Original Research

Total Prostate-Specific Antigen (tPSA) Outperforms Free PSA Percentage (fPSA%) in Detecting High-grade Prostate Carcinoma (PCa) in Patients Older than 60 Years of Age

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The PSA-based prostate cancer (PCa) screening remains a controversial topic. Total PSA (tPSA) levels along with % free PSA (fPSA%) still remain the most widely used screening markers for PCa in clinical practice. To assess tPSA and fPSA% screening performance and threshold to identify high-grade PCa, a large hospital-based cohort study is executed. A total of 853 patients who received 6 or 12 core prostate biopsies between January 2011 and August 2016 were included in the study and the tPSA and fPSA% were evaluated. The highest tPSA and lowest fPSA% levels within the prior 2 years of the biopsies were scrutinized. Both tPSA and fPSA% have the ability to discriminate patients with PCa from men without PCa. Intriguingly, only tPSA levels in patients older than 60 years showed a significant difference between men with and without PCa. More aggressive PCa also tends to occur in older patients (*Ptrend* = 0.045). With a level of tPSA > 20ng/mL, the likelihood ratio for detecting PCa with pathologic Gleason score ≥ 8 is 6.43, with 95% specificity and 30% sensitivity. fPSA% did not show a correlation with PCa histological grades or patients' age. Both tPSA and fPSA% have significant predictive values in PCa screening. The tPSA levels with the highest predictive value for PCa were achieved in patients older than 60 years in our cohort. Furthermore, a higher level of tPSA, such as 20 ng/mL rather than the widely adopted screening cutoffs (i.e. 4.0 or 10.0 ng/mL) is significantly associated with a high-grade PCa.
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INTRODUCTION

Prostate carcinoma (PCa) kills more than 31,000 men per year in United States, and there is significant morbidity in those who develop advanced disease.[1] The prostate specific antigen (PSA) test is the mainstay of early detection.[2-5] PSA is a serine protease that is regulated by testosterone and secreted by prostate epithelial cells. The normal function is to liquefy semen to facilitate sperm motility. PSA is secreted into the lumen of the duct as an inactive 244 amino acid polypeptide that is activated upon cleavage of 7 amino acids. Any additional cleavage inactivates the protein. PSA is secreted in smaller quantities by neoplastic epithelium. The higher serum levels associate with malignancy result from increased leakage of both the inactive and active forms of PSA into serum.[2] Once in the serum, either the active or inactive form may be free, or may be complexed to a number of serum proteins. "Total PSA (tPSA)" refers to any of these forms. "Free PSA (fPSA)" refers to an uncomplexed protein, which may be the inactive 244 amino acid form, the active 237 amino acid form, or inactive forms that have undergone additional cleavage in the prostate duct.[6]

Early detection of PCa is critical, as it is for any malignancy. In the United States, PSA was introduced to evaluate treatment response in 1987 but was soon widely adopted for screening.[2-5] There is clear evidence that screening with the PSA test can reduce the number of deaths from PCa. However, the diagnosis and treatment of cancer is unnecessary and potentially counterproductive if the disease will not limit longevity or curtail quality of life. Such "overdiagnosis" causes not only unnecessary treatment, but also needless patient anxiety. Current PSA testing suffers from poor sensitivity and specificity. Indeed, in 2012, the United States Preventive Service Task Force (USPSTF) recommended "against PSA based screening for PCa regardless age".[7] Early data suggest that this has been associated with a reduction in the diagnosis of low-risk disease but that the proportion of high-risk cases has increased.[8] Mathematical models and

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recent reports indicate that abandoning screening altogether would result in a doubling of patients presenting with metastatic disease and a 13-20% increase in PCa death by 2025.[9] Thus, completely eliminating screening is not a good option. Currently, both the American Urological Association and the American Cancer Society continue to recommend PSA screening for all men over age 50 whose life expectancy is considered to be 10 years or more.[10,11] In the years ahead, we will need to balance the proven benefits of screening and the early diagnosis it offers, with the risks of overtreating incidental, inconsequential cancer.

A variety of attempts have been made to "tweak" the PSA in an attempt to identify those tumors likely to be more aggressive, but these have been disappointing. There have been some suggestions for both an age-adjusted and a race-adjusted normal range for PSA. It seems to be "common sense" that younger men should have a lower PSA, and that African-Americans, in whom prostate carcinoma is more prevalent, should be followed more aggressively for the same PSA value.[2,4,5,12]

Detailed examination of fPSA fraction resulted in the identification of several distinctive fPSA forms, among which a mixture of precursor isoforms of prostate-specific antigen (pPSA or proPSA). ProPSA, which contains a seven amino acid pro leader peptide, is a molecular form of free PSA (fPSA) and is more likely to be associated with PCa. Truncated forms of proPSA also exist in serum, which contain five, four, or two more amino acids than PSA.[12,13] The [-2] proPSA (p2PSA) form has been identified in initial studies as the most prevalent form in tumor extracts, which suggests a role for these molecular forms of PSA for the early detection of PCa, and for possibly identifying aggressive PCa.[13,14]

Another approach, called the uPM3 test, uses nucleic acid sequence-based amplification to look for a non-coding RNA, prostate antigen 3 (PCA3), a gene that is overexpressed in prostate carcinoma, in the urine. PCA3 urine assay has been shown to be more specific than either total or free PSA in PCa screening, but it is less sensitive. Unlike PSA, urine levels of PCA3 are not dependent on the volume of the gland and will hopefully lead to fewer false positive from benign hyperplasia. While promising, PCA3 is far from ideal and seems likely to supplement, rather than replace, PSA.[15,16]

How to use screening and detection tool wisely to reduce morbidity and mortality from PCa at a lower cost and without causing undue harm is an urgent task. Most widely used screening markers, serum tPSA and %fPSA, lack clear thresholds balancing specificity and sensitivity for the early detection of PCa. In this study, we evaluated pre-procedure tPSA and fPSA% values in a cohort consisting of 863 biopsy proven PCa cases, and determined the predictive value of these two most commonly used screening markers.

Table 1. Cohort Basic Characteristics.

Categories	**Number (%)**
Age (years): Median = 63.4 (range: 41-91)	
<= 50	44 (5.2)
51-60	269 (31.5)
61-70	383 (44.9)
71-80	137 (16.1)
> 81	20 (2.3)
Total	853 (100.0)
Histology Diagnoses on Biopsies	
No Malignancy	437 (51.2)
Atypical Glands	33 (4.0)
High Grade PIN	21 (2.5)
Adenocarcinoma	360 (42.1)
Gleason 6	126 (35.1)
Gleason 3+4	94 (25.9)
Gleason 4+3	51(14.2)
Gleason 8 or more	89 (24.8)
Total	360 (100.0)
Other Cancers*	2 (0.2)
Total	853 (100.0)

* Including one urothelium carcinoma and one small cell carcinoma, which are excluded from further analysis.

METHODS

Study Cohort

We retrospectively scrutinized 1213 patients who had prostate biopsy were performed within a year from Jan 2011 to August 2016, at UMass Memorial Medical Center, Worcester, MA. Exclusion criteria included taking any medications that affected the patient's androgen status, or manipulations performed that might have affected the PSA concentration before obtaining the assay samples, or not having one or more total and free PSA determinations in our system before biopsy procedure. A total of 853 patients were included in our study cohort. The characteristics of the study population are summarized in **Table 1**. The median patient age was 63.4 years (range 41-91 years). Prostate biopsies were performed in all patients because of elevated tPSA level (> 4.0 ng/ml), or abnormal findings on either digital rectal examination or transrectal ultrasonography. Six to fourteen cores were obtained in prostate biopsies. In all 853 patients, 108 (12.7%) patients underwent repeat biopsampling. If different histological grades were revealed by the repeat biopsies, the highest grade was used for classification and further analysis. Consequently, biopsies from 437 patients (51.2%) only revealed benign prostate tissue; 33 (4.0%) patients were found to have some atypical glands; and 21 (2.5%) patients were diagnosed with high grade prostatic intraepithelial neoplasm (PIN). The remaining 362 patients were diagnosed with PCa. Among them, 360 (42.1%) patients were diagnosed with prostatic acinar adenocarcinoma, 1 (0.1%) with urothelial carcinoma, and 1 (0.1%) with small cell carcinoma. These two malignant cases other than acinar adenocarcinoma were excluded from further analysis. Among all diagnosed acinar adenocarcinoma cases, 126 (35.1%) patients had low grade or well differentiated disease (Gleason score 6), 94 (25.9%) and 51 (14.2%) patients had moderately differentiated disease of Gleason score 3+4 and Gleason score 4+3, respectively, and 89 (24.8%) patients had high grade or poorly differentiated disease (Gleason score 8 or more).

Assays for Total and Free PSA

We measured total and free PSA during the period from January 2011 to August 2016. Measurements of tPSA and fPSA were done by two-site "immunoenzymatic" sandwich assay on Beckman Access Immunoassay system. Both total and free PSA are determined in the same serum sample. Free PSA results were measured as ng/mL and reported as percentage of free PSA (fPSA%). fPSA% was calculated as the ratio of free to total PSA multiplied by 100.

Statistical Analysis

Patient characteristics were tested by the Fischer's exact test for categorical variables and by the Kruskal-Wallis test or the Mann-Whitney U test for continuous ones. All results for continuous variables are expressed as the median and range. The coefficient of variation was stratified by patient age, histology and the tPSA levels, the fPSA% using the Kruskal-Wallis test or the Mann-Whitney U test. The Spearman's rank test was used to analyze the correlation of the mean coefficient of variation between total and percent free PSA. All reported *P* values were obtained by the two-sided exact method. Receiver Operating Characteristic (ROC) curves of total and percent free PSA for their performance of diagnosing prostatic adenocarcinoma were obtained overall and individually for different histological categories and different age groups. These analyses were performed using Prism GraphPad (version 7.0; San Diego, CA)

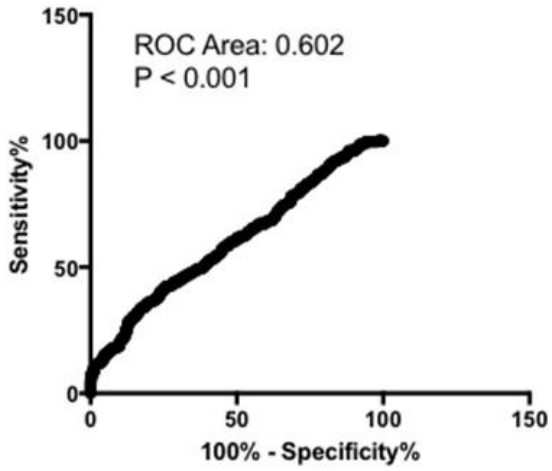

Figure 1. ROC Curve of total PSA.

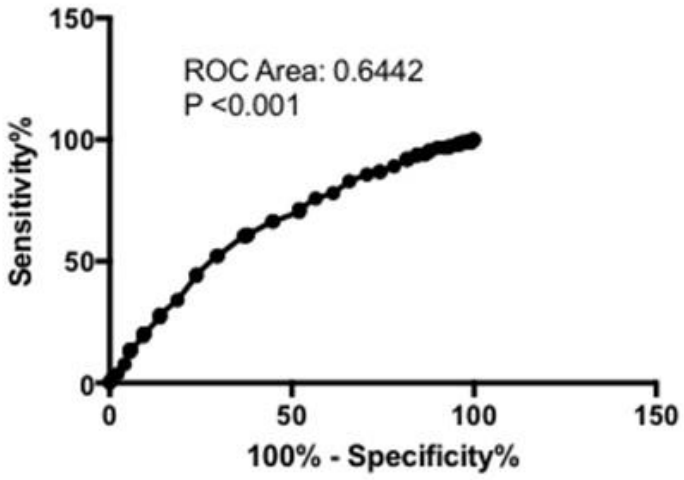

Figure 2. ROC Curve of Free PSA Percentage.

Table 2. Overall tPSA and fPSA% Performance in Prostate Biopsy Cohort.

	N (%)	Median (95%CI)	*P* value*
tPSA			
Cancer	360 (42%)	7.0 (6.4-7.8)	< 0.0001
Non-cancer	491 (58%)	5.7 (5.5-6.2)	
Total	851 (100%)		
Free PSA %			
Cancer	182 (40%)	12.0 (11-13)	< 0.0001
Non-cancer	269 (60%)	15.0 (14-16)	
Total	451 (100%)		

*Mann Whitney test

RESULTS

Overall, the median level of tPSA in our cohort is 6.4 ng/mL (N =853, 95% CI: 5.9-7.0). The median levels of tPSA in men with PCa diagnosis was 7.0 ng/mL (N = 360, 95%CI: 6.4-7.8), which is significantly higher than 5.7 ng/mL identified in men without biopsy proven prostate adenocarcinoma (N = 491, 95%CI: 5.5-6.2, $P < 0.0001$). In a subset 451 men who had concurrent fPSA% data, fPSA% appeared lower in patients with PCa (Median 12.0%, 95%CI: 11-13%) than in patients without biopsy proven cancer (Median 15.0%, 95%CI:14-16%; $P < 0.0001$) (**Table 2**). The tPSA level and fPSA% in patients with the biopsy diagnosis of atypical glands or HG PIN was not heterogeneous from group to only show "benign prostate tissue" in biopsy ($P > 0.05$, **Suppl Table 1**). Therefore these patients were combined into the "benign prostate tissue" group and referred as a "non-cancer" group for further analysis. In general, both tPSA level and fPSA% have acceptable performance in detecting PCa in our cohort, with areas under ROC curves of 0.602 and 0.644 respectively (**Figure 1**, **Figure 2** and **Suppl Table 2**, both $P < 0.001$).

Table 3. tPSA levels in Different Age Groups in Prostate Biopsy Cohort.

Age Group (years)	N (%)	Median tPSA level (95% CI)	*P* value*
<= 50	44	5.2 (4.1-6.4)	0.249
Non-cancer	29 (64%)	4.9 (3.6-6.4)	
Cancer	16 (36%)	5.9 (3.8-8.4)	
51-60	269	5.7 (5.1-6.2)	0.219
Non-cancer	181 (67%)	5.4 (5.0-6.2)	
Cancer	88 (33%)	6.0 (5.0-6.8)	
61-70	383	6.4 (5.9-7.0)	<0.001
Non-cancer	181 (67%)	6.0 (5.3-6.7)	
Cancer	88 (33%)	7.0 (6.3-8.7)	
71-80	137	7.8 (6.5-9.3)	0.018
Non-cancer	66 (48%)	6.7 (5.0-8.3)	
Cancer	71 (52%)	9.5 (7.1-11.8)	
> 81	20	8.9 (5.3-28.3)	0.027
Non-cancer	4 (20%)	4.5 (0.7-7.0)	
Cancer	16 (80%)	10.8 (7.4-58.9)	

*Mann Whitney tests

Suppl Table 1. Sub-classifications of Non-Cancer Cases.

	Total PSA		Free PSA percentage	
	N (%)	Median (95%CI)	N (%)	Median (95%CI)
No Malignancy	437 (51%)	5.7 (5.4-6.2)	243 (55%)	15.0 (14-16)
Atypical Glands	33 (4%)	6.8 (4.4-9.8)	15 (3%)	15.5 (8-27)
High Grade PIN	21 (3%)	5.6 (4.1-7.0)	11 (2%)	19.5 (13-30)
Adenocarcinoma	360 (42%)	7.0 (6.4-7.8)	182 (40%)	12.0 (11-13)
Total	851 (100%)		451 (100%)	

Suppl Table 2. Performances of tPSA and fPSA% in Detecting Prostate Carcinoma.

	Sensitivity (%)	95% CI	Specificity (%)	95% CI	Likelihood Ratio
tPSA (ng/mL)					
> 1.6	99	98-100%	6	4-9%	1.06
> 2.4	95	93-98%	12	9-15%	1.09
> 4.0	83	79-87%	26	22-30%	1.13
> 10.0	34	29-40%	81	78-85%	1.87
> 18.3	15	12-20%	95	93-97%	3.20
> 37.7	8	5-11%	99	98-100%	9.93
fPSA %					
< 37	99	96-100%	1	0-4%	1.00
< 25	95	91-98%	12	9-17%	1.08
< 6.5	8	4-13%	95	93-97%	1.88
< 4.5	2	0-5%	99	97-100%	2.22

tPSA levels increased steadily with age in populations with and without PCa (**Table 3**). In the group younger than 50 years old, the median tPSA level was 5.2 ng/mL. The median tPSA levels increased to 5.7 and 6.4 ng/mL in age groups 51-60 and 61-70 years, respectively and further increased to 7.8 and 8.9 ng/mL in age groups 71-80 and >81 years (P_{trend} < 0.0001), respectively. We also noticed that the pre-test probability of diagnosing PCa also increased with age. In our cohort, 33% of patients who underwent prostate biopsy were diagnosed with PCa in the group younger than 60 years old, while 48% patients were proven to have PCa in the age group older than 60 years (P < 0.001). Intriguingly, the significance in the difference between non-cancer and cancer groups seems mainly contributed by the elder population. There is no statistically significant difference of tPSA level in patients younger than 50 years (non-cancer group vs cancer group, median 4.9 vs 5.9 ng/mL, respectively, P = 0.249) or in the age group of 51-60 years (non-cancer group vs cancer group, median 5.4 vs 6.0 ng/mL, respectively, P = 0.219). In age groups of 61-70 and 71-80 years, tPSA level in people without cancer were significantly lower than PCa patients (6.0 vs 7.0 ng/mL and 6.7 vs 9.5 ng/mL in non-cancer and cancer groups; P < 0.001 and P = 0.018 respectively). In patients older than 81 years, the difference between non-cancer and cancer groups still appeared to be statistically significant (4.5 vs 10.8 in non-cancer vs cancer group; P = 0.027), even with limited study subject numbers (N = 16 and N = 4 respectively). However, we did not observe any statistical trend between age and fPSA% in our cohort (**Suppl Table 3**).

We also analyzed the correlations between patients' age, tPSA level, fPSA % and histologic tumor grade (Gleason score) in biopsy diagnosed PCa patients (**Table 4**). Higher grade PCa tended to occur in older patients (P_{trend} = 0.045). Mean age at diagnosis in patients with well differentiated PCa (Gleaseon score 6) was 63.1 years, whereas mean age at diagnosis of patients with poorly differentiated cancer (Gleason score >= 8) was 67.1 years. The tPSA level also steadily increases with worsening tumor grade (P_{trend} = 0.0001). Patients with low grade (Gleason 6) tumors had a median tPSA level of 5.8 ng/mL (95%CI: 5.0-6.8ng/mL), while the median tPSA of patients with Gleason 3+4 PCa was 6.5 (95%CI: 5.2-7.3), and with Gleason 4+3 PCa was 10.9 (95%CI: 8.9-12.7). Patients diagnosed with high grade tumor (Gleason >= 8) have a median tPSA level of 11.4 ng/mL (95%CI: 6.5-14.7). Similar trends among different grade PCas was not observed with fPSA% (P_{trend} = 0.9556). With a level of tPSA > 20ng/mL, the likelihood ratio for detecting PCa with pathologic Gleason score $\geq$ 8 was 6.43, with 95% specificity and 30% sensitivity.

Table 4. Association between tPSA and fPSA% and Histologic Grade and Patients' Age in Diagnosed Prostate Cancer Cases.

		Age		tPSA		fPSA%		
	N (%)	**Mean (yrs)**	***P* value***	**Median (95% CI)**	***P* value***	**N (%)**	**Median (95% CI)**	***P* value#**
Gleason 6	126 (35)	63.1	0.045	5.8 (5.0-6.8)	0.0001	73 (40)	13 (11-15)	0.9556
Gleason 3+4	94 (26)	64.5		6.5 (5.2-7.3)		49 (27)	12 (11-14)	
Gleason 4+3	51(14)	66.8		10.9 (8.9-12.7)		25 (14)	12 (11-14)	
Gleason>=8	89 (25)	67.1		11.4 (6.5-14.7)		35 (19)	11 (9-14)	
Total	360 (100%)					182 (100%)		

* Ordinary one-way ANOVA
\# Kruskal-Wallis non-parametric one-way ANOVA

Suppl Table 3. Free PSA Percentage Between Different Age Groups.

Age Group	Median (95% CI)	Mean +/- SD
<= 50 (N = 28)	14 (12-16)	16.86 +/- 8.46
51-60 (N = 136)	14 (13-15)	14.92 +/- 6.91
61-70 (N = 215)	14 (13-15)	15.36 +/- 6.67
71-80 (N = 61)	14 (13-19)	16.51 +/- 7.56
> 81 (N = 6)	11 (8-23)	13.38 +/- 6.12

DISCUSSION

The PCa screening controversy has reached a critical turning point.[2,4,17,18] On one hand, the screening is estimated to account for 45-70% reduction in PCa mortality in the United States.[3] On the other hand, PSA screening may result in significant harm, including unnecessary biopsies with potentially associated adverse effects, over-diagnosis and resultant overtreatment.[4,18] Though many attempts have been made to discover new biomarkers with better sensitivity and specificity in detecting lethal PCa cases, re-evaluation of currently widely-used and cost efficient tests, tPSA and fPSA% in a large commentary cohort is lacking.[5] In our current study, we retrospectively analyzed the association between prostate biopsies diagnoses with the pre-biopsy tPSA levels and free-PSA percentage performed in our institute in a 5-year period. To our best knowledge, it is the largest single institution cohort report to assess the performances of the most commonly used biomarkers tPSA and fPSA% in screening patients for PCa in recent years.

Presently, a common PSA threshold for biopsy has been greater than 4.0 ng/mL, a cut-off point which has been reported to be associated with a positive predictive value of about 30% in men aged 50 years or more, and a negative predictive value of about 85% in men of median age 69 years at biopsy.[3] In our present cohort of 853 patients that underwent prostate biopsies, tPSA seems to become more specific with a higher cutoff (i.e. tPSA ng/mL > 20.0 ng with 95% specificity), and it is with a higher positive predictive value in detecting PCa in patients older than 60 years. Furthermore, a higher tPSA level is positively associated with detecting more biologically aggressive disease. Though performance of fPSA% is similar to tPSA in detecting overall PCa, we did not observe an association between age or cancer grade and fPSA%.

Our study is unique in a few ways. First, it was a retrospective, observational association study in a contemporary cohort of a large number of patients who underwent prostate biopsies. Second, the biopsy candidates were recruited using the same criterion and collected in the same practice setting which ensured the homogeneity of cohort. Furthermore, all blood samples were centrally managed and uniformly analyzed according to large diagnostic laboratory guidelines, which avoid discrepancies and variation in testing between different laboratories.

The present study is not devoid of limits. First, due to the nature of the retrospective association study, a selection bias may have been applied when we chose patients without biopsy proven PCa as a control group. Second, typically only samples with a "grey zone" tPSA value (4 ng-10 ng) will have fPSA% ordered.[6] However, it is not a strict selection rule in this cohort. This selection bias will affect the analysis of the fPSA% performance. Third, there is no long-term follow-up data collected to correlate survival or disease outcome in the cohort of patients with a reported PCa diagnosis. A second large cohort, and a prospective study aiming to evaluate tPSA and fPSA% are warranted.

In this study, we wished to address how to better utilize the two most widely used PCa screening markers, tPSA and fPSA%. Both tPSA and fPSA% have significant predictive values in PCa screening. The tPSA levels with the highest predictive value for PCa were achieved in patients older than 60 years in our cohort. Furthermore, a higher level of tPSA, such as 20 ng/mL rather than the widely adopted screening cutoffs (i.e. 4.0 or 10.0 ng/mL) is significantly associated with a high-grade PCa. fPSA% did not show any significant difference in the different age groups or a better performance in distinguishing higher grade PCa in our cohort. Our data suggests it is necessary to have separate reference ranges for different age groups when using tPSA for PCa screening, and tradeoff with a higher cutoff of tPSA to result in high specificity in diagnosing high grade PCa.

CONFLICT OF INTEREST
None.

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