14–16]. On the other hand, however, other studies demonstrated that low levels of testosterone are associated with more aggressive disease [17–19].

The aim of the present study is to evaluate the association of TT density (TTD), which is defined as the ratio of serum TT on prostate volume, with high tumor load (HTL) in patients with low-risk PCA undergoing radical prostatectomy (RP).

Materials and methods

Study population

This is a single institution study. We obtained Institutional Review Board approval for this retrospective study, but the data were collected prospectively. Each patient provided informed-signed consent for data collection and analysis.

In a period ranging from November 2014 to March 2018, preoperative basal levels of TT and PSA were measured in 601 consecutive patients who were not under androgen deprivation therapy and elected to undergo RP with or without extended pelvic lymph node dissection (ePLND). Serum samples of TT and PSA were obtained from a cubital vein, at least 1 month after biopsies, between 8.00 and 8.30 a.m. Our lab analyzed all samples. Plasma levels of TT (ng/dL) and PSA (ng/mL) were determined by radioimmunoassay. Age (years), body mass index (BMI; kg/m^2), prostate volume (PV, mL) prostate weight (PW) and biopsy positive cores (BPC; percentage) were calculated for each case.

Clinical staging and surgery

Tumor, nodal and metastatic status was assessed according to the TNM system [20]. Pelvic lymph node staging (cN) was performed by axial imaging modalities. Enlarged pelvic nodes measuring more than 1 cm in diameter were staged as cN1 disease. The metastatic status was investigated by both axial imaging and total bone scan modalities. Patients were classified according to the EAU risk classification system [2]. Skilled and experienced surgeons performed RP with ePLND by the robot assisted (RARP) or by the open retro-pubic (RRP) approach. RARP was delivered by the da Vinci Robot System (Intuitive Surgical, Inc, Sunnyvale, CA, USA) and was performed through the transperitoneal approach with anterograde prostatic dissection [21]. RRP was performed according to the technique of Walsh [22]. The decision to perform an extended lymph node dissection was mainly based on preoperative nomograms showing a risk of lymph node invasion greater than 5% [23]. In low-risk patients, the decision to perform an ePLND was based on clinical factors indicating increased risk of tumor upgrading in the surgical specimen [24, 25]. Lymph nodes were sampled according to an anatomical template including bilateral external iliac (extending proximally to the crossing of the ureter), obturator, Marcille's, common iliac and Cloquet's nodal stations.

Assessment of prostate biopsies and surgical specimens

Biopsies performed elsewhere were assessed for the following features: (1) at least 12 biopsy cores; (2) the reported number of positive cores; and (3) measurement of prostate volume (mL). In our Institution, the 14-core trans perineal guided prostate biopsy technique was used. Prostate volume (mL) was measured by an ellipsoid formula [diameter (d)1 × d2 × d3 × 0.52] during ultrasound exam. Tumors were classified into grade groups according to the ISUP system

[3–5]. A dedicated pathologist assessed all specimens, which were processed according to the Stanford protocol [26]. ISUP grade group system was applied to classify tumors [3, 4]. Surgical margins were stated positive when cancer invaded the inked surface of the specimen. Nodal packets were grouped according to a standard template and submitted in separate packages. Lymph nodes were assessed for histopathology after hematoxylin and eosin staining. Immunohistochemical staining was performed when appropriate. In each case, the number of removed lymph nodes and lymph node invasion (LNI) was assessed. Prostate and nodal specimens were then staged according to the TNM system [20]. As suggested by EAU guidelines, tumor volume was also investigated and evaluated as percentage of prostate volume involved by cancer (PPI-PCA).

Study objectives and design

The primary aim of this study was to evaluate the association of TTD with features of aggressive disease, which were compared to PSA and PSA density (PSAD) values, in low-risk PCA. TTD was defined as the ratio of preoperative TT levels to PV (ng/dL mL). PSAD was defined as the ratio of total PSA on PV (ng/mL^2).

According to the aim of the study, clinical and pathological factors were assessed to determine if any association with features of UD and HTL in the low-risk PCA population was present. Tumor load was evaluated by PPI-PCA. Features defining UD included tumor upgrading, tumor upstaging or positive surgical margins. The strongest factors associated with UD were then selected to stratify the low-risk population. Associations of TT, TTD, PSA and PSAD with HTL were then investigated.

Statistical methods

Summary statistics and distributions of factors between groups were assessed in the low-risk population. Data on continuous variables are reported as medians with their respective interquartile ranges (IQR) and differences among groups were assessed using the test of Kruskall and Wallis. Data on categorical variables are presented as frequencies with percentages and differences among groups were assessed using Pearson's Chi squared test or Fisher exact test as appropriate. The receiver-operating characteristic (ROC) curve analysis was used to evaluate the association of significant factors with features of UD. The area under the curve (AUC) of each significant factor was evaluated. The factor showing the highest discriminant power (AUC) was used to stratify the low-risk population after an appropriate cut-off value predicting the risk of HTL for a percentage of at least 50%. Significant clinical factors associated with HTL were evaluated by ROC curves and AUC analysis. The association with the risk of HTL and significant clinical factors was also evaluated by binary logistic regression analysis. Finally, significant clinical factors were evaluated by correlation and linear regression analysis, which stratified patients according to the cut-off point of the parameter associated with HTL. The software used to run the analysis was IBM-SPSS version 20 (SPSS Inc., IBM Corp., Armonk, NY, USA). All tests were two-sided with $p < 0.05$ considered to indicate statistical significance.

Results

Overall, 144 cases were low risk (24%), 316 intermediate risk (52.6%) and 141 high risk/locally advanced (23.4%). RARP was performed in 521 cases (86.7%) and RRP in 80 subjects (13.3%). Extended pelvic lymph node dissection was performed in 332 RARP cases (63.7%) and 73 (91.2%) of RRP patients. Our analysis will focus on the low-risk subpopulation. The demographics of the low-risk population are reported in Table 1. The patient population showed a median age of 65 years with a median PSA of 6.2 ng/mL. The tumor was not evident on imaging or digital rectal exam in 111 cases (77.1%). Considering pathological features, median BPC was 25% and the median PPI-PCA was 15%.

In the surgical specimen, tumors were upgraded in 96 cases (66.7%), upstaged in 11 patients (7.7%) with extra-prostatic extension in eight subjects (5.6%) and seminal vesicle invasion in three patients (2.1%). Positive surgical margins were detected in 35 patients (25%).

Overall, 104 patients (72.2%) had at least one feature indicating UD, as indicated in Table 1. The only clinical factor that was significantly associated with UD was BPC, which was higher in patients with UD (median 27%) compared to the control group (median 17%). Other parameters did not show any significant difference between groups. Considering pathological factors, the subpopulation showing features of UD demonstrated greater PPI-PCA (27%) compared to the control group (only 5%); moreover, the UD group showed ISUP grade group > 1 in 96 cases (92.3%), extra-prostatic extension in 11 patients (10.6%) and positive surgical margins in 36 subjects (34.6%), as shown in Table 1.

On multivariate analysis, both BPC (odds ratio, OR = 1.030; 95% CI: 1.002–1.059; $p = 0.039$) and PPI-PCA (OR = 1.078; 95% CI: 1.024–1.135; $p = 0.004$) were associated with the risk of UD. Figure 1 shows receiver-operating characteristic (ROC) curves of factors associated with the risk of UD. According to the AUC, the discriminating power of PPI-PCA was higher (AUC = 0.775; 95% CI: 0.656–0.854; $p < 0.0001$) than BPC (AUC = 0.653; 95% CI: 0.557–0.749; $p = 0.004$). So far, PPI-PCA showed a fair discrimination power and thus was used to determine the cut-off point to divide the low population.

Table 1 Clinical and pathological factors associated with high load disease in low-risk prostate cancer (PCA) including at least one of tumor upgrading/upstaging or positive surgical margins

Low-risk PCA	Population	Tumor upgrading/upstaging or positive surgical margins		
		No	Yes	*p* values
n (%)	144	40 (27.8)	104 (72.2)	
Clinical factors				
Age (years); median (IQR)	65 (60–70)	64 (59–69)	65.5 (60–70)	0.561
BMI (kg/m^2); median (IQR)	25.9 (24.2–28.1)	25.5 (24–28)	26 (24.3–28.1)	0.621
PSA (ng/mL); median (IQR)	6.2 (4.7–7.5)	6.2 (4.1–4.8)	6.2 (4.7–7.4)	0.782
TT (ng/dL); median (IQR)	377 (291.5–474.2)	374.5 (317.7–538.3)	377 (283–466.7)	0.431
PV (mL); median (IQR)	41 (30–52)	43 (36.2–53)	40 (29–52)	0.166
PSAD (ng/mL^2); median (IQR)	0.14 (0.10–0.20)	0.12 (0.09–0.20)	0.14 (0.11–0.20)	0.26
TTD (ng/dl mL); median (IQR)	9.1 (6.3–13.9)	8.5 (6.6–12.9)	9.2 (5.7–14)	0.792
BPC (%); median (IQR)	25 (14–35)	17 (14–30.7)	27 (14–42)	0.005
cT *n* (%)				0.712
cT1 *n* (%)	111 (77.1)	30 (75)	81 (77.9)	
cT2a *n* (%)	33 (22.9)	10 (25)	23 (22.1)	
Pathological factors				
PW (g); median (IQR)	53 (40–69.5)	54 (46–68.2)	52 (40–69.7)	0.806
PPI-PCA (%); median (IQR)	15 (8–20)	5 (5–15)	15 (10–24.2)	< 0.0001
ISUP grade group *n* (%)				< 0.0001
1	48 (33.3)	40 (100)	8 (7.7)	
2	59 (41)		59 (56.7)	
3	30 (20.8)		30 (28.8)	
4	6 (4.2)		6 (5.8)	
5	1 (0.7)		1 (1.0)	
pT; *n* (%)				0.032
pT2	133 (92.4)	40 (100)	93 (89.4)	
pT3	11 (7.6)		11 (10.6)	
Surgical margins *n* (%)				< 0.0001
Negative	108 (75)	40 (100)	68 (65.4)	
Positive	36 (25)		36 (34.6)	

BMI body mass index, *PSA* prostate-specific antigen, *TT* total testosterone, *PV* prostate volume, *PSAD* PSA PSA density, *TTD* TT density, *BPC* biopsy positive cores, *cT* tumor clinical stage, *PW* prostate weight, *ISUP* International Sociaty of Urologic Pathology tumor grade group, *pT* pathological tumor stage, *PPI-PCA* percentage of prostate involved by PCA

Figure 2 depicts the cumulative risk curve of PPI-PCA that predicts the risk of UD. As shown in the diagram, the risk of UD of 50% or more (0.53) corresponded to a cut-off point of PPI-PCA = 20%, which was chosen as a parameter to divide the low-risk population.

The low-risk population was divided into two groups according to PPI-PCA ≥ 20% versus less than 20% (control group), as shown in Table 2. Considering pathological parameters, patients with PPI-PCA ≥ 20% had higher median values of PPI-PCA (25% versus 10%; $p < 0.0001$), higher rates of ISUP grade group > 1 (78.7% versus 67%; $p = 0.043$), extra-prostatic extension (14.9% versus 4.1%; $p = 0.023$) and positive surgical margins (44.7% versus 15.5%; $p < 0.0001$). Considering clinical parameters, factors associated with PPI-PCA ≥ 20% include smaller median PV (34 ml versus 44.8; $p = 0.001$) as well as higher median values of BPC (29% versus 21%; $p = 0.014$), PSAD (0.15 versus 0.13; $p = 0.032$) and TTD (11.7 versus 8.2; $p = 0.032$). Other clinical factors did not show any significant association. On univariate analysis, BPC (OR = 11.024; 95% CI: 1.004–1.044; $p = 0.108$), PSAD (OR = 2.750; 95% CI: 1.232–6.139; $p = 0.014$) and TTD (OR = 1.055; 95% CI: 1.002–1.111; $p = 0.042$) were associated with an increased risk of having PPI-PCA ≥ 20% (HTL). Because of the high correlation between PSAD and TTD, which is related to PV, two bivariate models were computed. In the first one, BPC (OR = 1.025; 95% CI: 1.004–1.045; $p = 0.018$) and PSAD (OR = 2.835; 95% CI: 1.237–6.497; $p = 0.014$) was associated with the risk of PPI-PCA ≥ 20%. In the second model, both BPC (OR = 1.024; 95% CI: 1.004–1.044; $p = 0.021$) and TTD (OR = 1.054; 95% CI: 1.000–1.110; $p = 0.049$) were associated with an increased risk of PPI-PCA ≥ 20%. As

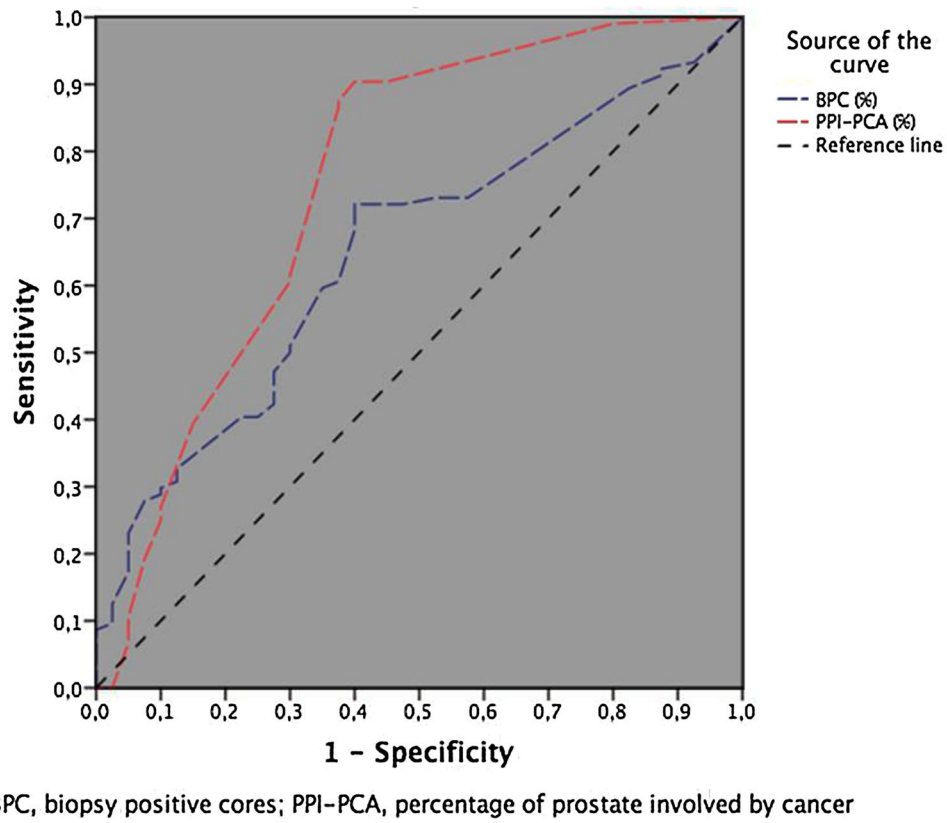

Fig. 1 Receiver-operating characteristic (ROC) curves of the factors associated with the contemporary risk of tumor upgrading, upstaging or positive surgical margins (unfavorable disease, UD) in low-risk prostate cancer (PCA). According to the area under the curve (AUC), the discriminating power of percentage of prostate involved by cancer (PPI-PCA) was higher (AUC = 0,775; 95% CI: 0.656–0.854; $p < 0.0001$) than percentage of biopsy positive cores (BPC; AUC = 0.653; 95% CI: 0.557–0.749; $p = 0.004$). PPI-PCA shows a fair discrimination power

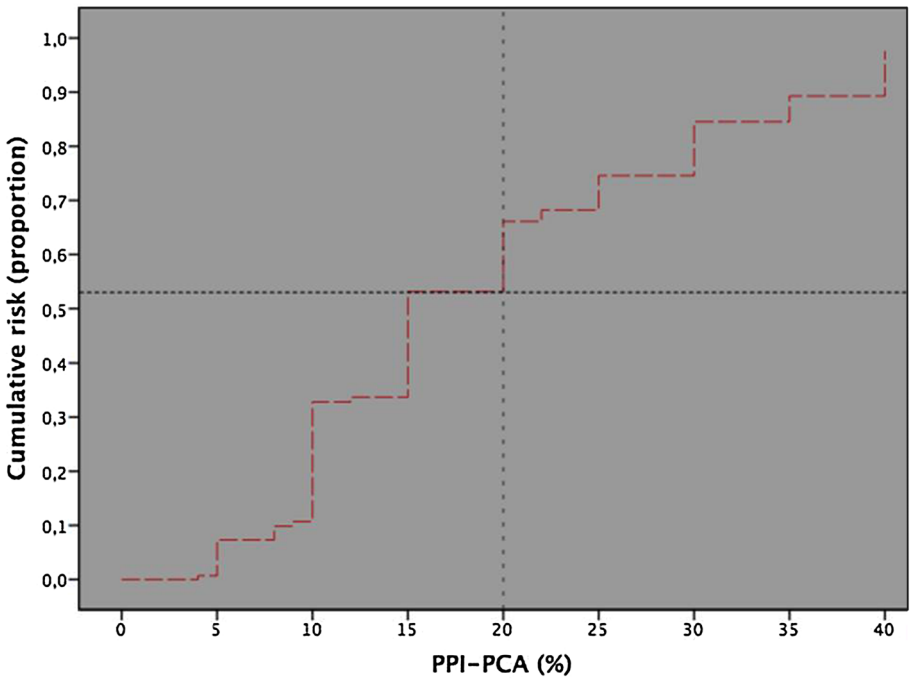

Fig. 2 Cumulative risk curve of percentage of prostate involved by cancer (PPI-PCA) that predicts the risk of tumor upgrading, upstaging or positive surgical margins (UD) in low-risk PCA. As shown in the diagram, the risk of more than 50% (0.53) corresponded to a cut-off point of PPI-PCA ≥ 20%, which was chosen as a parameter to divide the low-risk population

shown in Fig. 3, ROC curves of these factors were evaluated by AUC, which were equivalent for BPC (AUC = 0.628; 95% CI: 0.533–0.72; $p = 0.013$), PSAD (AUC = 0.611; 95% CI: 0.515–0.706; $p = 0.032$) and TTD (AUC = 0.610; 95% CI: 0.507–0.714; $p = 0.032$). After analysis, BPC, PSAD and TTD showed similar discrimination power.

Table 3 shows the correlations of the most relevant clinical and pathological factors with PSAD and TTD. As

Table 2 Clinical and pathological factors associated with percentage of prostate involved by cancer

Low-risk PCA	Population	Percentage of prostate involved by cancer		*p* values
		< 20%	≥ 20%	
n (%)	144	97 (67.4)	47 (32.6)	
Clinical factors				
Age (years); median (IQR)	65 (60–70)	66 (60–70)	64 (57–68)	0.401
BMI (kg/m^2); median (IQR)	25.9 (24.2–28.1)	26 (24.2–28.4)	25.5 (24.7–27.8)	0.478
PSA (ng/mL); median (IQR)	6.2 (4.7–7.5)	6.2 (4.8–7.5)	6.2 (4.5–7.4)	0.836
TT (ng/dL); median (IQR)	377 (291.5–474.2)	370 (295–495.4)	389 (286–463)	0.769
PV (mL); median (IQR)	41 (30–52)	44.8 (34.7–54.5)	34 (26–44)	0.001
PSAD (ng/mL^2); median (IQR)	0.14 (0.10–0.20)	0.13 (0.10–0.18)	0.15 (0.11–0.21)	0.032
TTD (ng/dl mL); median (IQR)	9.1 (6.3–13.9)	8.2 85.7–12.9)	11.7 (7.5–15.9)	0.032
BPC (%); median (IQR)	25 (14–35)	21 (14–33)	29 (20–47)	0.014
cT *n* (%)				0.241
cT1 *n* (%)	111 (77.1)	72 (74.2)	39 (83)	
cT2a *n* (%)	33 (22.9)	25 (25.8)	8 (17)	
Pathological factors				
PW (g); median (IQR)	53 (40–69.5)	57 (46.5–72)	46 (37–70)	0.001
PPI-PCA (%); median (IQR)	15 (8–20)	10 (5–15)	25 (20–35)	< 0.0001
ISUP grade group *n* (%)				0.043
1	48 (33.3)	38 (39.2)	10 (21.3)	
2	59 (41)	40 (41.2)	19 (40.4)	
3	30 (20.8)	17 (17.5)	13 (27.7)	
4	6 (4.2)	2 (2.1)	4 (8.5)	
5	1 (0.7)	0 (0.0)	1 (2.1)	
pT *n* (%)				0.023
pT2	133 (92.4)	93 (95.9)	40 (85.1)	
pT3	11 (7.6)	4 (4.1)	7 (14.9)	
Surgical margins *n* (%)				< 0.0001
Negative	108 (75)	82 (84.5)	26 (55.3)	
Positive	36 (25)	15 (15.5)	21 (44.7)	

BMI body mass index, *PSA* prostate-specific antigen, *TT* total testosterone, *PV* prostate volume, *PSAD* PSA PSA density, *TTD* TT density, *BPC* biopsy positive cores, *cT* tumor clinical stage, *PW* prostate weight, *ISUP* International Sociaty of Urologic Pathology tumor grade group, *pT* pathological tumor stage, *PPI-PCA* percentage of prostate involved by PCA

shown, PSAD is significantly correlated with PSA (Pearson's correlation coefficient, $r = 0.496$; $p < 0.0001$), PV ($r = -0.605$; $p < 0.0001$), PW ($r = -0.421$; $p < 0.0001$) and PPI-PCA ≥ 20% ($r = 0.200$; $p = 0.016$). In addition, TTD is correlated with BMI ($r = -0.269$; $p = 0.001$), TT ($r = 0.683$; $p < 0.001$), PSA ($r = -0.284$; $p = 0.001$), PV ($r = -0.670$; $p < 0.0001$), PW ($r = -0.533$; $p < 0.0001$) and PPI-PCA ≥ 20% ($r = 0.175$; $p = 0.036$). On univariate linear regression, PSA, PV, PW and PPI-PCA ≥ 20% (regression coefficient, $c = 0.032$; 95% CI: 0.006–0.058; $p = 0.016$) significantly predicted PSAD. Also, BMI, TT, PSA, PV, PW and PPI-PCA ≥ 20% ($c = 2.516$; 95% CI: 0.173–4.858; $p = 0.036$) significantly predicted TTD. On multivariate linear regression BMI ($c = -0.396$; 95% CI: −0.715 to −0.077; $p = 0.015$), PSA ($c = -0.765$; −1.291; −0.240; $p = 0.005$) and PPI-PCA ≥ 20% ($c = 2.284$; 95% CI: 0.066–4.503; $p = 0.044$) were independent predictors of TTD. The functional relation of PSAD and TTD with PSA, TT, and PPI-PCA are depicted in Figs. 4 and 5. Figure 4 shows the bivariate plot of PSAD as a function of PSA and PPI-PCA. The regression lines have been stratified by the cut-off value of PPI-PCA ≥ 20% versus < 20%. As shown, there is a significant difference of PSA mean values between PPI-PCA categories (higher when PPI-PCA < 20%) at each level of PSAD. As an example, considering PSAD median values of the population (PSAD = 0.14), PSA mean values are significantly lower for PPI-PCA ≥ 20% (PSA = 3.5 ng/mL) than PPI-PCA < 20% (PSA = 5.4 ng/mL). The bivariate plot of TTD versus TT is shown in Fig. 5, where the regression lines have been stratified by PPI-PCA (PPI-PCA ≥ 20% versus less than 20%). As shown, there are significant differences of mean TT levels for each group (significantly lower

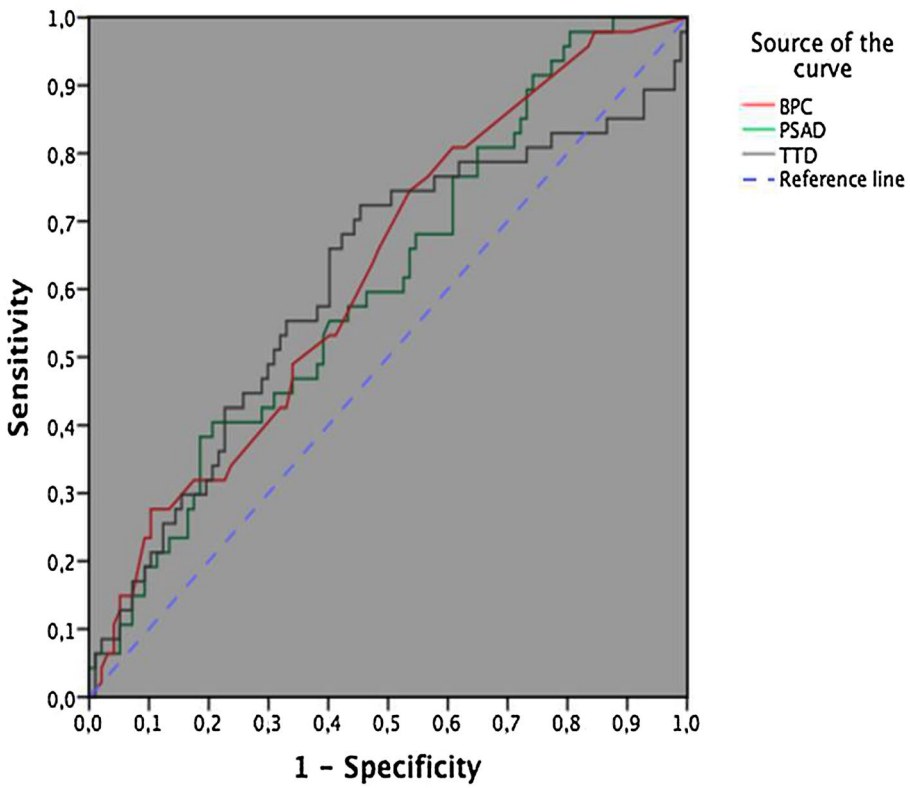

Fig. 3 Receiver-operating characteristic (ROC) curves of factors associated with the risk of detecting a percentage equal or greater than 20% (high tumor load, HTL) of percentage of prostate volume involved by cancer (PPI-PCA) in specimens of low-risk PCA. The values of the area under the curve (AUC) were equivalent for biopsy positive cores (BPC; AUC = 0.628; 95% CI: 0.533–0.72; $p = 0.013$), prostate-specific antigen density (PSAD; AUC = 0.611; 95% CI: 0.515–0.706; $p = 0.032$) and total testosterone density (TTD; AUC = 0.610; 95% CI: 0.507–0.714; $p = 0.032$). BPC, PSAD, and TTD show the same discriminating power

Table 3 Analysis of factors associated with PSAD and TTD

Factors	Statistics	Correlation analysis (*)		Univariate linear regression analysis (**)		Multivariate linear regression analysis (**)
		PSAD	TTD	PSAD	TTD	TTD
Age	Coefficient (95% CI)	−0.006	−0.136			
	p value	0.974	0.103			
BMI	coefficient (95%CI)	−0.026	−0.269			−0.396 (−0.715; −0.077)
	p value	0.754	0.001			0.015
PSA	Coefficient (95% CI)	0.496	−0.284	0.018 (0.013; 0.023)	−0.536 (−0.855; −0.217)	−0.765 (−1.291; −0.240)
	p value	< 0.0001	0.001	< 0.0001	< 0.0001	0.005
TT	Coefficient (95% CI)	−0.021	0.683		0.032 (0.027; 0.038)	
	p value	0.799	< 0.0001		< 0.0001	
PV	Coefficient (95% CI)	−0.605	−0.670		−0.270 (−0.320; −0.221)	
	p value	< 0.0001	< 0.0001		< 0.0001	
BPC	Coefficient (95% CI)	0.030	0.048			
	p value	0.719	0.566			
PW	Coefficient (95% CI)	−0.421	−0.533	−0.002 (−0.002; −0.001)	−0.180 (−0.227; −0.133)	
	p value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	
PPI− PCA ≥ 20%	Coefficient (95% CI)	0.200	0.175	0.032 (0.006; 0.038)	2.516 (0.173; 4.858)	2.284 (0.066; 4.503)
	p value	0.016	0.036	0.016	0.036	0.044

BMI body mass index, *PSA* prostate-specific antigen, *TT* total testosterone, *PSAD* PSA density, *TTD* total testosterone density, *PV* prostate volume, *BPC* biopsy positive cores, *PPI-PCA* percentage of prostate involved by cancer

*Pearson's correlation coefficient r reported

**Linear regression coefficients c reported with relative 95% CI

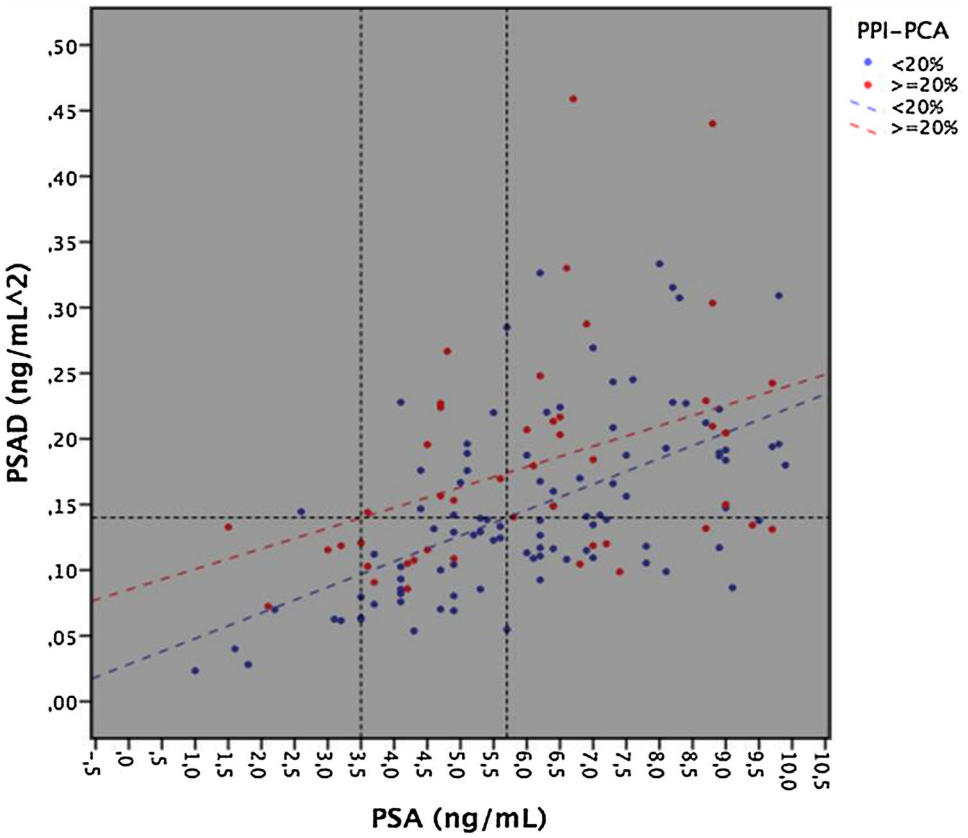

Fig. 4 Bivariate plot of prostate-specific antigen (PSA) density (PSAD) as a function of PSA. The regression lines have been stratified by the cut-off value of percentage of prostate involved by cancer (PPI-PCA ≥ 20% versus less than 20%). As shown in the figure, there is a significant difference of PSA mean values between PPI-PCA categories (higher when PPI-PCA < 20%) at each value of PSAD. As an example, considering PSAD median values of the population (PSAD = 0.14), PSA mean values are significantly lower for PPI-PCA ≥ 20% (PSA = 3.5 ng/mL) than PPI-PCA < 20% (PSA = 5.4 ng/mL)

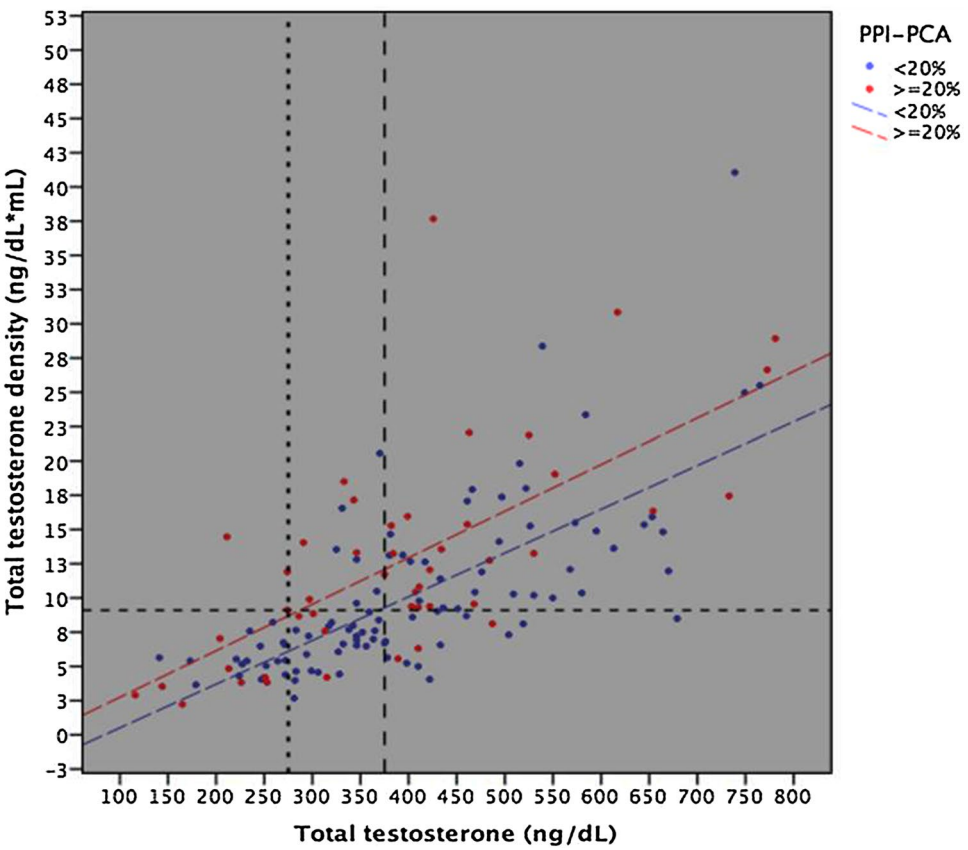

Fig. 5 Bivariate plot of total testosterone (TT) density (TTD) as a function of TT. The regression lines have been stratified by values of percentage of prostate involved by cancer (PPI-PCA ≥ 20% versus < 20%). As shown, there are significant differences of mean TT levels for each group and at each level of TTD (significantly lower HTL group with PPI-PCA ≥ 20%). As an example, considering the median PSAD of the low-risk population (TTD = 9.1), mean levels of TT are significantly lower for HTL (PPI-PCA ≥ 20%; TT = 270 ng/dL) than low tumor load (PPI-PCA < 20%; TT = 370 ng/dL)

in HTL group with PPI-PCA ≥ 20%) at each level of TTD. As an example, considering the median TTD of the low-risk population (TTD = 9.1), mean levels of TT are significantly lower for HTL (TT = 270 ng/dL) than for low tumor load (PPI-PCA < 20%; TT = 370 ng/dL).

Discussion

Low-risk PCA, as defined by EAU criteria, is an interesting and challenging subpopulation that may be treated by several options, which include watchful waiting (WW) and active surveillance (AS), surgery or radiotherapy [2]. However, low-risk PCA is a heterogeneous subpopulation that is clinically under-graded and under-staged, as shown by series treated by active surveillance or surgery [2]. This is a serious drawback that does not always allow the clinician to decide the appropriate treatment. Novel biological markers and mpMRI modalities are promising but do not have application in everyday practice [2]. Thus, we need additional clinical parameters predicting the risk of UD in low-risk PCA. Although BPC, percentage of cancer involving each core and PSAD have shown to predict the risk of HTL, these factors are not sufficient to define the natural history of the disease after treatment [2, 27, 28].

In our study, we have shown that the low-risk subpopulation, which included 24% of all patients undergoing surgery, was heterogeneous with at least one feature indicating UD (upgrading, upstaging and/or positive surgical margins) in 72.2% of cases. We showed that UD was predicted by both BPC and PPI-PCA with the latter showing higher discriminant power when compared to the former on ROC analysis (Fig. 1). A cut-off point of PPI-PCA ≥ 20% was chosen, because it corresponded to a risk of detecting UD in 50% of patients (Fig. 2). Independent clinical predictors associated with the risk of HTL (PPI-PCA ≥ 20%) were BPC, PSAD and TTD with equivalent discriminant power (Fig. 3). Tumor volume measurement by PPI-PCA is a simple and effective pathological parameter to assess tumor load, as suggested by ISUP [4]. Our study showed that BPC, PSAD and TTD are equivalent in predicting the risk of HTL in this category of patients undergoing surgery. BPC is a well-known parameter that has shown to be associated with aggressive disease [2]. In clinical practice, PSAD has long shown to be a useful parameter for distinguishing benign prostatic hypertrophy (BPH) and PCA [2]. Freedland and associates showed further application of this parameter in clinical practice when they demonstrated that pathological PSAD was a strong predictor of advanced pathology and biochemical failure after surgery [29]. Our study confirmed these findings using PV, which is a parameter that we detected clinically before planning any treatment. The novelty of our study is that we identified TTD as a new parameter for predicting HTL, which predicts UD in the low-risk population; moreover, TTD discriminant power was equivalent to other well-assessed parameters including BPC and PSAD. The best of our knowledge, this is the first study showing the association between TTD and the risk of aggressive disease in low-risk PCA.

In clinical investigations, the association of TT with PCA is a subject that has been extensively investigated but no definitive conclusions have been made, because evidence remains controversial and controlled studies have not been carried out [30]. Controversy related to this topic concerns the association between preoperative levels of TT and prostate cancer biology in patients treated with RP [30, 31]. Indeed, there are studies reporting some association, both positive as well as negative, and studies that do not report any association at all [30, 31]. In clinical practice, TT biology is a critical issue that has been recently outlined by a EAU position paper that describes TT as a pivotal sex hormone, which is closely related to men's health and prostate biology [2]. However, we need clinical studies that will let us understand the complex relationship between TT and prostate disease including BPH and PCA, which are both present in the aging male. Periodic evaluations of TT hormone levels will probably allow a better understanding of the interactions with prostate physiology [32]. In our opinion, a rigorous methodology for approaching the subject is missing in the reported literature [30, 31]. In this study, we have introduced a completely new parameter in evaluating PCA in the low-risk population. This group of patients is heterogeneous, since it includes low-risk PCA patients and patients who have high tumor load associated with unfavorable disease. As shown by the ROC curves, our study demonstrated that TTD together with PSA and BPC is associated with the risk of HTL and thus with PCA biology. This study is the first one showing evidence between TTD and PCA biology; moreover, new findings relating TT with PCA biology are being discovered.

Biology of prostate diseases, which include BPH and PCA, are closely related to variations in volumes of the gland. Gland size variations are a result of hormonal changes that occur over the lifetime of aging patients who have variations in their BMI, which correlates with both TT and PV [2].

Elevated PSA levels may be related to benign prostate growth as well as PCA, which is hormone dependent [2]. PSA, TT, PV and BMI are factors closely associated with PCA biology. PSAD is a factor that expresses the interaction between the gland size and its product, which is represented by PSA levels. As such, this parameter is able to distinguish between BPH and PCA, which often coexists due to the aging process [33]. In our study, we have shown new insights regarding PSAD and PCA biology. As shown in Fig. 4, patients who have similar levels of PSAD have lower

mean PSA values for high tumor load than patients who have low tumor load, because the former had significantly lower PV than the latter. Thus, high tumor load is associated with small gland size and lower PSA levels in low-risk PCA. This finding explains why PSA alone did not predict the risk of high tumor load among low-risk PCA patients. The negative association between PV and PCA biology has extensively been demonstrated by Freedland and associates who showed that patients who have smaller prostates have an increased risk of having features of more aggressive biology such as higher grade cancers, more advanced stage disease and higher risk of progression after surgery than patients who have larger glands [12]. Patients with equal values of PSAD may have more aggressive biology for smaller prostates with lower PSA values in low-risk PCA. Although this feature represents a new finding in the subject dealing with PSAD and PCA biology, confirmatory studies are needed.

In the present study, we introduced a new clinical parameter that combines basal TT and PV into a ratio, which we defined as TTD. This ratio was found to be positively associated with HTL with a discriminant power that was equivalent to BPC and PSAD that have been demonstrated to have an important role in the prediction of PCA biology. Low-risk prostate cancer is an inhomogeneous category that can have different biological behaviors. In this context TTD has to be considered a tool that should be evaluated in addition to BPC and PSAD to correctly assess the risk of UD in low-risk PCA patients.

The study of this factor allowed new insights into PCA biology, which have not been shown by previous studies [30, 31]. As shown in Fig. 5, equivalent TTDs were associated with different tumor loads in the low-risk population. Indeed, at each TTD level, patients with HTL (PPI-PCA $\geq 20\%$) showed significantly lower mean TT levels when compared to cases with low tumor load (PCI-PCA $< 20\%$). This result is quite amazing and could explain all the controversies that have been reported by the literature addressing this subject [30–32]. These findings might be explained by considering the close associations between BMI, PV and TT in the natural history of prostate diseases. As previously reported, there is evidence that smaller prostates are associated with features of more aggressive PCA [12]. Smaller prostates are associated with lower androgenic activity in the prostate microenvironment, which is associated with more aggressive cancers [12, 34, 35]. These features were demonstrated by our study, which showed that, at each level of TTD, patients with HTL had smaller prostates and lower TT levels than cases with low tumor load (PPI-PCA $< 20\%$).

It is known that many chronic diseases can influence TT serum levels in males. Among these obesity has a pivotal role [36]. Interestingly, several studies investigated the relationship between BMI, metabolic syndrome and PCA and reported controversial results. Particularly, it has been shown that BMI is a risk factor for aggressive PCA by several biologic tumor mechanisms that facilitate progression [37, 38] as well as high grade intraoperative complications after RARP [39]. Interestingly, a recent meta-analysis showed that diabetes is not related with the risk of biochemical recurrence in PCA patients [40]; however, on the other hand, diabetes mellitus has been associated with short PSA doubling time after radical prostatectomy [41]. Furthermore, in a Korean population of 880 PCA patients, Koo et al. demonstrated that obese and overweight patients had lower Gleason score and a reduced risk of biochemical recurrence compared to normal weight patients underlining an interference of external and racial factors [42].

Our study has limitations and strengths. Considering the limitations, several issues should be listed. First, it was a retrospective study and such that it suffers from bias due to studies of this nature. Second, TT was measured only once, which cannot be considered as a parameter reflecting the overall testosterone status in each patient. Third, TT was measured by commercially available analytic methods and not by gas chromatography–mass spectrometry, which is considered the most effective method for evaluating TT levels [43]. Fourth, we did not assess the hypothalamic–pituitary–gonadal axis. Fifth, we acknowledge the potential for variations of TT levels related to seasonal changes, which have been documented [44]. Sixth, prostate volume was not always measured in our institution; however, when the volume was evaluated at an outside institution, it was done by TRUS method, which is the standard worldwide. Seventh, our dedicated pathologist did not review biopsies, which were performed outside our institution. Eight, data were retrospectively evaluated. However, our study has also several strengths, which should be considered. First, it was single center and as such, the patient population was homogenous for TT measurements at the same laboratory. Second, all TT samples were collected in the morning between 8.00 and 8,30 am. Third, the dedicated pathologist assessed all specimens. Fourth, data were prospectively collected.

Conclusions

In patients with low-risk PCA who underwent surgery, TTD is associated with the risk of high tumor load, which predicts the risk of unfavorable disease including upgrading, upstaging or positive surgical margins. At each level of TTD, mean TT levels were significantly lower in patients with high tumor load. TTD is a simple and effective and low cost tool that should be evaluated in low-risk PCA patients who elect to undergo active treatment.

Author contributions ABP: project development, data analysis and interpretation, manuscript writing. AT: project development, data collection, data analysis and interpretation, manuscript writing. MS, MP, TP, NA, and RR: data collection. AS: data collection and language and critical revision. MB, FM, SS, and WA: other (supervision and critical revision).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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