Complexed prostate specific antigen: better test in the diagnosis of prostate cancer for the clinically relevant 2.5-4 ng/ml total PSA range

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Background: *Data on utilizing complexed prostate specific antigen (cPSA) offering increased diagnostic performance over other available clinical parameters in diagnosis of prostate cancer is still controversial. Our objective was to determine diagnostic performance of cPSA compared to total prostate specific antigen (tPSA) and corresponding ratios for possible routine application.*
Methods: *In a prospective study including overall 315 consecutive men, 177 patients with suspicious digital rectal examination, and/or tPSA value ≥ 2.5 ng/ml underwent prostate biopsy. Serum samples for tPSA, cPSA and free PSA were analyzed using automated chemiluminometric technology.*

Results: *Area under the curve (AUC) for cPSA, although greater, was not statistically different compared to that of tPSA (p = 0.253). AUCs of f/c, f/t and c/t ratios were all found significantly inferior. At clinically relevant 2.37 ng/ml threshold, cPSA performed with 85% sensitivity and significantly higher specificity of 63.1%, compared to same sensitivity and specificity of 57.2% at a 3.00 ng/ml cut off for tPSA.*
Conclusions: *Utilizing automated assay systems at predetermined cut off value for cPSA we would be able to save 27.1% of the biopsies while missing 13.4% of the cancers. Therefore, results of this study indicate higher discriminatory power of cPSA in diagnosis of prostate cancer for clinically relevant 2.5-4 ng/ml tPSA range.*

Key Words: biomarkers, complexed prostate specific antigen, prostate cancer, prostate specific antigen

Introduction

Early diagnosis of prostate cancer is of paramount importance because both the disease is curable only when confined to the gland and cancers detected by screening are frequently organ confined.[1] Although not devoid of drawbacks, prostate specific antigen (PSA) based screening as introduced two decades ago, has emerged as the most effective means of accomplishing this goal.[2] Since then, in order to enhance the diagnostic power of PSA, various concepts have been used, such as age-related reference values, PSA density and PSA velocity, as well as the quantitative determination of the different PSA isoforms and their ratios.[3-5]

Following identification of the major immunoreactive forms of PSA in serum, it was also observed that the complex of PSA and α_1-antichymotrypsin (PSA-ACT) was present in higher proportions in the serum of prostate cancer patients.[4,6] This finding, although subsequently not confirmed by all investigators, led to rapid development of different monoclonal and polyclonal antibodies that specifically recognize PSA isoforms. The main purpose of these efforts was to achieve a higher specificity in prostate cancer detection. However, the search for better concordance between the serum PSA isoforms and the

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actual disease state was further complicated by the physiological properties of the PSA molecule itself and the issues related to different commercial assay and method standardization.[7] Accurate measurement of PSA-ACT complex appeared to be difficult because of high concentration of free ACT found in serum and its complex formation with other proteases, which may cross react in assays designed to measure primarily PSA-ACT. Thus, a critical need was raised for a common consensus that will overcome the discrepancies between the different studies concerning the diagnosis of prostate cancer. One of the most important steps in this account was the approval of the Stanford 90:10 PSA Standard, developed by Stamey et al, by the NCCLS Subcommittee on PSA Reference Material Specifications on December 1, 1995.[8] This led to the advancement of the commercial assays measuring total PSA (tPSA) and the derivatives on equimolar basis, helping to increase the accuracy of the tests for diagnosing prostate cancer.

A fully automated immunoassay for specifically measuring the complexed PSA (cPSA) was described by Allard and colleagues a decade ago and standardized against highly purified PSA-ACT reference material with proposed improved ability in diagnosing prostate cancer as a single high specific noninvasive marker, and made available for *in vitro* diagnostic use.[9,10] We were interested if this test will essentially improve the diagnosis of prostate cancer patients and avoid the unnecessary biopsy rates in our patient population. This prospective clinical study was designed to determine the possible improved diagnostic performance of the cPSA assay alone compared to tPSA assay, free to total PSA (f/t), complexed to total PSA (c/t) and free to complexed PSA (f/c) ratios separately or in combination, and the potential utilization of this noninvasive technique for successfully diagnosing prostate cancer in our routine clinical practice.

Materials and methods

Patient and sample design

We designed a prospective clinical study in which overall 315 consecutive men (median age 60; range 34-92 years) with lower urinary tract symptoms were enrolled. Patients generally constituted a population of men who were examined in the Department of Urology at the Marmara University Hospital during the period of April 2006-October 2007. The study fulfilled the guidelines required by the local Ethics Committee. Written consent was obtained from all included patients. The study subjects underwent digital rectal examination (DRE) strictly after blood sample collection. Patients with a previous history of elevated tPSA, established benign prostatic hyperplasia (BPH), chronic prostatitis or prostate cancer, those on testosterone or finasteride therapy, or underwent prior resection of prostate and those who rejected the biopsy procedure were excluded from the study. From a total of 315 patients who fulfilled the inclusion criteria, patients (n = 177) with a suspicious DRE and/or a PSA value $\geq$ 2.5 ng/ml, underwent transrectal ultrasound (TRUS) guided systematic 12-core and lesion (if present) directed prostate needle biopsy. During sagittal scanning of the prostate gland by a biplanar 7.5 MHz ultrasound probe, biopsy samples from each patient with an 18-gauge biopsy needle, driven by a spring-loaded biopsy gun, were obtained. The total and transitional zone volumes of the prostate glands of all biopsy subjected patients were calculated by the ellipsoid formula (volume = 0.52 x length x weight x height) and recorded. The remaining patients (n = 138) with normal DRE and PSA levels < 2.5 ng/ml were considered the control group. According to histopathological results of the undertaken biopsy, patients were divided into two groups: prostate cancer group (n = 44); benign group (n = 133); either benign prostatic hyperplasia or chronic prostatitis.

In an attempt to analyze the possible age related changes, patients were further substratified into four different age interval groups: $\leq$ 49; 50-59; 60-69 and $\geq$ 70 years of age.

The collection and handling of serum samples were performed according to the NCCLS recommendations and were frozen and stored at -85º C until analysis. Any kind of transrectal or transurethral manipulation was deferred until after the sample collection. For each sample, testing for the tPSA and isoforms were performed in double within the same 24 hour freeze-thaw cycle.

Analytical methods

The samples obtained from patients and the control group, were analyzed in duplicate for the levels of tPSA and cPSA (ADVIA Centaur, Bayer Corporation, Tarrytown, NY), and tPSA and free PSA (fPSA; Elecsys2010, Roche Diagnostics, Mannheim) using chemiluminometric technology. Also, fPSA values obtained by extracting cPSA from tPSA, both measured on ADVIA Centaur system and cPSA values obtained by extracting fPSA from tPSA, both measured on Elecsys2010 system were calculated. In the distinctive cPSA assay of interest, fPSA is prevented from reacting with the PSA antibodies by preliminary incubating the sample with fPSA specific monoclonal mouse antibodies (termed MM1 antibodies) that block the fPSA, rendering it nonreactive in the assay.

Statistical analysis

Recorded data was analyzed using the statistical software packages Analyze-it Method Validation Edition for Microsoft Excel 2007 (version 1.62) and SPSS for Windows (version 15; SPSS Inc, Chicago). Performed assay measurement values were tested for normal distribution by Shapiro-Wilk test. Mann-Whitney U-test, Kruskal-Wallis nonparametric ANOVA, linear regression and Spearman's rank correlation analyses were performed in order to compare the two histopathologically established study groups, the control group and the predetermined four subgroups for age, prostate gland volume and the measured assay values. The diagnostic accuracy, sensitivity, specificity and cut off values of the analyzed assays were determined for the whole patient population and for the four age interval groups separately and further evaluated by the receiver operating characteristic (ROC) curve analysis, a global measure of the diagnostic performance of a test.[11] Z-test was performed in order to compare areas under the curve (AUC) for the different assays obtained from ROC analysis. A statistical difference of $p < 0.05$ was considered significant.

Results

We principally followed the instructions for evaluating and reporting studies of diagnostic accuracy of medical tests, published previously.[12] We evaluated results of all 315 consecutive patients including entire tPSA range (0.1 ng/ml-348 ng/ml), followed by assessment of the four predetermined age subgroups.

TABLE 1. **Demographic, histopathological and biochemical characteristics of study groups**

		Prostate cancer group[a] (n = 44)	**Benign group[b] (n = 133)**	**Control group[c] (n = 138)**	**Significance (two tailed p)**
Age (years)	Mean ± SD Median [95%CI]	65 ± 7.2 65 [62-70]	64.3 ± 9.1 63 [61-65]	55.1 ± 9.7 54 [52-55]	$p^{ab} = 0.463$ $p^{ac} < 0.0001$ $p^{bc} < 0.0001$
Prostate volume (ml)	Mean ± SD Median [95%CI]	42.3 ± 20.6 38.1 [32-50]	54.3 ± 32.6 50.4 [42-57]	—	p = 0.025
tPSA (ng/ml)	Mean ± SD Median [95%CI]	25.3 ± 58.9 7.33 [6.04-11.24]	8.53 ± 14.28 5.24 [4.54-6.14]	1.50 ± 0.74 1.37 [1.29-1.48]	$p^{ab} = 0.009$ $p^{ac} < 0.0001$ $p^{bc} < 0.0001$
cPSA (ng/ml)	Mean ± SD Median [95%CI]	19.4 ± 50.6 5.85 [4.32-9.14]	4.99 ± 4.11 3.87 [3.15-4.64]	0.80 ± 0.57 0.74 [0.59-0.84]	$p^{ab} = 0.003$ $p^{ac} < 0.0001$ $p^{bc} < 0.0001$
fPSA (ng/ml)	Mean ± SD Median [95%CI]	3.47 ± 5.07 1.41 [1.17-2.08]	2.73 ± 8.52 1.18 [0.98-1.40]	0.28 ± 0.25 0.21 [0.18-0.26]	$p^{ab} = 0.118$ $p^{ac} < 0.0001$ $p^{bc} < 0.0001$
f/t PSA	Mean ± SD Median [95%CI]	0.24 ± 0.23 0.18 [0.14-0.26]	0.64 ± 0.97 0.36 [0.32-0.47]	0.49 ± 0.22 0.46 [0.42-0.54]	$p^{ab} = 0.011$ $p^{ac} < 0.0001$ $p^{bc} < 0.0001$
c/t PSA	Mean ± SD Median [95%CI]	0.76 ± 0.17 0.80 [0.75-0.83]	0.68 ± 0.17 0.73 [0.68-0.76]	0.51 ± 0.22 0.54 [0.47-0.58]	$p^{ab} = 0.011$ $p^{ac} < 0.0001$ $p^{bc} < 0.0001$
f/c PSA	Mean ± SD Median [95%CI]	0.43 ± 0.53 0.25 [0.20-0.34]	0.64 ± 0.97 0.36 [0.32-0.47]	1.49 ± 1.51 0.85 [0.72-1.15]	$p^{ab} = 0.011$ $p^{ac} < 0.0001$ $p^{bc} < 0.0001$

tPSA, total PSA; cPSA, complexed PSA; fPSA, free PSA; f/t PSA, free to total PSA; c/t PSA, complexed to total PSA; f/c PSA, free to complexed PSA; CI, confidence interval; SD, standard deviation

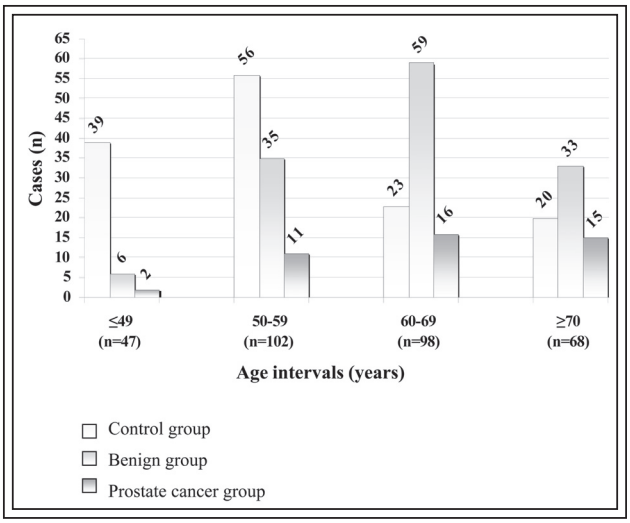

Figure 1. Age interval associated distribution of the disease states.

Demographic, histopathological and biochemical characteristics of study groups

Of the 177 patients who underwent biopsy, 44 were found positive for prostate cancer (24.9%) with median age of 65 years (95% CI: 62-70) and the remaining (n = 133) were histopathologically diagnosed to have BPH, CP or both with median age of 63 (95% CI: 61-65). The age comparison revealed no significance between the former two groups (p = 0.463) in contrast to the control group with significantly lower median age of 54 (95% CI: 52-55) compared to previous two (p < 0.0001). The prostate volume of the prostate cancer patients was found to be significantly smaller than the patients with benign disease (38.1 ml versus 50.4 ml, respectively, p = 0.025), Table 1. Classification of patients according to the predetermined age interval sub groups revealed 47 subjects belonging to ≤ 49, 102 to 50-59, 98 to 60-69 and 68 to ≥ 70 age groups. The highest prostate cancer rate was seen in the ≥ 70 age patient group (22.1%), whereas the benign disease was seen mostly in the 60-69 year group (60.2%) and finally the majority of patients in the ≤ 49 group were free of disease (82.9%, Figure 1).

Analytical performance of the PSA assays

The Bayer and Roche tPSA, cPSA and fPSA measured values for the overall study groups ranged between 0.1 ng/ml-348 ng/ml, 0.06 ng/ml-200 ng/ml, 0.02 ng/ml-318 ng/ml and 0.02 ng/ml-80 ng/ml, respectively. The values above the measurable limit which was 100 ng/ml for tPSA assays and 50 ng/ml for fPSA, were obtained by an automatic or if needed manual predilution of the relevant samples. The reproducibility of the assays was evaluated by intra-assay and inter-assay runs at two different concentrations, Table 2. Method comparison analysis between Bayer and Roche tPSA assays and Roche fPSA plus Bayer cPSA in relation to Bayer tPSA assays revealed Spearman's rank correlation values (r) of 0.93 (95%CI: 0.91-0.94) and 0.96 (95%CI: 0.95-0.97), respectively. Obviously, Bayer and Roche PSA assays were well correlated and made it possible for interchangeably calculate f/t and f/c PSA ratios. In general, the non-Gaussian distribution of the different PSA assay values, with low Shapiro-Wilk W scores ranging between 0.17-0.31, high skewness (7.35-13.25) and kurtosis (62.74-200.63) values lead us to perform nonparametric tests in order to compare medians. Except for the fPSA assay, for which values were not significantly different between the benign and prostate cancer groups, all of the other analyzed assay and ratio data were found statistically different among the three groups, with highest median values for the prostate cancer and lowest for the control groups when considering tPSA, cPSA and c/t ratio; and vice versa for the f/t and f/c ratios, Table 1. The distribution of

TABLE 2. **Reproducibility data of tPSA, cPSA and fPSA assays at two different concentrations**

	tPSA (ng/ml)		cPSA (ng/ml)		fPSA (ng/ml)	
Intra assay run						
Mean; % CV	3.24; 1.79	10.65; 2.08	2.90; 1.15	8.18; 1.86	0.83; 2.61	1.56; 3.19
Median [range]	3.23 [3.15-3.32]	10.70 [10.20-10.97]	2.89 [2.85-2.95]	8.16 [7.90-8.39]	0.81 [0.79-0.87]	1.52 [1.48-1.65]
Inter assay run						
Mean; % CV	3.34; 3.15	11.99; 5.11	3.18; 2.67	8.95; 3.67	0.62; 5.54	1.21; 7.62
Median [range]	3.39 [3.18-3.45]	12.23 [10.5-12.54]	3.20 [2.95-3.25]	8.86 [8.60-9.81]	0.71 [0.59-0.78]	1.19 [1.08-1.50]

tPSA, total PSA; cPSA, complexed PSA; fPSA, free PSA; CV, coefficient of variation

the three PSA assay values among the groups is shown on Figure 2. The individual analysis of demographic, histopathological and biochemical characteristics of the predetermined age interval groups are summarized in Table 3. In general they were analogous to the overall study data and reflected the same patterns as described above. The tPSA, cPSA and fPSA assays revealed direct correlation to age (r = 0.47, r = 0.46, r = 0.50) however, such a relationship was not found for the three ratios. Moreover, the median and 95% CI values for the tPSA, cPSA, fPSA assays, c/t and f/c ratios were consistent with reference values that were previously introduced in to the clinical practice (age dependant for the former three and non-age related for the last two), whereas f/t ratio appeared to have higher medians in our patient group.[3]

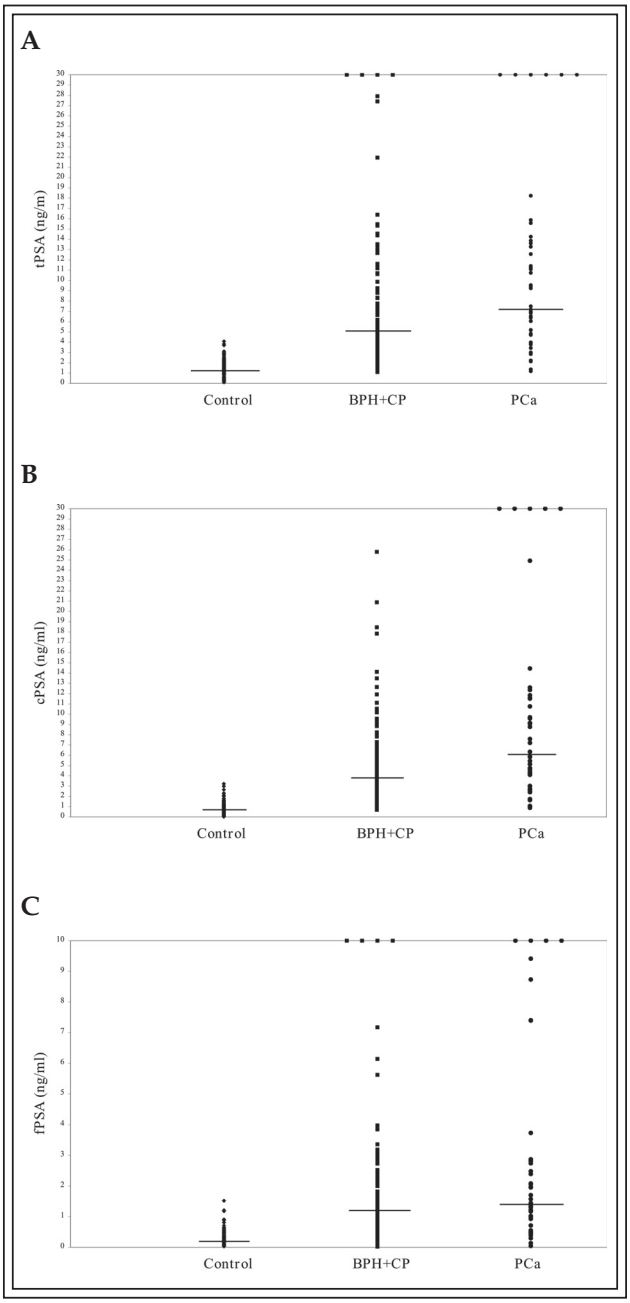

Figure 2. Scatter plots of total PSA (A), complexed PSA (B) and free PSA (C) values among the study groups (Median values of the respective groups are shown as horizontal lines; Control, control group; BPH+CP, benign group; PCa, prostate cancer group. Values ≥ 30 ng/ml for tPSA and cPSA and ≥ 10 ng/ml for fPSA are presented as single dots over one straight line on the top of each chart.)

Diagnostic performance of the PSA assays and ratios

With a median tPSA of 7.79 ng/ml (95%CI: 5.03-10.55), we assumed that our study population in general was a satisfactory statistical representative of the clinically significant grey zone in order to assess the diagnostic performances of the PSA assays and ratios. The area under the curve (AUC) for the cPSA assay (0.812, 95%CI: 0.752-0.873), although greater, was not statistically different compared to that of tPSA assay

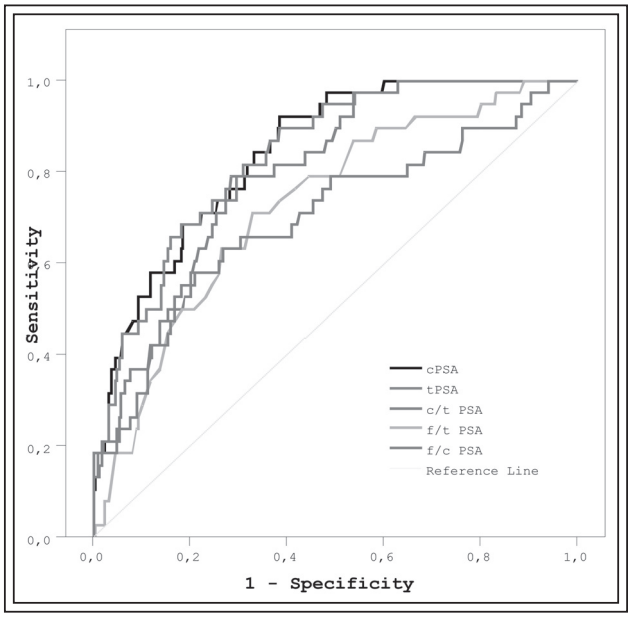

Figure 3. Diagnostic performance analysis of total PSA, complexed PSA, free to complexed PSA, free to total PSA and complexed to total PSA ratios.

The correspondent areas under the curve (AUCs) are 0.80 (95%CI: 0.735-0.864; p = 0.253), 0.812 (95%CI: 0.752-0.873), 0.724 (95%CI: 0.645-0.804; p = 0.039), 0.674 (95%CI: 0.560-0.782; p = 0.031) and 0.632 (95%CI: 0.533-0.731; p = 0.026), respectively.

TABLE 3. **Demographic, histopathological and biochemical characteristics of the predetermined age interval**

	≤ 49 (n = 47)			50-59 (n = 102)		
	PCaG (n = 2)	**BG (n = 7)**	**CG (n = 39)**	**PCaG (n = 11)**	**BG (n = 35)**	**CG (n = 56)**
Age (years)						
Mean ± SD		44.7 ± 4.3			54.6 ± 2.9	
Median [95%CI]		46 [43-47]			55 [54-56]	
Prostate volume (ml)						
Mean ± SD	35.6 ± 27	70 ± 34	—	36 ± 14.5	42 ± 19.1	—
Median [95%CI]	35.6 [12.3-59]	57 [41-85]		34.8 [19-60]	40.1 [32-48]	
tPSA (ng/ml)						
Mean ± SD	9.2 ± 2.4	12.9 ± 10	1.3 ± 0.6	7.7 ± 4.2	4.9 ± 3.3	1.4 ± 0.7
Median [95%CI]	9.2 [7.2-11.2]	9.96 [2.02-27.9]	1.3 [1-1.5]	6.8 [3-13.6]	3.9 [3.3-5]	1.4 [1.2-1.6]
cPSA (ng/ml)						
Mean ± SD	7.1 ± 2.3	9.2 ± 6.3	0.7 ± 0.5	6.6 ± 3.8	3.5 ± 2.9	0.8 ± 0.6
Median [95%CI]	7.1 [5.1-9.1]	8.96 [1.82-17.8]	0.6 [0.4-0.9]	6.3 [2.4-11.6]	2.6 [2.0-4.0]	0.7 [0.5-0.9]
fPSA (ng/ml)						
Mean ± SD	2.7 ± 0.2	3.25 ± 4.44	0.2 ± 0.1	1.1 ± 0.7	0.97 ± 0.84	0.27 ± 0.21
Median [95%CI]	2.7 [2.5-2.8]	1.64 [0.23-12.1]	0.19 [0.12-0.23]	1.2 [1.1-2]	0.80 [0.49-0.98]	0.19 [0.16-0.28]
f/t PSA						
Mean ± SD	0.24 ± 0.06	0.24 ± 1.15	0.49 ± 0.23	0.16 ± 0.1	0.32 ± 0.19	0.51 ± 0.22
Median [95%CI]	0.24 [0.19-0.28]	0.27 [0.04-0.39]	0.48 [0.37-0.62]	0.17 [0.07-0.25]	0.27 [0.20-0.34]	0.47 [0.39-0.61]
c/t PSA						
Mean ± SD	0.76 ± 0.06	0.76 ± 0.15	0.51 ± 0.23	0.84 ± 0.09	0.68 ± 0.19	0.49 ± 0.22
Median [95%CI]	0.76 [0.72-0.81]	0.73 [0.61-0.96]	0.52 [0.38-0.63]	0.84 [0.76-0.93]	0.73 [0.66-0.80]	0.53 [0.40-0.61]
f/c PSA						
Mean ± SD	0.31 ± 0.10	0.35 ± 0.26	1.47 ± 1.38	0.21 ± 0.14	0.68 ± 0.93	1.73 ± 1.81
Median [95%CI]	0.31 [0.23-0.40]	0.39 [0.05-0.63]	0.91 [0.58-1.61]	0.20 [0.07-0.33]	0.37 [0.25-0.52]	0.90 [0.64-1.53]

tPSA, total PSA; cPSA, complexed PSA; fPSA, free PSA; f/t PSA, free to total PSA; c/t PSA, complexed to total PSA; f/c PSA, control group

(0.80, 95%CI: 0.735-0.864; p = 0.253). However, the AUCs of f/c ratio (0.724, 95%CI: 0.645-0.804; p = 0.039), f/t ratio (0.674, 95%CI: 0.560-0.782; p = 0.031) and c/t ratio (0.632, 95%CI: 0.533-0.731; p = 0.026) were all found significantly inferior to cPSA assay, displaying decreased diagnostic efficacy, Figure 3.

Test performances in terms of sensitivity and specificity of the PSA assays and isoforms at different decision levels were evaluated. At 4.69 ng/ml decision level, tPSA assay had a sensitivity of 75% and specificity of 72.3%. Equivalent to the same sensitivity, at 3.00 ng/ml cut off point, cPSA assay revealed 70.5% specificity, which was not superior to tPSA assay, Table 4. However, at a lower 2.37 ng/ml cut off cPSA assay had a sensitivity of 85% and significantly higher specificity of 63.1% compared to the same level of sensitivity and specificity of 57.2% at a 3.00 ng/ml cut off for tPSA assay. Table 5 shows the diagnostic performance characteristics of all assays and ratios at this optimal preset limit of 63.1% specificity. At this point, f/t (61.8 %) and c/t (58.8 %) ratios had the lowest sensitivities, following the f/c ratio (72.7 %). A reverse comparison of the remaining variables, separately or in combination, did not revealed better performance than cPSA assay alone at 85% sensitivity. At this optimal sensitivity and specificity values, tPSA assay would have avoided the need for biopsy in 16.5% of patients, and missed 13.4% of the cancers, whereas cPSA assay would have missed the same number of cancers but higher number (27.1%) of unnecessary biopsies would have been saved. Furthermore, at 90% and 95% sensitivity values although tPSA had better specificity compared to cPSA assay, the difference was not significant, and because none of the ratios had higher specificity values, neither tPSA nor cPSA assay alone or in combination with any of the ratios, appeared to have improved

TABLE 3 (cont'd). **groups**

60-69 (n = 98)			≥ 70 age (n = 68)		
PCaG (n = 16)	**BG (n = 59)**	**CG (n = 23)**	**PCaG (n = 15)**	**BG (n = 33)**	**CG (n = 20)**
	63.7 ± 2.9 63 [62-65]			75.1 ± 4.5 74 [73-76]	
45 ± 27 37.7 [24-80]	60.2 ± 41.8 55.2 [41-70]	—	46.7 ± 14.4 49.7 [24.8-69.3]	57.8 ± 21.4 57.4 [39-75]	
14.4 ± 19.5 8.1 [3-15.9]	9.9 ± 19.5 5.7 [4.5-7]	2.0 ± 0.9 2.1 [1.4-2.5]	52 ± 95.1 7.5 [4.7-70.7]	9.1 ± 10.3 6.2 [4.5-9.9]	1.5 ± 0.7 1.4 [1.1-1.7]
11.3 ± 16.3 6.7 [2.6-7.4]	5.3 ± 4.7 4.0 [3.2-4.7]	1.2 ± 0.7 1.0 [0.8-1.6]	39.2 ± 83.2 4.8 [4.2-39]	5.3 ± 4.0 5.3 [3.2-6.4]	0.8 ± 0.4 0.7 [0.5-1.0]
2.7 ± 3.7 1.3 [0.5-2.4]	3.5 ± 11.5 1.3 [1.0-1.6]	0.43 ± 0.34 0.34 [0.28-0.48]	6.2 ± 7.2 2.8 [1.3-9.4]	3.1 ± 7.0 1.5 [1.2-2.1]	0.35 ± 0.33 0.22 [0.09-0.55]
0.24 ± 0.16 0.20 [0.13-0.37]	0.30 ± 0.16 0.27 [0.24-0.32]	0.43 ± 0.19 0.43 [0.28-0.59]	0.31 ± 0.20 0.28 [0.17-0.45]	0.32 ± 0.17 0.29 [0.22-0.35]	0.48 ± 0.21 0.43 [0.36-0.63]
0.77 ± 0.16 0.80 [0.63-0.87]	0.70 ± 0.16 0.73 [0.68-0.76]	0.57 ± 0.19 0.58 [0.41-0.72]	0.29 ± 0.20 0.72 [0.55-0.83]	0.68 ± 0.17 0.72 [0.65-0.78]	0.52 ± 0.21 0.57 [0.37-0.64]
0.39 ± 0.44 0.25 [0.15-0.60]	0.63 ± 1.04 0.36 [0.32-0.47]	0.99 ± 0.78 0.74 [0.39-1.47]	0.64 ± 0.73 0.38 [0.20-0.81]	0.66 ± 0.99 0.40 [0.29-0.55]	1.44 ± 1.45 0.75 [0.56-1.69]

free to complexed PSA; CI, confidence interval; SD, standard deviation; PCaG, prostate cancer group; BG, benign group; CG,

diagnostic performance. Moreover, although ROC analysis of the diagnostic performances of the PSA assays and ratios for the predetermined age groups was also performed, none of the four groups presented with sufficient sample number to achieve a satisfactory statistical power (data not shown).

Discussion

The original studies of Brawer et al; presented cPSA assay as satisfactory enough to replace the tPSA and fPSA assays and their ratios in diagnosis of prostate cancer.[10] Subsequently, other authors argued against such optimistic expectations and suggested that the differentiation of prostate cancer from benign state could be equally improved with f/t or c/t, whereas cPSA alone does not have any additional discriminatory power.[13,14] Over the years, there have been several single and multicenter reports demonstrating the improved clinical value of cPSA alone as a discriminatory tool in patients that should be referred for biopsy.[15-21]

Our data indicated that concerning the clinically relevant cut offs, at 85% sensitivity cPSA assay alone is moderately superior to tPSA, with reasonably superior specificity compared to tPSA assay (63.1% and 57.2%, respectively; Table 4). However, no further increase in specificity was observed with either cPSA or tPSA assays, separately or in combination with the ratios, at the all remaining clinically important decision limits. Since the diagnostic enhancement of cPSA over tPSA assay was observed at lower levels of thresholds (at 2.37 ng/ml for cPSA and 3.00 ng/ml for tPSA), it can be hypothesized that utilizing cPSA assay within the 2.5-4 ng/ml tPSA range could improve detection of prostate cancer. Actually our suggestion is consistent

TABLE 4. **Diagnostic validity parameters of complexed PSA assay and complexed to total (c/t), free to total (f/t) and free to complexed (f/c) PSA ratios according to clinically relevant sensitivity values for total PSA assay**

	total PSA		**complexed PSA**		**f/c**		**f/t**		**c/t**	
AUC (%) [95%CI]	**80 [73.5-86.4]**		**81.2 [75.2-87.3]**		**72.4 [64.5-80.4]**		**67.4 [56-78.2]**		**63.2 [53.3-73.1]**	
Sn (%)	Cut off (ng/ml)	Sp (%) [95%CI]	Cut off (ng/ml)	Sp (%) [95%CI]	Cut off (ng/ml)	Sp (%) [95%CI]	Cut off (ng/ml)	Sp (%) [95%CI]	Cut off (ng/ml)	Sp (%) [95%CI]
75 [57.2-88.2]	4.69	72.3 [66.6-77.6]	3.00	70.5 [64.7-75.8]	0.48	58.7 [52.6-64.6]	0.33	46.3 [38-54.7]	0.99	51 [42.2-59.3]
85 [68.9-95]	**3.00**	**57.2 [51.4-63.5]**	**2.37**	**63.1 [57.1-68.9]**	**0.60**	**44.3 [38.3-50.4]**	**0.37**	**35.4 [27.7-43.7]**	**0.55**	**27.9 [20.8-35.9]**
90 [76.3-98.1]	2.83	55 [48.8-61]	1.63	53.9 [47.7-59.9]	0.82	33.6 [28-39.5]	0.41	27.9 [20.8-35.9]	0.47	20.4 [14.2-27.8]
95 [80.3-99.3]	2.13	46.1 [40.1-52.3]	1.05	41.7 [35.8-47.8]	1.49	20.3 [15.7-25.6]	0.65	4.8 [1.9-9.6]	0.29	10.2 [5.8-16.3]

AUC, area under the curve; CI, confidence interval; Sn, sensitivity; Sp, specificity

TABLE 5. **Diagnostic performance characteristics of PSA assays and ratios for all study groups considering the biopsy saved rates**

Variable	**Specificity (%)**	**Sensitivity (%)**	**Absolute value**	**PPV**	**NPV**	**(+) LR**	**(-) LR**	**Biopsy saved**
tPSA	63.1	81.8	3.55 ng/ml					
cPSA	**63.1**	**85**	**2.37 ng/ml**					
c/t	63.1	58.8	1.28					
f/t	63.1	61.8	0.24					
f/c	63.1	72.7	0.42					
tPSA	57.6	85	3.00 ng/ml	0.25	0.96	2.01	0.26	16.5%
cPSA	**63.1**	**85**	**2.37 ng/ml**	**0.27**	**0.96**	**2.31**	**0.24**	**27.1%**
c/t	27.9	85	0.55	0.22	0.89	1.18	0.53	13.5%
f/t	35.4	85	0.37	0.23	0.91	1.32	0.42	14.3%
f/c	44.3	85	0.60	0.20	0.95	1.53	0.34	15.8%

tPSA, total PSA; cPSA, complexed PSA; f/t, free to total PSA; c/t, complexed to total PSA; f/t, free to complexed PSA; PPV, positive predictive value; NPV, negative predictive value; (+) LR, positive likelihood ratio; (-) LR, negative likelihood ratio

with other previous studies proposing utilization of single cPSA assay for the questionable tPSA range.[18,22] Additional enhancement of specificity was not demonstrated in our study with any of the ratios, which was consistent with previous findings that when considered separately, none of the ratios is better than cPSA or tPSA.[13]

Interestingly, although with low statistical power, analyses of diagnostic performances of PSA assays and ratios in the different age groups seemed to further augment the value of cPSA. The AUC of the assays and the ratios appeared to be different for the different age intervals, Table 6. Because of the small number of prostate cancer cases in the $\leq$ 49 age group (only 2 out of 47 patients), highest AUC values of all the assays that was observed was considered misleading, but alternatively, it can be speculated that even small increases in the levels of any of the three PSA isoforms can warrant further investigation with regards to prostate cancer in this

TABLE 6. **The area under the curve (AUC) values of the PSA assays and ratios for the predefined age groups**

	tPSA	cPSA	c/t	f/t	f/c
≤ 49 age group (n = 47)	0.92	0.92	0.81	0.84	0.84
50-59 age group (n = 102)	0.85	0.89	0.87	0.81	0.83
60-69 age group (n = 98)	0.68	0.69	0.68	0.63	0.65
≥ 70 age group (n = 68)	0.76	0.74	0.61	0.54	0.61

tPSA, total PSA; cPSA, complexed PSA; fPSA, free PSA; c/t, complexed to total PSA; f/t, free to total PSA; f/c, free to complexed PSA

young age group. In the 60-69 year age group cPSA, tPSA and c/t ratio had very similar AUC values revealing no clinical diagnostic enhancement over each other. Similarly, no diagnostic superiority was observed for cPSA over tPSA in the ≥ 70 year age group within the clinically relevant ranges (data not shown), although still with higher AUC value consistent with increased performance. Generally, the diagnostic performances of both cPSA and tPSA assays over the age of 60 were significantly lower compared to the whole population data. This could be explained with the increased incidence of BPH with advancing age coupled with a high frequency of inflammatory changes in tissue, causing increase in both cPSA and tPSA levels, that negatively contribute to the differentiation of prostate cancer from BPH.[23] Finally, cPSA assay alone had a larger AUC in comparison to all the other assays and ratios in the 50-59 year age group, reflected mostly as a considerable increase in sensitivity and similar specificity of the assay compared to tPSA. For this age group, we were able to show that at optimal threshold of 2.3 ng/ml cPSA has the highest discriminatory power, achieving sensitivity and specificity of 90.9% and 78%, respectively. This is an important observation especially due to the sharp increase in frequency of BPH above the age of 50 also indicating the well known need for age-related adjustment of the optimal cPSA cut off.[24] Although there are studies emphasizing the significance of age related cut offs, to our knowledge, this is the first attempt to define a specific age related threshold using ROC analysis.[24,25]

However, the lower statistical power of the age related diagnostic performance analyses prevented us from any firm conclusions except to recommend further reproduction of findings with larger number of patient samples for each particular age group. It is possible that reports of moderate superiority of cPSA alone over the other PSA variables could have been improved if age adjusted diagnostic performance analyses were to be applied.

Finally, we want to mention some of the weaknesses of our study that could have contributed negatively to our findings. Although the inclusion criteria were tried to be optimally defined some of the non-biopsy performed control patients could still have under diagnosed prostate cancer. Because principally we were not interested in utilization of any invasive technique tPSA and cPSA density parameters for the biopsy performed group, found to be higher in comparison to tPSA or cPSA assays in some preliminary studies, though calculated, were not analyzed for their diagnostic validity.[26] ROC analyses were performed including the entire tPSA range and not specific determined gray zone interval such as 2-4, 4-10 or 2-20 ng/ml tPSA which did not reached sufficient sample number.

Analysis of our data indicated moderate superiority of cPSA to tPSA assay considering the sensitivity of 85%, corresponding to the 2.5-4 ng/ml tPSA range. We were not able to show such superiority for the other clinically relevant ranges. However, the results are quite promising for cPSA with its ability to avoid major portion of the biopsies in the benign group and missing small fraction of cancers within this range. Owing to the fact that none of the remaining measured assays and ratios, either separately or in combination, performed better than cPSA alone, it is possible to speculate that application of follow up cPSA velocity could provide helpful in further lowering the false negative rate. Finally, a question for different diagnostic performances of the PSA assays within different age intervals has emerged that needs to be elucidated.

Conclusions

Although still actively debated, it appears that cPSA alone may be a step forward to improve further the diagnostic ability of tPSA, but the search for easily performed, noninvasive and highly reliable marker, exclusively for low tPSA range utilization, still remains a challenge. □

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