



Utility of Prostate-Specific Antigen Isoforms and Prostate Health Index in the Diagnosis of Metastatic Prostate Cancer

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Abstract

Objective The current study was undertaken to investigate the utility of total prostate-specific antigen (tPSA), its isoform [-2] proPSA (p2PSA), and prostate health index (PHI) in the diagnosis of metastatic prostate cancer (PCa).

Materials and Methods This study was conducted from March 2016 to May 2019. Eighty-five subjects who were diagnosed with PCa for the first time, following transrectal ultrasound-guided prostate biopsy, were included in the study. The prebiopsy blood samples were analyzed in Beckman Coulter Access-2 Immunoanalyzer for tPSA, p2PSA, and free PSA (fPSA), and the calculated parameters included %p2PSA, %fPSA, and PHI. Mann–Whitney's *U* test was used as test of significance, and *p*-value less than 0.05 was considered statistically significant.

Results Of the 85 participants, 81.2% (*n* = 69) had evidence of metastasis, both clinically and pathologically. The median tPSA (ng/mL), p2PSA (pg/mL), %p2PSA, and PHI were significantly higher in the group with evidence of metastasis (46.5 vs. 13.76; 198.0 vs. 35.72; 3.25 vs. 1.51; 237.58 vs. 59.74, respectively). The sensitivity (%), specificity (%), negative predictive value (%), and positive predictive value (%) to diagnose metastatic PCa of tPSA at a cutoff of 20 ng/mL, PHI at a cutoff of 55, and %p2PSA at a cutoff of 1.66 were 92.7, 98.5, and 94.2; 37.5, 43.7, and 62.5; 54.5, 87.5, and 71.4; and 86.4, 88.3, and 91.5, respectively.

Conclusion Using tests such as %p2PSA and PHI in the standard armamentarium for the diagnosis of metastatic PCa in addition to PSA will help in selecting the appropriate treatment strategy, including active surveillance.

Keywords

- ▶ prostate cancer
- ▶ prostate health index
- ▶ prostate-specific antigen
- ▶ PSA isoforms

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Introduction

Prostate cancer (PCa), the second most frequently diagnosed cancer accounting for more than 1.4 million new cases in 2020, and the fifth leading cause of mortality worldwide, poses major challenges in prevention and management.^{1,2} Despite advances in diagnosis and treatment, the incidence of PCa has seen a significant increase in recent years and is the third most common cancer among Indian males.^{3,4} According to the report from the National Cancer Registry Program, the cumulative risk of developing PCa is 1 in 125 with a projected incidence of 41,532 cases in 2020.³ Changes in lifestyle, increased screening, and increase in life expectancy are the major reasons for the rise in PCa incidence.^{5,6}

In early stages, PCa can run an indolent course, emphasizing the need to dissociate diagnosis from treatment and consider active surveillance (AS) for men with low- or intermediate-risk disease.⁷ Hence, the major challenge in PCa treatment is to detect high-risk individuals who could not be managed with AS but require immediate aggressive treatment within the window of curability.⁸ In this context, measurement of serum total prostate-specific antigen (tPSA) plays a crucial role in the diagnosis of high-risk PCa.^{9,10}

The National Comprehensive Cancer Network (NCCN) defines high-risk PCa as PSA greater than 20 ng/mL or grade group 4 or 5 or clinical T3a.¹¹ The different treatment options available for high-risk PCa include radical prostatectomy (RP),¹² external beam radiotherapy (EBRT) with androgen deprivation therapy (ADT) or EBRT plus brachytherapy and ADT,^{13,14} all of which have their own benefits but may be associated with posttreatment unwarranted effects on the quality of life. Hence, management of high-risk PCa involves making a choice between AS and all the available treatment options, and this is usually a shared decision taken by the clinical team and the patient/patient caregivers or family. Making this decision based on only few variables such as PSA levels, biopsy findings, and clinical staging may prove to be difficult. Hence, there is a need for more multivariable approach using several informative markers. This is the key for the development of newer markers for diagnosis of high-risk metastatic PCa.

In the blood, PSA is predominantly (70–90%) bound to serum protease inhibitors, and around 10 to 30% exist in free state—free PSA (fPSA).¹⁵ Among the fPSA forms, isoform [-2] proPSA (p2PSA), primarily found in tumor extracts,⁸ plays a key role in early detection and in the prediction of aggressive disease.¹⁶ Consequently, the prostate health index (PHI) that combines all the three forms of PSA (tPSA, fPSA, and p2PSA) to give a single score using the formula: $(p2PSA/fPSA) \times \sqrt{fPSA}$ ¹⁷ has been studied across the globe for its usefulness as a diagnostic and prognostic test of PCa.^{8,17–19} In multiple prospective international trials,¹⁷ PHI was shown to outperform tPSA and fPSA measurements in early diagnosis of PCa. PHI is also associated with an increase in Gleason score following RP, hence useful as an additional test during AS.^{8,12,20}

Although several studies across different ethnic groups in various countries have unequivocally demonstrated the reliability of PHI for diagnosis of high-risk metastatic PCa, there is paucity of information with reference to the Indian popu-

lation. The current study was therefore undertaken to investigate the utility of measuring PSA, p2PSA (%p2PSA), and PHI in the diagnosis of metastatic PCa.

Materials and Methods

The present study was performed between March 2016 and May 2019, in a Joint Commission International accredited Hospital in Bengaluru, Karnataka, India, whose laboratory services were accredited by the National Accreditation Board for Testing and Calibration Laboratories (NABL). The study protocol was approved by the Institutional Ethics Committee Clinical Studies (vide letter # 001/02-16 dated February 29, 2016).

Patient Selection and Evaluation

All consecutive patients ($n = 85$) diagnosed with adenocarcinoma of the prostate following a transrectal ultrasound-guided prostate biopsy for the first time were included for the study after obtaining informed written consent. Patients receiving 5- α -reductase inhibitors and those with previous history of prostatic surgery for any prostatic condition were excluded from this study. The diagnosis of adenocarcinoma of prostate was done by a qualified oncologist based on detailed clinical examination as well as on histopathological report that included the Gleason score and International Society of Urological Pathology (ISUP) grade provided by a qualified histopathologist. Further, metastasis was confirmed by the oncologist with the help of diagnostic imaging scans such as magnetic resonance imaging and ultrasound in addition to immunohistochemical analyses using a targeted panel of antibodies. Based on the final diagnosis, the study participants were divided into two groups: patients presenting with metastasis (clinical stage T3 and above; ISUP grade 4 and above) and those without evidence of metastasis.

Sample Collection

Blood samples were drawn prior to prostate biopsy using standard aseptic precautions in red-topped blood collection evacuated serum separator tubes manufactured by Becton Dickinson Company, as specified by the kit manufacturer. Samples were allowed to clot at room temperature and centrifuged at 3,500 rpm for 10 minutes. The sera were analyzed for tPSA immediately and the remaining sera aliquoted, labeled, and stored at -80°C until analysis for other parameters.

Biochemical Analysis

Upon confirmation of diagnosis of adenocarcinoma, the sera of the study subjects were used for the estimation of fPSA (pg/mL) and p2PSA (pg/mL) in Beckman Coulter Access-2 Immunoanalyzer using Access Hybritech p2PSA reagents (Beckman Coulter, Inc.). The prebiopsy tPSA (ng/mL) values were used to calculate the following:

1. %p2PSA was calculated using the formula $(p2PSA/fPSA) \times 100$.
2. %fPSA was calculated using the formula $(fPSA/tPSA) \times 100$.
3. PHI was calculated using the formula $([p2PSA/fPSA] \times \sqrt{fPSA})$.¹⁷

Table 1 Age group distribution of the subjects in the study cohort

Age (y)	With metastasis	No evidence of metastasis
51-60	8 (11.5%)	2 (12.5%)
61-70	23 (33.3%)	4 (25%)
71-80	28 (40.5%)	8 (50%)
> 80	10 (14.4%)	2 (12.5%)
Total	69	16

The biochemical parameters were analyzed as per the manufacturer’s guidelines. The Beckman Coulter Access Hybritech tPSA, p2PSA, and fPSA assays are all two-site chemiluminescent immunoenzymatic (sandwich) assays used for the quantitative determination of the respective parameter.^{21,22} Internal quality controls and external quality assurance programs were run to ensure the quality of the test results. Trilevel quality controls from Biorad Company were used for tPSA on a daily basis and on sample processing day for fPSA, whereas for p2PSA, three levels of kit controls provided by Beckman Coulter were run on the day of analysis. A standard deviation of ± 1 was considered to be acceptable. In-house precision was done prior to analysis as per the guidelines of the Clinical and Laboratory Standards Institute. The coefficient of variation for all the analytes was less than 5%.

Statistical Analysis

The data were analyzed using SPSS version 22. Categorical data are presented in the form of frequencies and percentages. Continuous data are presented as mean and standard deviation or as median with interquartile ranges. Mann-Whitney’s U test was used as test of significance to identify the mean difference between two quantitative variables with skewed distribution.²³ A p-value of less than 0.05 was considered to be statistically significant.

Results

Of the 85 patients, 81.2% (n = 69) presented (identified) with evidence of metastasis and the remaining (n = 16) did not show any evidence of metastasis at the time of diagnosis of PCa.

The distribution of the patients in different age groups is given in ▶Table 1. The age group of 71 to 80 years had maximum cases of metastasis accounting for 40.5% of the total 69 cases of metastatic PCa. Interestingly, this age group has also recorded maximum cases of nonmetastatic PCa accounting for 50% of this cohort (▶Table 1).

The median tPSA (ng/mL), p2PSA (pg/mL), %p2PSA, and PHI values in all the quartiles were significantly higher in the group with evidence of metastasis. The median %fPSA values in all quartiles was significantly lower in the group which showed evidence of metastasis (▶Table 2).

The validity indicators, namely, sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV) were calculated for the ability of the tests to

Table 2 Comparison of the variables in the two groups in our study cohort

Test parameters	Evidence of metastasis						No (n = 16)			Mann-Whitney U test	p-Value	
	Yes (n = 69)			No (n = 16)			Mean	Median	Std. Dev			
	Mean	Median	Std. Dev	Q1	Q3	Quartiles						
tPSA (ng/mL)	230.71	46.5	411.35	15.69	222.40	5.86	46.82	13.76	54.86	89.91	344.5	0.020 ^a
p2PSA (pg/mL)	1,080.80	198.0	1,700.78	34.44	1,159.50	17.16	508.67	35.72	1,069.67	200.58	368.5	0.039 ^a
%p2PSA	7.15	3.25	8.64	1.79	8.60	0.89	3.60	1.51	4.44	4.96	333	0.014 ^a
%fPSA	11.56	10.09	7.97	5.51	16.29	11.59	18.36	18.23	9.15	23.40	293	0.004 ^a
PHI	1,317.24	237.58	2,410.57	78.29	1,270.16	24.27	341.24	59.74	580.43	375.38	322	0.010 ^a

Abbreviations: fPSA, free prostate-specific antigen; PHI, prostate health index; p2PSA, isoform [-2] proPSA; Std. Dev, standard deviation; tPSA, total prostate-specific antigen.
^ap-Value is significant.

Table 3 Validity indicators and 2 × 2 contingency tables for tPSA at a cutoff value of 20 ng/mL, PHI at a cutoff value of 55, and %p2PSA at a cutoff value of 1.66

	Metastasis	No evidence of metastasis	Total	Sensitivity%	Specificity%	NPV%	PPV%
tPSA (ng/mL)							
≤ 20	5 (FN)	6 (TN)	11	92.7	37.5	54.5	86.4
> 20	64 (TP)	10 (FP)	74				
Total	69	67	85				
PHI							
≤ 55	1 (FN)	7 (TN)	8	98.5	43.7	87.5	88.3
> 55	68 (TP)	9 (FP)	77				
Total	69	16	85				
%p2PSA							
< 1.66	4 (FN)	10 (TN)	14	94.2	62.5	71.4	91.5
≥ 1.66	65 (TP)	6 (FP)	71				
Total	69	16	85				

Abbreviations: FN, false negative; FP, false positive; NPV, negative predictive value; PHI, prostate health index; p2PSA, isoform [-2] proPSA; PPV, positive predictive value; TN, true negative; TP, true positive; tPSA, total prostate-specific antigen.

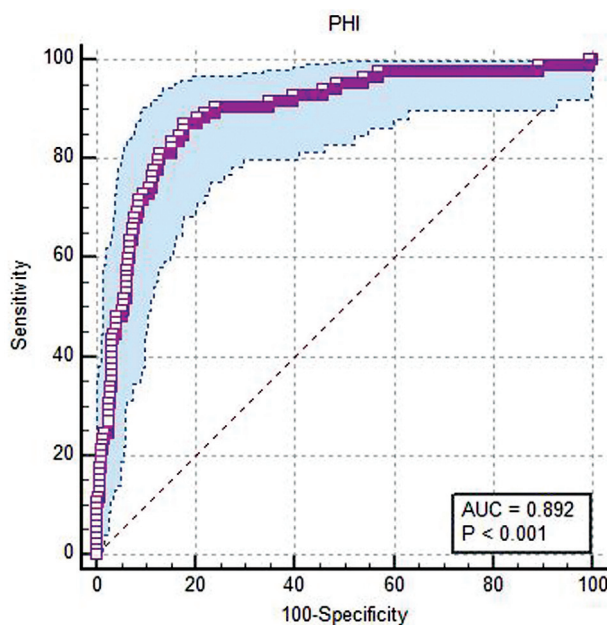
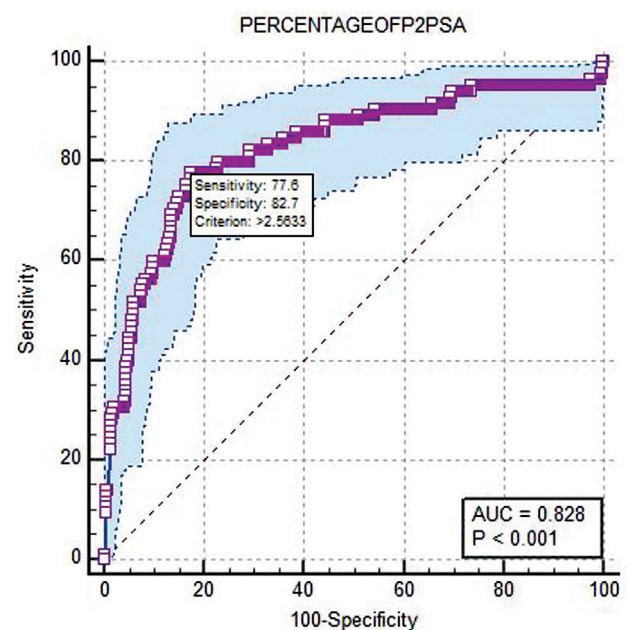
diagnose metastatic high-risk PCa in comparison to the histopathological and imaging studies which acted as the gold standard based on which the presence of metastasis had been confirmed (►Table 3).

In our study, when we used a cutoff value of 20 ng/mL for tPSA based on the NCCN criterion for diagnosis of high-risk PCa,¹¹ tPSA had a sensitivity of 92.7% and specificity 37.5% with NPV of 54.5% and PPV of 86.4% for diagnosis of metastatic PCa.

For PHI, when 55 was considered as cutoff based on previous studies,²⁴ PHI had a sensitivity of 98.5% and specificity of 43.7%, while NPV was 87.5% and PPV was 88.3% for

diagnosis of metastatic PCa. The area under the receiver operating curve (AUC) was 0.892 with *p*-value less than 0.001 (►Fig. 1). The Youden index *J* was 0.69 when the criterion of PHI greater than 90.16 was used with a sensitivity of 87.06 and specificity of 82.2 with 95% confidence interval (CI) of 78.0 to 93.4 and 78.1 to 85.8, respectively.

When we used a threshold value of 1.66 for %p2PSA,²⁵ it had a sensitivity of 94.2% and a specificity of 62.5% with NPV of 71.4% and PPV of 91.5%. The AUC for %p2PSA is shown in ►Fig. 2. The AUC was 0.828 with a *p*-value of less than 0.001. The Youden index was found to be 0.60 when the criterion of %p2PSA greater than 2.56 was used with a

**Fig. 1** Area under the receiver operating curve (AUC) for prostate health index (PHI).**Fig. 2** Area under the receiver operating curve (AUC) for percentage of isoform [-2] proPSA (%p2PSA).

sensitivity of 77.65 and specificity of 82.68 with 95% CI of 67.3 to 86.0 and 78.7 to 86.2, respectively.

Discussion

The purpose of this study was to investigate the utility of estimating PSA isoforms and PHI in the diagnosis of metastatic high-risk PCa, in a cohort of Indian population. Majority of the patients in our study showed evidence of metastasis and the medical records of these patients revealed that the sites of metastasis include regional lymph nodes, bone, distant lymph nodes, lungs, and liver in descending order of frequency. Most of these patients belonged to the age group 71 to 80 years similar to findings documented in the literature.²

All the biomarkers evaluated in this study were significantly elevated in the group which showed evidence of metastasis. Previous studies^{26,27} have shown that among the PSA isoforms, the production of p2PSA is selectively increased in cancer and is significantly associated with high-grade PCa at RP, and that higher %p2PSA may be regarded as a diagnostic marker for clinically significant PCa.²⁸ In our study, %p2PSA was significantly increased in patients who displayed evidence of metastasis when compared with patients without metastasis. Moreover, %p2PSA with a cutoff of 1.66 turned out to be a better marker both in terms of sensitivity and specificity for metastatic PCa when compared with tPSA and PHI (► **Table 3**). In a previous meta-analysis study,²⁶ which showed that %p2PSA along with PHI could detect more aggressive PCa at the initial prostate biopsy, the AUC for %p2PSA was 0.54. We speculate that a value of 0.82 for the AUC of %p2PSA obtained in the present study could be because majority of the patients presented with metastasis and for the same reason, the %p2PSA turned out to be a better biomarker in terms of specificity of metastatic PCa than tPSA and PHI.

The PHI developed by Beckman Coulter, Inc. is a mathematical formula and uses three biomarkers, p2PSA, fPSA, and tPSA to give an index number. Although the PHI test has regulatory approval in more than 50 countries worldwide, its use in India is limited and used sparingly for either screening or in predicting the aggressiveness of PCa.²⁷ Discrepancies exist between clinical cancer staging and pathological staging.²⁹ There are few studies which have used PHI in this clinical context, but some studies have shown that among patients with biochemical recurrence, p2PSA and PHI were significantly higher in men with metastatic PCa compared with those without clinical metastasis.^{17,29} In our study also, PHI was significantly elevated in patients presenting with metastasis compared with those without any evidence of metastasis.

There has been no consensus on the most appropriate cutoff value for the %p2PSA and PHI in cancer detection or for predicting clinically significant PCa due to the use of different study designs.³⁰ So, when we used a threshold of 30 for PHI as recommended for Asian population,³¹ the specificity of PHI in our study decreased to 25%, with 98% sensitivity and hence

according to our study results, a cutoff value of 55 for PHI as recommended worldwide would be better suited for Indian population. When compared with tPSA, PHI was more specific at this cutoff value and may be useful as an adjunct marker for risk stratification at initial diagnosis and in predicting the possibility of metastasis.

This study, which is one of its kind performed among the Indian population adds to the information that is available on %p2PSA and PHI besides underscoring the potential utility of these novel biomarkers in understanding the impact of age, ethnicity, and extent of disease. However, this study is relatively small sized, and there are limitations to generalize the findings to the entire population. Future studies are necessary to further evaluate %p2PSA and PHI for their biological reference ranges and validate their role with appropriate cutoff values in the management of more advanced disease.

Conclusion

Since many treatment options are available including AS for the management of PCa especially when there is a possibility of metastasis, using a multimodal approach to take treatment decisions will be useful rather than based on only pathological and clinical staging. Using many noninvasive or minimally invasive tests such as %p2PSA and PHI as a part of the standard armamentarium will give additional information that will help in the decision-making process for treatment selection for PCa.

Authors' Contribution

G.N.L. contributed to study concept, design, acquisition analysis and interpretation of data, drafting the article, and revising it critically for intellectual content. P.P.B. drafted the article and revised it critically for intellectual content. S.N. guided, designed, and approved the final version to be published.

Conflict of Interest

None declared.

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