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Original Article

Low levels of serum testosterone in middle-aged men impact pathological features of prostate cancer

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ABSTRACT

Background: Serum testosterone deficiency increases with aging. Age is also a major risk factor for prostate cancer (PrCa) and PCa tumors are more frequently diagnosed among men >65 years old. We evaluated the relationship between preoperative serum testosterone and clinical/ pathological features of PrCa in middle-aged and elderly patients.

Methods: A total of 605 PrCa patients who underwent robotic-assisted radical prostatectomy between September 2010 and January 2013 at the University of Pennsylvania, and who had serum testosterone levels measured using Elecsys Testosterone II Immunoassay were included in this IRB-approved protocol. Androgen deficiency was determined as serum free testosterone (FT) <47 pg/ml and total testosterone (TT) <193 ng/dl. Demographic, clinical and tumor characteristics of men with low vs. normal TT or FT were compared using t-test or chi-square tests. Logistic regression was used to determine associations of clinical and pathological variables with FT or TT levels.

Results: Among middle-aged men (45–64 years; $n = 367$), those with low FT and low TT had, on average, a higher BMI (29.7 vs. 27.4, $P < 0.01$; and 32.2 vs. 27.6; $P < 0.01$, respectively) and higher proportion of Gleason 8–10 PrCa (13.3% vs. 4.8%, $P = 0.011$; and 19.2% vs. 5.1%, $P = 0.012$) compared to men with normal FT and normal TT values. Patients with low FT had also higher number of positive cores on biopsy (3.9 vs. 3.1 $P = 0.019$) and greater tumor volume (7.9 ml vs. 6.1 ml, $P = 0.045$) compared to those with normal FT. Among men ≥65 years ($n = 135$) there was no difference in prostatectomy specimens of PrCa between patients with low or normal FT or TT.

Conclusion: Among men aged 45–64 years low serum pretreatment FT and TT predicted more aggressive features of PrCa in prostatectomy specimens. In middle-aged patients low testosterone levels measured pre-operatively may indicate more aggressive disease parameters.

1. Introduction

Serum total testosterone (TT) measurements are used to assess the androgen status in men. Free testosterone (FT) is the clinically relevant fraction of TT, and serum levels of FT depend on the interplay between sex hormone binding globulin (SHBG) levels and its affinity for testosterone (T). Alterations in the complex T–SHBG interaction are commonly found in obese[1] and elderly[2] patients. The preferred method for androgen evaluation in these men is the measurement of serum FT levels. In addition, SHBG levels and function can be altered by several comorbidity conditions including liver disorders, thyroid disorders, diabetes mellitus, and hypo- or hyperalbuminemia.

The dependence of prostate cancer (PrCa) on serum androgen levels was demonstrated for the first time in 1941 by Huggins and Hodges[3] when they were treating patients with metastatic disease. Since then, various studies have analyzed the relationship between serum levels of T and risk of PrCa with conflicting results.[4–13] Although the association between T levels and overall PrCa risk is

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inconclusive, some studies have reported that low levels of preoperative T increases the risk of higher Gleason score PrCa,[4,5] advanced tumor stage,[6–9] and biochemical PrCa recurrence following radical prostatectomy (RP).[10,11]

Serum T deficiency is a complex multifaceted issue and the prevalence increases with age, making it more frequent in men over 65 years of age in comparison with middle-aged men.[14,15] The incidence of PrCa also increases with aging, and PrCa tumors are more frequently diagnosed in men over the age of 65 years in the USA.[16,17] Current treatment options for low-risk PrCa patients involve surgery, radiation, or active surveillance for the appropriately selected patient. It is imperative to decipher among the men with low-risk clinical stage who will overall benefit from a curative intent.

Currently, there is no study evaluating associations between prediagnostic FT and TT levels and pathological features of PrCa separately in middle-aged and elderly patients. We examined associations between serum T levels, and clinical and pathological features of PrCa in a cohort of PrCa patients who underwent prostatectomy, and then conducted stratified analysis in middle-aged versus elderly patients.

2. Materials and methods

2.1. Patients and data collection

We retrospectively reviewed medical records of 968 patients diagnosed with clinically localized PrCa (cT1–cT2) who underwent robotic-assisted laparoscopic RP performed by a single surgeon (D.I.L.) at the University of Pennsylvania between September 2010 and January 2013. All patients' information was collected in a prospective registry that was approved by our Institutional Review Board. A total of 605 patients who had their TT and FT levels measured using Testosterone II Immunoassay were screened for participation in the study. In order to evaluate the androgen status uniformly, patients were excluded from this study if their T levels were measured using an alternative assay ($n = 324$), were not collected preoperatively ($n = 39$), or if they had incomplete clinical data ($n = 11$). In addition, patients who had preoperative prostate resection (Transurethral resection of the prostate (TURP), $n = 10$), diabetes mellitus ($n = 62$), or hypo- or hyperthyroidism ($n = 20$) were excluded, because of potential T level variations in these diseases. No patient had hypo- or hyperalbuminemia, or prior use of androgen deprivation therapy. A total of 502 patients met the inclusion criteria.

Demographic, clinical, and pathological data that were collected from medical records and analyzed included reports on the age at surgery, body mass index (BMI; kg/m^2), race, preoperative prostate-specific antigen (PSA), clinical stage, serum TT and FT levels, prostate biopsy, and surgical pathology. Clinical stage was determined using the Union for International Cancer Control (UICC) 2002 tumor-node-metastasis (TNM) system. All pathological specimens were reviewed at our institution using standard pathology procedures.

2.2. T measurement

Serum levels of TT and FT were measured as part of the pre-RP evaluation protocol, and the time of collection was between 7 AM and 5 PM (during the work hours of outpatient laboratory testing). Both TT and SHBG were measured with the Elecsys 2010 analyzer by ARUP Laboratories (Salt Lake City, UT, USA) using the electrochemiluminescence immunoassay provided by Roche Diagnostics (Indianapolis, IN, US). The lower limits of detection of SHBG and TT were 1 nmol/L and 3 ng/mL, respectively. FT levels were calculated based on a mathematical model of the TT, SHBG, and albumin-binding equilibria using a novel spreadsheet method,[18] which provides an acceptable assessment compared with the gold standard of equilibrium dialysis. The threshold for hypogonadism was adjusted in accordance with patients' age and defined as FT levels <47 pg/mL and TT levels <193 ng/dL, using the definitions of ARUP Laboratories.

2.3. Statistical analyses

Demographic, clinical, and tumor characteristics between patients with low and normal FT or TT levels were compared using Student t tests for continuous normally distributed variables or Wilcoxon sign-rank test for continuous non-normally distributed variables, and Chi-square tests for categorical variables. We carried out these analyses among all patients as well as among middle-aged (45–64 years) and elderly (65 years or older) patients. For demographic, clinical, and pathological variables that were associated with low FT or TT levels in univariate analysis ($P < 0.05$), we fitted multivariate logistic regression models to examine associations in middle-aged and elderly patients. All tests were two sided using a significance level of $\alpha = 0.05$. Statistical analysis was performed using STATA version 13.0 (Stata Statistical Software: Release 13. College Station, TX: StataCorp LP).

3. Results

Of the 502 patients included in the study, 102 (20.3%) and 33 (6.6%) of men had preoperatively low serum FT and TT levels, respectively. In Table 1, we compared middle-aged patients with elderly ones and found that elderly patients presented a greater total Gleason score on biopsy ($P < 0.01$) or in surgical specimens ($P = 0.004$), a higher prostate volume ($P = 0.002$), and a higher pathological stage ($P = 0.03$).

The results of univariate analysis of characteristics among all patients with low T compared with those with normal T are presented in Table 2. Both patients with low FT levels and those with low TT levels were more likely to have an elevated BMI (FT groups: 29.4 vs. 27.4; $P < 0.01$; TT groups: 31.2 vs. 27.6; $P < 0.01$) and a greater percentage of positive lymph nodes (FT groups: 5.7% vs. 1.0%; $P = 0.002$; TT groups: 9.1% vs. 1.5%; $P = 0.003$) in comparison with the normal groups. For all patients, those with low FT levels were significantly more likely to have a greater number of positive cores on biopsy (3.9 vs. 3.1; $P = 0.014$) and a greater tumor volume (TV) in prostatectomy specimens (8.0 vs. 6.4; $P = 0.049$). All other pathologic findings were not substantially different between the two comparison groups.

We further examined demographic and clinical characteristics of patients stratified by age (i.e., middle-aged vs. older men). Table 3 shows a univariate comparison of low versus normal FT and TT levels in middle-aged patients (age 45–64 years). There were statistically significant differences between low ($n = 75$) and normal ($n = 292$) serum FT groups regarding BMI (29.7 vs. 27.4; $P < 0.01$), the number of positive cores (3.9 vs. 3.1; $P = 0.018$), total Gleason score on pathological report ($P = 0.011$), TV (7.9 vs. 6.1, $P = 0.045$), and positive lymph node rates (6.7% vs. 1%, $P = 0.003$). Among patients with low and normal serum TT levels, there were statistically significant differences regarding BMI (32.2 vs. 27.6, $P < 0.01$), total Gleason score on pathological report ($P = 0.012$), and positive lymph node rates (11.5% vs. 1.5%, $P = 0.001$).

Multivariate logistic regression was performed to analyze factors associated with a greater total Gleason score, a higher pathological stage, and positive lymph nodes in surgical specimens. Table 4 shows the predictors of a more aggressive total Gleason score in surgical specimens using a multinomial logistic regression model. Compared with patients with a Gleason score of 6, those with a Gleason score of 7 had increased preoperative PSA [Gleason

Table 1
Demographic and clinical characteristics of all PrCa patients (middle aged and elderly).

	Overall ($n = 502$)	Middle aged ($n = 367$)	Elderly ($n = 135$)	P[a)]
Age, mean (SD)	59.7 (7.2)	56.8 (4.9)	68.6 (3.1)	<0.01
BMI, mean, (SD)	27.8 (4.0)	27.9 (4.2)	27.5 (3.8)	0.35
Race, *n* (%)				
Caucasian	450 (89.6)	332 (90.5)	118 (87.4)	0.14
African American	43 (8.6)	31 (8.4)	12 (8.9)	
Asian	9 (1.8)	4 (1.1)	5 (3.7)	
Preoperative PSA, median (IQR)	4.7 (2.7)	4.7 (2.8)	5.0 (2.5)	0.34[b)]
Total testosterone, *n* (%)				
Low	33 (6.6)	26 (7.1)	7 (5.2)	0.45
Normal	469 (93.4)	341 (92.9)	128 (94.8)	
Free testosterone, *n* (%)				
Low	102 (20.3)	75 (20.4)	27 (20)	0.91
Normal	400 (79.7)	292 (79.6)	108 (80)	
Clinical stage, *n* (%)				
T1	438 (87.2)	323 (88)	115 (85.2)	0.39
T2	64 (12.7)	44 (12)	20 (14.8)	
Prostate biopsy				
Total cores, mean (SD)	12 (0.3)	12 (0.3)	12 (0.3)	0.72
Positive cores, mean (SD)	3.3 (2.3)	3.2 (2.4)	3.5 (2.3)	0.32
Gleason score, *n* (%)				
≤6	266 (53)	210 (57.2)	56 (41.5)	<0.01
7	186 (37)	124 (33.8)	62 (45.9)	
≥8	50 (10)	33 (9)	17 (12.6)	
Surgical specimen				
Gleason score, *n* (%)				
≤6	154 (30.7)	120 (32.7)	34 (25.2)	0.04
7	307 (61.1)	223 (60.8)	84 (62.2)	
≥8	41 (8.2)	24 (6.5)	17 (12.6)	
Prostate volume, mean (SD)	47 (18.2)	45.4 (16.8)	51.9 (21)	0.002
Tumor volume, mL (SD)	6.7 (5.8)	6.5 (5.9)	7.6 (5.7)	0.055
Stage, *n* (%)				
T2	388 (77.3)	293 (79.8)	95 (70.4)	0.03
T3	114 (22.7)	74 (20.2)	40 (29.6)	
Positive surgical margins, *n* (%)	96 (19.1)	67 (18.3)	26 (19.3)	0.79
Positive lymph nodes, *n* (%)	10 (2)	8 (2.2)	2 (1.5)	0.62

BMI, body mass index; IQR, interquartile range; PrCa, prostate cancer; PSA, prostate-specific antigen; SD, standard deviation.
[a)] Comparing middle-aged and elderly patients only.
[b)] We used Wilcoxon sign-rank tests given the non-normal distribution.

score 7: odds ratio (OR) = 1.2, 95% confidential interval (CI): 1.08−1.33] and a greater number of positive cores on biopsy (OR = 1.52, 95% CI: 1.3−2.59), while patients with Gleason scores of 8−10 had increased preoperative PSA (OR = 1.32, 95% CI: 1.16−1.5), low FT levels (OR = 3.18, 95% CI: 1.38−2.12), low TT levels (OR = 4.25, 95% CI: 1.05−17.31), a greater number of positive cores on biopsy (OR = 1.73, 95% CI: 1.39−2.14), and a higher clinical stage (OR = 1.71, 95% CI: 1.38−2.12). Logistic regression assessing independent predictors of positive lymph nodes in surgical specimens is shown in Table 5. Increased preoperative PSA (OR = 1.18, 95% CI: 1.04−1.34), low FT levels (OR = 9.45, 95% CI: 1.52−58.69), and low TT levels (OR = 12.35, 95% CI: 2.1−72.49) were associated with positive lymph nodes. Independent predictors of a higher pathological stage were also assessed using binary logistic regression (Table S1). Increased preoperative PSA (OR = 1.09, 95% CI: 1.01−1.18), a greater total Gleason score on biopsy (OR = 1.98, 95% CI: 1.07−3.67; OR = 3.59, 95% CI: 1.49−8.63), and a greater number of positive cores on biopsy (OR = 1.25, 95% CI: 1.11−1.39) were associated with a higher pathologic stage. While neither FT nor TT was associated with a higher tumor stage.

In elderly patients (aged 65 years and above), the low TT group was presented with a higher Gleason score on biopsy compared with the normal TT group (42.8% vs. 10.9%, $P = 0.035$). This finding was not reproduced when we compared low versus normal TT regarding their Gleason scores in the specimen report, which is more accurate. Three of seven cases (43%) were downgraded to Gleason scores 7 and 6 (Table S2). Overall, 165 (33%), 265 (53%), and 71 (14%) patients had their biopsy Gleason score upgraded, the score remained the same, and the score was downgraded in the specimen report, respectively.

4. Discussion

As the US population ages, there is a growing concern about the increasing prevalence of both T deficiency[14,15] and the risk of PrCa.[16,17] Concurrently, PSA as a screening tool has raised concerns about overdiagnosis and overtreatment of PrCa. While a conservative approach may be reasonable for men with low-grade PrCa, higher-grade PrCa should be treated more aggressively.[19,20]

In our study, we evaluated preoperative serum levels of TT and FT with subsequent clinical and pathological features of PrCa among middle-aged and elderly men. Interestingly, in our study, the prevalence of low FT and low TT was 20% and 7%, respectively. This is consistent with prior studies reporting a greater proportion of patients with low FT levels compared with those with low TT levels.[2,14,15] Furthermore, a similar prevalence of low FT and TT levels was reported by Schnoeller et al[6] using the laboratory normal range for serum TT and FT levels. While studying a homogenous Caucasian cohort ($n = 137$), they correlated higher pathological Gleason scores in patients with low pretreatment TT levels (30% vs. 5.6%; $P = 0.018$) and more advanced disease (pT3−4 and/or positive lymph nodes) with pretreatment low FT levels. The number of positive cores found on prostate biopsy and TV in surgical specimens were not reported. In our study, including a larger non-homogenous patient population (Afro-American = 8.6%), we did not find the same correlation. Instead, the univariate analyses of the

Table 2
Demographic and clinical characteristics of all PrCa patients stratified by free and total testosterone levels.

	FT			TT		
	Low FT ($n=102$)	Normal FT ($n=400$)	P	Low TT ($n=33$)	Normal TT ($n=469$)	P
Age, mean (SD)	60.5 (6.6)	59.9 (6.9)	0.423	59.3 (6.4)	60.1 (6.9)	0.50
BMI, mean (SD)	29.4 (5.1)	27.4 (3.6)	<0.01	31.2 (5)	27.6 (3.9)	<0.01
Race, *n* (%)						
Caucasian	92 (90.2)	358 (89.5)	0.78	29 (87.9)	421 (89.8)	0.56
African American	9 (8.8)	34 (8.5)		4 (12.1)	39 (8.3)	
Asian	1 (1)	8 (2)		0	9 (1.9)	
Preoperative PSA, median (IQR)	5.0 (2.3)	4.7 (2.7)	0.43[a)]	5.5 (2.6)	5.8 (4.6)	0.48
TT, mean (SD)	237 (76.4)	422 (136.1)	<0.01	162 (26.2)	401 (138.7)	<0.01
FT, mean (SD)	38.8 (6.7)	68.5 (16.1)	<0.01	36 (9.1)	64 (18)	<0.01
Clinical stage, *n* (%)						
T1	90 (88.2)	343 (85.8)	0.52	28 (84.8)	410 (87.4)	0.67
T2	12 (11.8)	57 (14.2)		5 (15.2)	59 (12.6)	
Prostate biopsy						
Total cores, mean (SD)	12.1 (0.54)	12 (0.16)	0.59	12.1 (0.56)	12.0 (0.25)	0.63
Positive cores, mean (SD)	3.9 (2.7)	3.1 (3.0)	0.014	3.9 (2.8)	3.2 (2.3)	0.20
Gleason score, *n* (%)						
≤6	46 (45.1)	220 (55)	0.09	13 (39.4)	253 (53.9)	0.14
7	41 (40.2)	145 (36.2)		14 (42.4)	172 (36.7)	
≥8	15 (14.7)	35 (8.8)		6 (18.2)	44 (9.4)	
Surgical specimen						
Gleason score, *n* (%)						
≤6	25 (27)	129 (32.3)	0.15	9 (27.3)	145 (30.9)	0.31
7	64 (61)	243 (60.7)		19 (57.6)	288 (61.4)	
≥8	13 (12)	28 (7)		5 (15.1)	36 (7.7)	
Prostate volume, mean (SD)	47.4 (18.8)	46.4 (15.9)	0.58	45.5 (16.5)	47.3 (18.4)	0.56
Tumor volume, mL (SD)	8.0 (7.6)	6.4 (5.2)	0.049	8.4 (7.3)	6.7 (5.7)	0.19
Stage, *n* (%)						
T2	76 (74.5)	312 (78)	0.45	24 (72.7)	364 (77.5)	0.52
T3	26 (25.5)	88 (22)		9 (27.3)	105 (22.5)	
Positive surgical margins, *n* (%)	17 (16)	76 (19)	0.58	5 (15.2)	88 (18.8)	0.61
Positive lymph nodes, *n* (%)	6 (5.7)	4 (1)	0.002	3 (9.1)	7 (1.5)	0.003

BMI, body mass index; FT, free testosterone; IQR, interquartile range; PrCa, prostate cancer; PSA, prostate-specific antigen; SD, standard deviation; TT, total testosterone.
[a)] We used Wilcoxon sign-rank tests given the non-normal distribution.

Table 3
Demographic and clinical characteristics of middle-aged PrCa patients stratified by free and total testosterone levels.

	FT			TT		
	Low FT ($n=75$)	Normal FT ($n=292$)	P	Low TT ($n=26$)	Normal TT ($n=341$)	P
Age, mean (SD)	57.4 (4.4)	56.7 (5.0)	0.19	56.8 (4.5)	56.9 (4.9)	0.96
BMI, mean (SD)	29.7 (5.2)	27.4 (3.7)	<0.01	32.2 (4.5)	27.6 (3.9)	<0.01
Race, *n* (%)						
Caucasian	67 (89.3)	265 (90.8)	0.92	23 (88.5)	309 (90.6)	0.73
African American	7 (9.3)	24 (8.2)		3 (11.5)	28 (8.2)	
Asian	1 (1.4)	3 (1)		0	4 (1.2)	
Preoperative PSA, median (IQR)	5.05 (2.55)	4.6 (2.72)	0.08	4.7 (2.2)	4.7 (2.8)	0.77
TT, mean (SD)	232 (74.6)	410 (129.6)	<0.01	163 (27.4)	390 (132)	<0.01
FT, mean (SD)	39.6 (6.1)	69.1 (16)	<0.01	37.4 (8.5)	65.1 (17.9)	<0.01
Clinical stage, *n* (%)						
T1	65 (86.7)	258 (88.3)	0.71	22 (84.6)	301 (88.3)	0.60
T2	10 (13.3)	34 (11.7)		4 (15.4)	40 (11.7)	
Prostate biopsy						
Total cores, mean (SD)	12.1 (0.6)	12.0 (0)	0.16	12.12 (0.6)	12.01 (0.2)	0.38
Positive cores, mean (SD)	3.9 (2.9)	3.1 (2.2)	0.019	3.9 (2.9)	3.2 (2.3)	0.26
Gleason score, *n* (%)						
≤6	37 (49.3)	173 (59.2)	0.18	12 (46.2)	198 (58.1)	0.49
7	28 (37.3)	96 (32.9)		11 (42.3)	113 (33.1)	
≥8	10 (13.3)	23 (7.9)		3 (11.5)	30 (8.8)	
Surgical specimen						
Gleason score, *n* (%)						
≤6	18 (24)	102 (34.9)	0.011	7 (26.9)	113 (38.6)	0.012
7	47 (62.7)	176 (60.3)		14 (53.9)	209 (56.3)	
≥8	10 (13.3)	14 (4.8)		5 (19.2)	19 (5.1)	
Prostate volume, mean (SD)	45.2 (14.4)	45.5 (17.4)	0.84	46.3 (16.5)	45.4 (16.8)	0.78
Tumor volume, mL, (SD)	7.9 (7.3)	6.1 (5.4)	0.045	9.07 (7.7)	6.27 (5.7)	0.09
Stage, *n* (%)						
T2	59 (78.7)	234 (80.1)	0.77	19 (73.1)	274 (80.4)	0.37
T3	16 (21.3)	58 (19.9)		7 (26.9)	67 (19.6)	
Positive surgical margins, *n* (%)	13 (17.3)	54 (18.4)	0.82	4 (15.4)	63 (18.5)	0.69
Positive lymph nodes, *n* (%)	5 (6.7)	3 (1)	0.003	3 (11.5)	5 (1.5)	0.001

BMI, body mass index; FT, free testosterone; IQR, interquartile range; PrCa, prostate cancer; PSA, prostate-specific antigen; SD, standard deviation; TT, total testosterone.

Table 4
Multinomial logistic regression assessing independent predictors of pathologic Gleason score.

	Univariate analysis						Multivariate analysis					
	Gleason score 7 vs. 6[a)]			Gleason score 8–10 vs. 6[a)]			Gleason score 7 vs. 6[a)]			Gleason score 8–10 vs. 6[a)]		
	Odds ratio	Confidential interval (95%)	*P*	Odds ratio	Confidential interval (95%)	*P*	Odds ratio	Confidential interval (95%)	*P*	Odds ratio	Confidential interval (95%)	*P*
Age	1.05	1.00–1.09	0.051	1.1	1.00–1.21	0.05	Excluded			Excluded		
Preoperative PSA	1.23	1.12–1.36	<0.01	1.35	1.19–1.52	<0.01	1.2	1.08–1.33	0.001	1.32	1.16–1.50	<0.01
BMI	1.01	0.96–1.06	0.76	1	0.89–1.11	0.99	Excluded			Excluded		
Free testosterone												
Low vs. normal	1.51	0.83–2.75	0.17	4.05	1.56–10.5	0.004	1.2	0.63–2.29	0.58	3.18	1.38–2.12	0.031
Total testosterone												
Low vs. normal	1.08	0.42–2.76	0.87	4.25	1.22–14.77	0.023	1.02	0.37–2.83	0.96	4.25	1.05–17.31	0.043
No. of positive cores	1.58	1.35–1.85	<0.01	1.89	1.54–2.32	<0.01	1.52	1.3–2.59	<0.01	1.73	1.39–2.14	<0.01
Clinical stage												
T2 vs. T1	0.87	0.43–1.76	0.7	3.12	1.1–8.84	0.032	1.19	0.55–2.58	0.66	1.71	1.38–2.12	0.005

Race and biopsy Gleason scores presented sparseness in the data and were not included in the logistic regression model.
BMI, body mass index; PSA, prostate-specific antigen.
[a)] Gleason score 6 is the reference category.

Table 5
Logistic regression assessing independent predictors of lymph node involvement.

	Univariate analysis			Multivariate analysis		
	Odds ratio	Confidential interval (95%)	*P*	Odds ratio	Confidential interval (95%)	*P*
Age	0.96	0.84–1.11	0.62	Excluded		
Preoperative PSA	1.19	1.07–1.24	0.002	1.18	1.04–1.34	0.011
BMI	1.1	0.96–1.26	0.17	Excluded		
Race			0.23	Excluded		
White	Ref	Ref				
Asian	6.91	0.77–61.91				
African American	1.32	0.16–10.77				
Free testosterone						
Low vs. normal	6.88	1.61–29.48	0.009	9.45	1.52–58.69	0.016
Total testosterone						
Low vs. normal	8.76	1.97–38.98	0.004	12.35	2.1–72.49	0.005
No. of positive cores	1.46	1.19–1.78	<0.01	1.27	0.99–1.62	0.053
Clinical stage				Excluded		
T1	Ref	Ref				
T2	1.05	0.13–8.74	0.96			

Biopsy Gleason scores presented sparseness in the data and were not included in the logistic regression model.
BMI, body mass index; PSA, prostate-specific antigen; Ref, reference.

entire cohort revealed that low FT levels are associated with an increased number of positive cores found on prostate biopsy and a trend toward increased TV in prostatectomy specimens. It is believed that the physiologic decline of T levels with aging induces prostate gland atrophy, which may lead to the survival of prostatic cells independent of androgen levels and subsequent development of more aggressive PrCa.[21] We speculate that low androgen levels reflected by the reduced serum FT levels may thus contribute to this pathogenesis.

In an earlier study, Hoffman et al[4] reported similar results to our study regarding the relationship between the number positive cores and FT levels. Analyzing 117 patients with PrCa, they found an increased percentage of positive cores (43% vs. 22%, $P = 0.013$) and a Gleason score of 8 or greater in those with low FT levels (7 of 64 vs. 0 of 48, $P = 0.025$). In patients who had prostatectomy (57 from the previous 117 patients), an increased percentage of positive cores was related to the low pretreatment FT levels only (47% vs. 28%, $P = 0.018$). Our cohorts are similar in regard to the age of patients. The number of positive cores is an important factor in estimating PrCa progression and has been reported as a strong predictor of reclassification at repeat biopsy in patients included in an active surveillance protocol,[22] as well as an independent predictor of biochemical PrCa recurrence after RP.[23]

4.1. Middle-aged versus older patients

One theory proposed on why decreased T levels may occur in PrCa patients is the suppression theory. In simplified terms, a negative feedback loop from the PrCa cells decreases secretion from the pituitary gland.[24,25] Miller et al[24] found a statistically significant increase in serum TT, FT, estradiol, luteinizing hormone, and follicle-stimulating hormone levels and a decrease in dihydrotestosterone (DHT) levels at 1 year following RP compared with the preoperative levels. The age of patients included in this study (range: 43–67 years) was similar to the middle-aged group in our study. This study shows a correlation between low T levels and more aggressive features of PrCa.

Our study failed to find more aggressive features of PrCa in surgical specimens in older patients with low TT or FT levels. This may have been caused by confounding variables in elderly men, which influenced the levels of T as compared with middle-aged men. These would include advanced age itself, an increasing prevalence of comorbidities, and medications that may be associated with hypogonadism. Further complicating the picture is the possibility of differing relationships between androgen status and PrCa itself in varying age groups. Pierorazio et al[26] reported, in a longitudinal study, that high levels of FT in the elderly were related

to more aggressive PrCa. They found that chances of high-risk PrCa were doubled when the FT index (FT index = TT/SHBG) was increased per unit of 0.1 (CI: 1.01–4.23, $P = 0.047$). On the contrary, the trend of association between FT index and high-risk PrCa was inverse in middle-aged patients (<65 years old), reaching no statistical significance ($P = 0.9$). In a later study of Albisinni et al[12] in 812 patients who underwent prostate biopsy, those with a Gleason score of ≥7 ($n = 185$) had greater %FT levels (%FT = FT/TT). Their median age was 71 years (interquartile range: 65–76), similar to our older patient group. There is experimental and clinical evidence about the growth-enhancing role that T plays in PrCa tumor. While increased levels of T may not harm the prostate in middle-aged men, in the elderly population where histological changes of prostate tissue occur, increased levels of T could be a risk factor promoting the proliferation of PrCa cells.

We found a statistically significant correlation between low preoperative serum T (FT and TT) levels and a higher pathological Gleason score in middle-aged patients. These findings have previously been described by other investigators and are further corroborated in our study. Lane et al[5] studied 455 patients with a median age of 59 years (interquartile range: 54–63) and found on multivariate analysis that low preoperative levels of TT were associated with an increased dominant Gleason pattern 4-5 PrCa in prostatectomy specimens. There are similarities between their study and the present one. First, both of them used the low T definition provided by the manufacturer based on the assay. Second, the percentage of men reported with low T levels (5.5% vs. 7.1% in our middle-aged group) and the age of their patients (75% of them were 63 years old and younger) were comparable. However, low T levels were not an independent predictor of PrCa recurrence in localized disease in that study. While a prognostic value of the low T levels was not found, they hypothesized that the grading of PrCa could be an effect of a low-androgen environment, or the bimodal effect that T may have first on initiation and then on PrCa progression. FT levels were not reported in this study.

Massengill et al[7] looked at a more similar cohort of patients ($n = 879$) to ours (37.4% were 65 years of age or older), and subsequently found no relation between pretreatment TT levels and Gleason score. Additionally, they found a significant correlation between low TT levels and advanced pathological stage (pT3–4; $P = 0.046$). No FT was recorded.

Some limitations of our study are related to the data collection from a single center, which may introduce selection/referral bias, solitary preoperative T measurements, and time variability of T measurements between 7:00 AM and 5:00 PM (even though the evening fall of T was spared).[27,28] Studies have shown the importance of T measurement at 8 AM in young patients; however, there is no significant diurnal variability in elderly patients.[29]

In addition, all patients were treated uniformly by a single surgeon, all pathological specimens were analyzed in our institution in a standardized fashion, data were collected in a prospective database by the research staff independently from the treating team, and all blood samples collected for T measurement were assessed during the preoperative evaluation. FT was included in the analysis, which reveals better information about the amount of intraprostatic T.

Elderly patients with low T levels preoperatively did not present with more aggressive PrCa on biopsy and in prostatectomy specimens. By contrast, middle-aged patients with low levels of T preoperatively were presented with a higher Gleason score on the final pathologic report. Furthermore, those presented with low FT levels were found to have a greater number of positive cores on biopsy and TV in the final pathologic specimen. If included in predicting models, the measurement of serum T levels in middle-aged men undergoing RP can improve the prediction of PrCa features in prostate biopsy and prostatectomy specimens, which further help in counseling those patients. Further prospective studies are needed to corroborate these data.

Conflicts of interest

All authors have no conflict of interest to declare.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.prnil.2016.12.003.

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