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ORIGINAL ARTICLE

ProPSA and the Prostate Health Index as predictive markers for aggressiveness in low-risk prostate cancer—results from an international multicenter study

I Heidegger[1], H Klocker[1], R Pichler[1], A Pircher[2], W Prokop[3], E Steiner[1], C Ladurner[4], E Comploj[4], A Lunacek[5], D Djordjevic[6], A Pycha[4], E Plas[5], W Horninger[1] and J Bektic[1]

BACKGROUND: One of the major challenges in prostate cancer (PCa) treatment is distinguishing insignificant PCa from those forms that need active treatment. We evaluated the impact of PSA isoforms on risk stratification in patients with low-risk PCa as well as in active surveillance (AS) candidates who underwent radical prostatectomy.

METHODS: A total of 112 patients with biopsy confirmed Gleason score (GS) 6 PCa of four different international institutions were prospectively enrolled in the study. Blood withdrawal was performed the day before radical prostatectomy. In addition, patients were classified according to the EAU and NCCN criteria for AS candidates. PSA, free PSA (fPSA) and proPSA were measured using dual monoclonal antibody sandwich immunoassays. In addition, the Prostate Health Index (PHI = proPSA/fPSA × √PSA) was calculated. Final histology of the radical prostatectomy specimens was correlated to PSA, its isoforms and PHI.

RESULTS: Serum proPSA levels were significantly elevated in those patients with an upgrade in final histology (GS ⩾ 7). In addition, higher proPSA levels were predictive for extraprostatic extension (⩾ pT3a) as well as for positive surgical margins. Interestingly, PHI had an even higher predictive power when compared with proPSA alone concerning GS upgrading, extraprostatic extension and surgical margins in both the total and the AS patient group.

CONCLUSION: We showed in a multicenter study that proPSA is a valuable biomarker to detect patients with aggressive PCa in a cohort of GS 6 patients, who would benefit from active tumor therapy. Combining proPSA with the standard markers PSA and fPSA using PHI further increases the predictive accuracy significantly. Moreover, our data support the use of PHI for monitoring PCa patients under AS.

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INTRODUCTION

Measurement of PSA levels in blood is widely used as diagnostic, predictive and prognostic marker of prostate cancer (PCa).[1–3] In addition to total PSA, selective detection of molecular forms of PSA has been suggested to increase the informative value of PSA testing.[4,5] In general, PSA is a serine-protease produced and released by epithelial cells of the prostate. PSA is secreted as an inactive proenzyme (proPSA) into seminal fluid and activated by the kallikrein-related peptidase 2 and other endopeptidases produced in the prostate. PSA itself occurs in several different molecular forms in serum: free PSA (fPSA, composed of several subtypes, proPSA, cleaved PSA and others) and complexed PSA (cPSA).[1]

Currently fPSA/tPSA ratio is widely used to differentiate BPH from cancer as PCa was detected in biopsies of 56% of men with fPSA/PSA $<$ 10 ng/ml but only in 8% of patients with a high fPSA/PSA ratio.[6,7]

In addition, several studies identified the PSA isoform proPSA as a predictor of significant PCa.[4,8,9] However, only few studies including our own work, were able to demonstrate that the amount of proPSA is associated with aggressive forms of PCa.[9–11]

The calculated factor Prostate Health Index (PHI) that combines PSA, fPSA and proPSA, outperforms tPSA in discriminating the presence of PCa from noncancerous prostatic diseases.[12,13] Previous unicentric studies were able to show that PHI is able to predict PCa aggressiveness, however, to the best of our knowledge no multicenter study evaluated the impact of PHI as predictor of aggressive PCa using prospectively collected patient samples before radical prostatectomy (RP).[11,14]

A major challenge in PCa treatment is to distinguish those forms of PCa that become metastatic and thus lethal without active therapy from slow growing indolent forms of the disease that can undergo an active surveillance (AS) regime.[15]

Existing guidelines regarding definition and inclusion criteria of AS candidates vary widely among the different societies, however, most of them like the European Society of Urology (EAU) guidelines 2016 as well as according to the National Comprehensive Cancer Network (NCCN) guidelines from 2014, state that AS can be offered to patients with the lowest risk of cancer progression in particular clinical stage T1-2a, PSA $<$ 10 ng ml, biopsy Gleason score (GS) ⩽ 6 (at least 10 biopsy cores taken), and only one or two positive biopsies with $>$ 50% tumor involvement in a biopsy core.[16]

[1]Department of Urology, Medical University Innsbruck, Innsbruck, Austria; [2]Department of Internal Medicine V, Hematology and Oncology, Medical University Innsbruck, Innsbruck, Austria; [3]Department of Laboratory and Chemical Medicine, Medical University Innsbruck, Innsbruck, Austria; [4]Department of Urology, Hospital of Bolzano, Bolzano, Italy; [5]Department of Urology, Hanusch Hospital Wien, Vienna, Austria and [6]Department of Urology, Klinicki centar Srbije Beograd, Belgrade, Serbia. Correspondence: Associate Professor J Bektic, Department of Urology, Medical University Innsbruck, Anichstreet 35, Innsbruck 6020, Austria.
E-mail: Jasmin.bektic@tirol-kliniken.ac.at
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However, we recently showed that 41.6% of patients who meet the selection criteria for AS were upgraded after analysis of RP specimens when RP was performed in these patients.[17] Thus, one of the most important challenges in PCa research is the identification of biomarkers to distinguish patients who need definitive therapy from those who are real candidates for AS. Therefore, the aim of the present multicenter study was to evaluate the impact of proPSA and PHI as predictive markers of PCa aggressiveness in patients with biopsy GS 6 PCa as well as to determine their value in the management of candidates for AS and as a predictive biomarker during AS.

MATERIALS AND METHODS

Patients and data acquisition

This international multicenter study has been approved by the Ethic Review Committee Innsbruck (Austria) (UN 5219, 329/4.3). In addition, each participating institution obtained an additional ethic approval by their local Ethic Committees. Informed consent was obtained from all patients. The patients were recruited between May 2012 and June 2014 at two urological centers in Austria (Medical University Innsbruck, Hanusch Hospital Vienna), one center in Italy (Hospital Bolzano) and a study center in Serbia (Klinicki centar Srbije Beograd).

Sample preparation and PSA isoforms measurement

All serum samples were prospectively collected from patients scheduled for RP because of a biopsy diagnosed GS 6 PCa as part of the pre-operative blood withdrawal. Within 1 h after collection, the samples were centrifuged at 3200 U min for 6 minutes. Immediately afterwards, the serum was stored at − 80 °C and thawed only once for study analysis. Total PSA and fPSA levels were determined by Access Hybritech PSA and fPSA assays (Beckman Coulter, Fullerton, CA, USA).[18] The immunoassay for the detection of proPSA was a commercial dual monoclonal antibody sandwich assay (Access Hybridech pPSA assay, Beckman Coulter) in a microtiter plate format using a biotinylated capture anti-PSA monoclonal antibody and Europium-labeled proPSA-specific monoclonal antibody for detection with a Victor 1420 multi-label counter (PerkinElmer, Gaithersburg, MD, USA). The assays were performed according to the manufacturer's instructions.

In addition, PHI was calculated using the formula PHI = proPSA/fPSA × √PSA as described previously.[13]

Readout and statistical evaluation

We evaluated the usefulness of the PSA isoform proPSA and PHI in predicting PCa aggressiveness defined as GS upgrade from GS 6 to ⩾ GS 7 and/or histology upgrade from organ confined (⩽pT2c) to extraprostatic (⩾pT3a) disease. In addition, we calculated the impact of proPSA and PHI in prediction of positive surgical margins. Moreover, we analyzed the impact of proPSA and PHI in patients eligible for AS according to the EAU and NCCN criteria, that can be offered to patients with the lowest risk of cancer progression defined as cT1/2, GS ⩽6, ⩽2 positive cores, PSA ⩽10 ng ml and ⩽50% cancer involvement per biopsy core.

Using logistic regression, the influence of PSA, fPSA, proPSA and PHI on the outcome was investigated; odds ratio was given as a measure of this. In addition, these factors were considered by multivariate log regression in the multivariate context. The *P*-values below 0.05 are considered significant (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). Box plots (Figures 1 and 2) show median, 25 and 75 percent quartiles, as well as outliers, moreover results are presented as scatter blots (Supplementary Figure 1).

RESULTS

Patient characteristics

A total of 112 patients with biopsy diagnosed GS 6 cancer were prospectively enrolled in the study. Patient characteristics are shown in Table 1. Nineteen patients (17%) had a first-degree relative with PCa.

Evaluating final histology of RP specimens, we found that 75/112 (66.7%) of patients had a histological upgrade (⩾GS 7). Detailed pathology report of RP specimens is shown in Table 2.

In line with previous studies, PSA and fPSA predicted GS upgrading, adverse pathology (⩾pT3a) as well as positive surgical margins in our patient collective (Table 3).[3,5,7] Evaluating proPSA in predicting PCa aggressiveness, we found that proPSA outperformed PSA and fPSA in predicting aggressive PCa (GS upgrading and adverse pathology) as well as positive margins (Table 3, Figures 1 and 2, Supplementary Figure 1).

As proPSA was highly predictive for PCa aggressiveness in our patient collective, we aimed to add this marker to the routinely used values PSA and fPSA. For this, we calculated the PHI incorporating proPSA, PSA and fPSA. We found that PHI has an even higher predictive power when compared with proPSA alone concerning GS upgrading ($P = 0.004$), extraprostatic extension ($P < 0.001$) and surgical margins ($P = 0.051$). Thus, we conclude that the PHI incorporating PSA, fPSA and proPSA is able to predict PCa aggressiveness as well as positive surgical resection status with highest accuracy compared with each of the single markers (Table 4, Figures 1 and 2, Supplementary Figure 1).

Next, we tested the impact of proPSA and PHI in a multivariate logistic regression analysis including patient characteristics like age, body mass index, prostate volume, PSA density or the number of positive cores. However, we could not emphasize any of the factors to influence significantly the outcome of the findings in a multivariate context.

Among our patient collective, 44 patients met the criteria for AS according to the EAU and NCCN criteria termed in the following as 'AS candidates'. Just as for the entire patient group, PSA and its isoforms fPSA and proPSA were predictive for PCa aggressiveness and local cancer expansion in the AS candidate cohort (Table 5). Again proPSA outperformed PSA and fPSA.

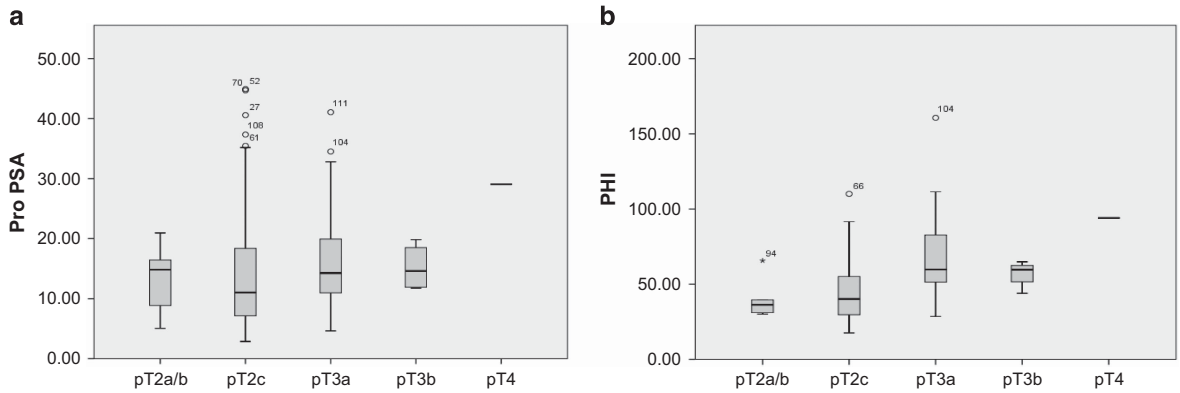

Figure 1. Box plots showing pT stage of radical prostatectomy specimens in relation to proPSA (**a**) and Prostate Health Index (PHI) (**b**) levels.

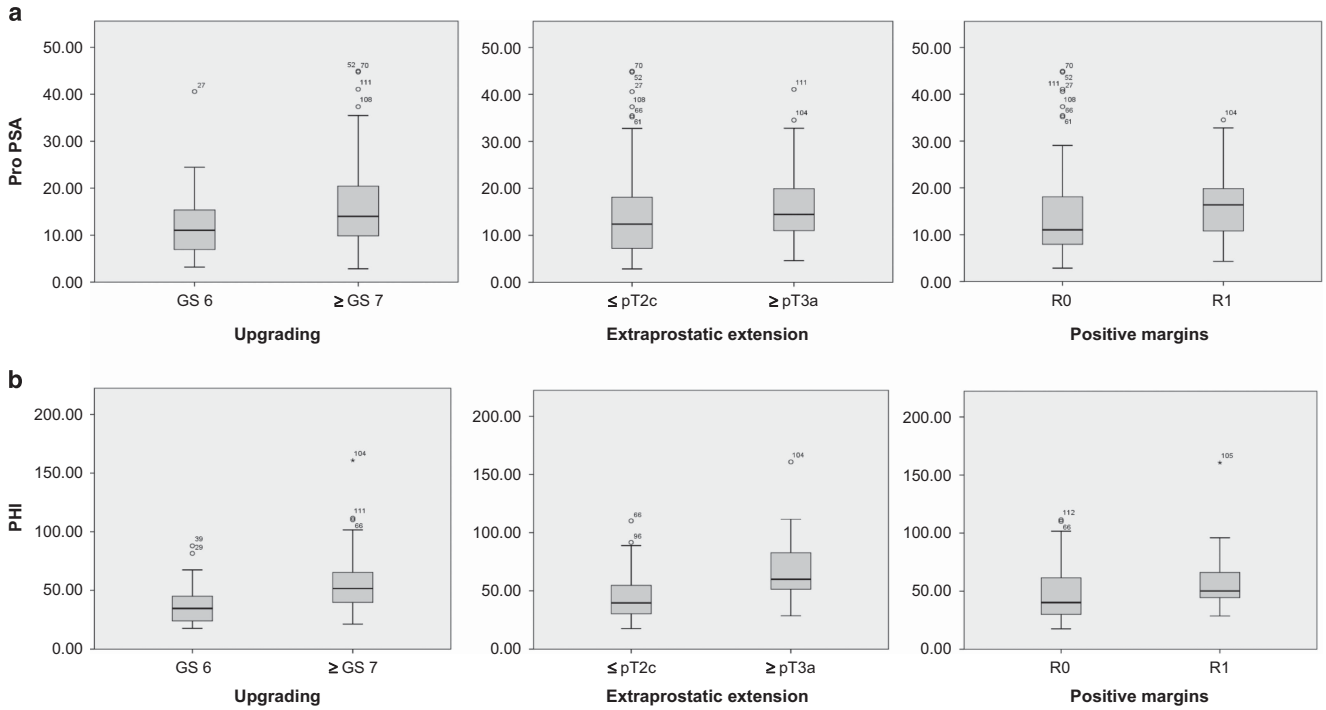

Figure 2. Box plots showing Gleason score (GS) upgrading, extraprostatic extension (⩽ pT2c vs ⩾ pT3a) and surgical margins (R0 vs R1) in relation to proPSA (**a**) and Prostate Health Index (PHI) (**b**) levels.

Table 1. Patient characteristics

Parameter	*Mean*	*Median*	*Range*
Age	61.57	63	40–76
BMI	26.45	26.49	18.82–38.86
Transrectal prostate volume (ml)	43.56	39.5	14–101
PSA density (tPSA/prostate volume, ng/ml)	0.15	0.13	0.04–1.01
Positive cores (%)	26%	21%	8.3%–100%
PSA (ng/ml)	5.87	5.38	1.15–25.41
fPSA (%)	13.19	12.58	2.37–30.6
ProPSA (ng/ml)	16.41	12.72	2.85–176.86
PHI	54.96	46.08	17.61–500.49

Abbreviations: BMI, body mass index; fPSA, free PSA; PHI, Prostate Health Index; PSA, total PSA.

Table 2. Final histology of upgraded patients in the RP specimens ($n = 75$) according to the International Society of Urological Pathology and Gleason classifications

ISUP (GS)	n *(%)*
ISUP 2 (GS 3+4)	57 (76%)
ISUP 3 (GS 4+3)	14 (18.7%)
ISUP 4 (GS 4+4)	2 (2.7%)
ISUP 5 (GS 5+4)	2 (2.7%)

Abbreviations: GS, Gleason score; ISUP, International Society of Urological Pathology; RP, radical prostatectomy.

Interestingly, PHI was able to predict extraprostatic extension ($P = 0.001$) and positive margins ($P = 0.043$), in our subgroup of AS candidates (Table 6).

Therefore, we conclude that PHI is a useful marker for the prediction of PCa aggressiveness and local extension in candidates for AS and in patients undergoing AS.

Table 3. Impact of PSA, fPSA and proPSA on histology

Parameter	*PSA*	*fPSA*	*ProPSA*
Upgrading (GS 6 vs ⩾ GS 7)			
P-value	0.028	0.957	0.022
Odds ratio	1.211	0.264	1.063
Extraprostatic extension (⩽ pT2c vs ⩾ pT3a)			
P-value	< 0.001	0.001	0.029
Odds ratio	1.339	0.810	1.033
Positive margins (R0 vs R1)			
P-value	0.019	0.066	0.031
Odds ratio	1.179	0.918	1.027

Abbreviations: GS, Gleason score; fPSA, free PSA.

DISCUSSION

Both proPSA and PHI have a significant role in PCa detection.[4,8,19,20] In contrast to most other studies in the present study, we did not focus on the detection of PCa, rather we focused on the identification of aggressive tumors defined as GS ⩾7 and/or extraprostatic extension (⩾ pT3a). We recently showed in a retrospective study that proPSA and its combination with PSA and fPSA is a marker for PCa aggressiveness.[10] In the present study, we were able to confirm these data in a international multicenter study of prospectively collected patient samples. In addition, we demonstrate that combining these parameters using PHI further improves the diagnostic accuracy compared with single PSA isoforms.

Our study supports the results of a recent work of Cantiello *et al*.[14] who evaluated patients with biopsy-proven localized PCa treated by RP. They concluded that PHI predicts GS, extracapsular extension as well as seminal vesicles involvement.[14]

Table 4. Impact of PHI on histology

Parameter	*PHI*
Upgrading (GS 6 vs ⩾GS 7)	
P-value	0.004
Odds ratio	1.039
Extraprostatic extension (⩽pT2c vs ⩾pT3a)	
P-value	< 0.001
Odds ratio	1.046
Positive margins (R0 vs R1)	
P-value	0.051
Odds ratio	1.018

Abbreviations: GS, Gleason score; PHI, Prostate Health Index.

Table 5. Impact of PSA, fPSA and proPSA on histology in the active surveillance subgroup

Parameter	*PSA*	*fPSA*	*ProPSA*
Upgrading (GS 6 vs ⩾GS 7)			
P-value	0.212	0.760	0.310
Odds ratio	0.1097	0.988	1.021
Extraprostatic extension (⩽pT2c vs ⩾pT3a)			
P-value	0.002	0.001	0.129
Odds ratio	1.346	0.028	1.033
Positive margins (R0 vs R1)			
P-value	0.021	0.072	0.148
Odds ratio	0.141	0.920	1.030

Abbreviations: GS, Gleason score; fPSA, free PSA.

Table 6. Impact of PHI on histology in the active surveillance subgroup

Parameter	*PHI*
Upgrading (GS 6 vs ⩾GS 7)	
P-value	0.412
Odds ratio	1.06
Extraprostatic extension (⩽pT2c vs ⩾pT3a)	
P-value	< 0.001
Odds ratio	1.048
Positive margins (R0 vs R1)	
P-value	0.043
Odds ratio	1.020

Abbreviations: GS, Gleason score; PHI, Prostate Health Index.

One of the most important challenges in PCa research is the identification of biomarkers for distinguishing significant from insignificant forms of PCa to avoid overdiagnosis and over-treatment on the one hand, and on the other hand to early treat those patients who would progress to metastatic and thus lethal forms of the disease. Numerous serum and urine biomarkers have been evaluated in preclinical and clinical studies including PSA density, urinary TMPRSS2-ERG or PCA3 mRNA, however, their clinical application is progressing slowly (reviewed in refs 21,22). Consequently, PSA remains the main marker for monitoring cancer progression in an AS regime.

In recent years, multiparametric magnetic resonance imaging has been proposed for monitoring these patients.[18,23] Despite this, the patients are forced to undergo regularly re-biopsies of the prostate for discovering a potential change from a low-risk to a significant disease that needs active treatment. The use of proPSA and PHI in monitoring AS has not been evaluated adequately so far.[11,24] Therefore, we sub-analyzed our patient collective fulfilling the EAU and NCCN criteria for AS with the aim to test biomarkers that are on one hand feasible in clinical routine and on the other hand noninvasive comparing with prostate biopsy.

We identified proPSA and PHI as reliable markers for the identification of significant PCa requiring definitive treatment. These findings are in line with a retrospective study from Tosoian *et al*.[24] investigating men undergoing AS who also found that baseline and longitudinal values of proPSA and PHI predicted reclassification towards high grade cancer.

Also other studies found that both PHI and proPSA predict aggressive pathology in RP specimens.[11,14,25] In the present study, we were able to confirm these findings in an international multicentric study cohort in univariate, but not in multivariate setting.

The strength of our study is the international multicenter prospective character. Drawbacks are the relatively low number of patients included and the lack of a reference pathologist. Pathological analyses were performed by the local uro-pathologists.

From the clinical practical point of view, we believe that especially PHI has an important status in the decision of undergo AS or active treatment, however, we would like to emphasize that in the decision process additional factors like patients' age, performance status and comorbidities, patient's psychological situation or PSA velocities have to be incorporated. In addition, we would like to point out the impact of multiparametric magnetic resonance imaging in AS.[26,27]

CONCLUSION

In a prospective multicenter study, we identified proPSA and PHI as useful markers for PCa aggressiveness and local tumor extension in GS 6 cancers.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

IH was involved in planning and organizing the study, ethics, sample collection in Innsbruck, funding, manuscript and preparation of the revised manuscript. RP was involved in sample collection and preparation in Innsbruck, manuscript. AP contributed towards the ethics, sample collection and preparation in Innsbruck, manuscript, preparation of the revised manuscript. WP was involved in the laboratory analyses. ES was involved in the statistics and preparation of the revised manuscript. AP, EC and CL were involved in the sample collection in Bolzano. AL and EP was involved in the sample collection in Vienna. DD was involved in the sample collection in Belgrad. WH, HK and JB contributed towards the general idea and supervision.

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Supplementary Information accompanies the paper on the Prostate Cancer and Prostatic Diseases website (http://www.nature.com/pcan)