VILNIUS UNIVERSITY

Marija
BARISIENĖ

Significance of new prostate-specific antigen isoforms for early diagnosis of prostate cancer

SUMMARY OF DOCTORAL DISSERTATION

Medicine and Health Sciences,
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This dissertation was written between 2015 and 2020 at the Center of Urology, Clinics of Gastroenterology, Nephrourology and Surgery, Clinical Medicine Institute, Faculty of Medicine, Vilnius University.

Academic supervisor
Prof. Dr. Feliksas Jankevičius (Vilnius University, medicine and health sciences, medicine – M 001).

This doctoral dissertation will be defended in a public meeting of the Dissertation Defence Panel:

Chairman
Prof. Dr. Marius Miglinas (Vilnius University, Medicine and Health Sciences, Medicine, M 001).

Members
Assoc. Prof. Dr. Vilnis Lietuvietis (Riga Stradins University, Medicine and Health Sciences, Medicine, M 001).
Prof. Dr. Daimantas Milonas (Lithuanian University of Health Sciences, Medicine and Health Sciences, Medicine, M 001).
Prof. Habil. Dr. Kęstutis Strupas (Vilnius University, Medicine and Health Sciences, Medicine, M 001).
Assoc. Prof. Dr. Gilvydas Verkauskas (Vilnius University, Medicine and Health Sciences, Medicine, M 001).

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VILNIAUS UNIVERSITETAS

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Naujų prostatos specifinio antigeno izoformų reikšmė ankstyvajai prostatos vėžio diagnostikai

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Mokslinis vadovas
prof. dr. Feliksas Jankevičius (Vilniaus universitetas, medicinos ir sveikatos mokslai, medicina, M 001).

Gynimo taryba
Pirmininkas – **prof. dr. Marius Miglinas** (Vilniaus universitetas, medicinos ir sveikatos mokslai, medicina, M 001).

Nariai
doc. dr. Vilnis Lietuvietis (Rygos Stradinio universitetas, medicinos ir sveikatos mokslai, medicina, M 001).
prof. dr. Daimantas Milonas (Lietuvos sveikatos mokslų universitetas, medicinos ir sveikatos mokslai, medicina, M 001).
prof. habil. dr. Kęstutis Srupas (Vilniaus universitetas, medicinos ir sveikatos mokslai, medicina, M 001).
doc. dr. Gilvydas Verkauskas (Vilniaus universitetas, medicinos ir sveikatos mokslai, medicina, M 001).

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CONTENTS

ABBREVIATIONS

AUC - area under the curve
CI - confidence interval
DRE - digital rectal examination
fPSA - free prostate-specific antigen
HPIN - high-grade prostatic intraepithelial neoplasia
ISUP - International Society of Urological Pathology
NPV - negative predictive value
OR - odds ratio
PB - prostate biopsy
PC - prostate cancer
PCA3 - prostate cancer antigen 3
PHI - Prostate Health Index
PHID - Prostate Health Index density
PPV - positive predictive value
PSA - prostate-specific antigen
PSAD - PSA density
pTNM - pathologic TNM stage
PV - prostate volume
RBREC - Regional Biomedical Research Ethics Committee
ROC - receiver operating characteristic
RP - radical prostatectomy
TNM - tumor, node, metastasis
%fPSA - tPSA and fPSA ratio
%p2PSA - [-2]proPSA and fPSA ratio

1. INTRODUCTION

1.1. Relevance of the topic

Prostate cancer (PC) is the second most common cancer in males worldwide and the fifth leading cause of cancer mortality among men. It is the most frequently occurring cancer among males and the second major cause of cancer-related deaths in Lithuania (1).

More than three dacades ago, the detection of prostate-specific antigen (PSA; total PSA: tPSA) in blood serum was introduced into clinical practice for PC diagnosis, and it still remains the most commonly performed diagnostic PC test worldwide (2,3). While PSA is an organ-specific, not cancer-specific biomarker. Due to low specificity in determing PC in males with PSA level below 10 ng/mL, the risk of PC in males with PSA between 4.1 and 9.9 ng/mL and negative digital rectal examination (DRE) is about 20% with 85% probability, respectively, these cancers would be organ confined (4,5). On the other hand, some males may harbor PC despite very low tPSA levels (<2.00 ng/ml) (5).

The widespread use of PSA testing and the implementation of PSA-based PC programs have led to the significant increase in PC incidence worldwide mainly due to the detection of clinically insignificant PC forms (2,3). Despite significant positive long-term outcomes of PC screening programs, such as the reduction of incidence of metastatic PC forms and PC mortality rates (2,6,7), PC screening programs based on tPSA are criticised for their potential harm, such as psychological distress, false-positive results following subsequent prostate biopsy (PB) and possible complications, as well as over-diagnosis and over-treatment of clinically-insignificant indolent disease, including treatment complications along with negative impact on a male's quality of life (8–10).

In up to 25% of all PB, premalignant condition, namely, high-grade prostatic intraepithelial neoplasia (HPIN), is diagnosed

(11,12). Molecular biomarkers that could predict HPIN in PB specimens are not currently used in clinical practice.

To this day, PSA remains the primary blood serum biomarker used for PC diagnosis, the assessment of treatment efficacy, and for the follow-up of patients undergoing active suveilance strategy. Due to limitations of PSA, there is a considerable interest in new diagnostic biomarkers, their combinations, or clinical PC characteristics and/or risk factors and biomarkers combinations that could be used for early PC diagnosis and accurately identify clinically singnificant PC forms, especially in males who are classified as "grey zone" patients, based on serum PSA level, and who have normal findings on DRE.

The introduction of high-performance technology platforms has accelerated the emergence of new biomarkers. In the 1990's, with the introduction of the selective immunodetection method, unbound forms of serum PSA, called free PSA (fPSA), were detected (13). Most recent clinical studies focuses on fPSA isoform, called [-2]proPSA, which predominates in PC epithelium (14). It have been suggested that this PSA isoform can be successfully used for early PC diagnosis, as well as for the detection of aggressive PC forms (15,16). As recently, multivariable approach for improved PC detection was advocated (17), [-2]proPSA derivatives, such as [-2]proPSA and fPSA ratio (%p2PSA), Prostate Health Index (PHI) and PHI density (PHID) have demonstrated greater sensitivity and specificity for PC diagnosis in comparison to PSA (18–29).

1.2. Aim of the study

The aim of this dissertation is to evaluate the diagnostic potential of molecular serum biomarker [-2]proPSA and its derivatives %p2PSA, PHI and PHID for early PC diagnosis in males with serum PSA levels of 2.00 – 10.00 ng/mL and negative DRE.

1.3. Tasks of the study

1. To evaluate the incidence of PC and its clinical forms in study cohort males with blood serum PSA levels of 2.00 – 10.00 ng/mL and negative DRE.
2. To evaluate the sensitivity, specificity, positive predictive value (NPV) and negative predictive value (PPV), as well as diagnostic accuracy of molecular serum biomarker [-2]proPSA and its derivatives %p2PSA, PHI and PHID for overall and clinically significant PC detection.
3. To assess whether the use of [-2]proPSA and its derivatives %p2PSA, PHI and PHID for early PC diagnosis may help to reduce unnecessary PBs.
4. To assess the predictive value of [-2]proPSA and its derivatives %p2PSA, PHI and PHID in combination with demographic, clinical parameters and other blood serum molecular biomarkers in detection of overall and clinically significant.
5. To determine the net benefit of [-2]proPSA and its derivatives %p2PSA, PHI and PHID in clinical decision making.
6. To evaluate the incidence of HPIN in study cohort and to assess the diagnostic ability of [-2]proPSA and its derivatives %p2PSA, PHI and PHID to predict this precancerous lesion at biopsy.

1.4. Novelty of the study

For the first time in Lithuania, the concentration of molecular biomarker [-2]proPSA was measured in blood serum and its derivatives %p2PSA, PHI and PHID were calculated for males who were consulted by a urologist due to suspicion of PC. The study cohort consisted of patients with serum PSA levels of "grey zone" ranging from 2.00 to 10.00 ng/mL and who had no PC specific findings on DRE. These patients represent the most debatable group of population in making decision on PB. The evaluation of diagnostic potential of [-2]proPSA and its derivatives to predict

overall and clinically significant PC was based not only on PB histology, but also on the final RP pathology. With the intention to improve and individualize the diagnostics of PC, logistic regression models composed of investigated blood serum biomarkers and its derivatives, therefore, clinical and demograPHIc charakteristics were concluded to predict the overall and clinically significant forms of PC at PB. The diagnostic accuracy of logistic regression models was assessed and compared to the diagnostic characteristics of its individual components. The ability of [-2]proPSA and its derivatives %p2PSA, PHI and PHID to discriminate HPIN at biopsy was evaluated. Decision curve analysis was used to determine the "net benefit" of single biomarkers in guiding clinical decision-making on PB and RP.

1.5. Practical significance

New molecular blood serum biomarkers may help to more accurately estimate the indications for PB and improve timely diagnostics of clinically significant PC, thus it may help to reduce unnecessary PB, clinically insignificant PC detection rate, overtreatment with its potential side effect profile and economic costs, as a result, to maintain the quality of male life.

New molecular blood serum biomarkers may help to more accurately predict clinical course of PC and to individualize the treatment strategy.

1.6. Statements to be defended

1. The molecular blood serum biomarker [-2]proPSA and its derivatives %p2PSA, PHI and PHID have sufficient diagnostc characteristics and can be used for an early PC diagnosis in males with serum PSA ranging from 2.00 to 10.00 mg/mL and normal DRE.

2. Molecular blood serum biomarkers in combination with other demograPHIc and clinical parameters may improve the prediction of the overall and clinically significant PC.
3. The molecular blood serum biomarker [-2]proPSA and its derivatives %p2PSA, PHI and PHID may help in guiding clinical decision-making on PB and RP.

2. THE STUDY METHODOLOGY

The prospective cohort study was approved by the Regional Biomedical Research Ethics Committee (RBREC) and the permit No. 158200-14-759-273 was obtained on December 9, 2014. The supplement No. 158200-759-PP1-06 to this permit was granted on February 9, 2016. The study has been performed at Vilnius University Hospital Santaros Klinikos and the National Cancer Institute from January 1, 2015 till December 31, 2016. The study was carried out in accordance with the 1975 Declaration of Helsinki and its subsequent amendments.

2.1. The study sample

Males who were consulted by a urologist at Vilnius University Hospital Santaros Klinikos and the National Cancer Institute due to suspicion of PC and met the inclusion creteria were invited to partcipate in the clinical study. Each patient was provided with detailed information about the study and all questions related to the study were answered. All patients confirmed their participation in the study by signing the Personal Information and Informed Consent Form approved by RBREC.

The participation in the study did not affect the patients' availability for treatment, its timely iniciation, the choice of the treatment method and follow-up.

2.2. Inclusion criteria

The following criteria were used to enroll patients into the study:

- Males 50 years old and over;
- Non-PC specific findings on DRE;
- Blood serum PSA concentration <10.00 ng/mL;

- Systematic transrectal ultrasound-guided PB biopsy is indicated.

2.3. Exclusion criteria

Patients were excluded from the study based on the following criteria:

- Prior history of PC;
- Final blood serum Hybritech PSA concentration < 2.00 ng/mL or > 10.00 ng/mL;
- < 12 biopsy cores were taken during systematic transrectal ultrasound-guided PB;
- > 6 months elapsed between DRE and blood draw;
- > 6 months elapsed between blood draw and PB;
- Use of 5α-reductase inhibitors (Dutasteride, Finasteride) at any time prior to the study;
- Use of androgen replacement therapy in the 3 months preceding blood draw;
- PB, or other transrectal or transurethral procedure which might elevate the serum PSA concentration, performed prior to blood draw;
- Open prostatectomy or transurethral resection of prostate for benign prostate hyperplasia was performed;
- Symptomatic urinary tract infection, including symptoms of acute prostatitis, at blood draw or PB;
- Equivocal PB results (i.e. cannot determine if cancer is present or not);
- Blood serum samples stored frozen for > 5 years.

2.4. Study protocol

2.4.1. Patients' study protocol

The data on patients' age, urinary disorders, comorbidities, concomitant medications, previous sergical interventions, PB number, and family history of PC were collected during an interview.

All patients underwent DRE. Only patients with normal DRE were included into the study.

One peripheral blood sample was taken from each patient prior to transrectal prostate ultrasound and PB. Special preparation of the patients prior to the blood draw was not needed.

Prostate height, width and length were assesed by transrectal ultrasound before PB in all patients. Prostate volume (PV) was calculated using ellipsoid formula: prostate length (cm) x prostate height (cm) x prostate width (cm) x 0.52.

Systematic transrectal ultrasound-guided PB was performed to a patient in a lateral position. Chlorheksidine/Lidocaine 20 mg + 0,5 mg/g 12,5 g gel was instilled into the rectum immediately prior to the biopsy. An automatic biopsy device and disposable 18 G 20 cm length biopsy needle was used for each procedure. PBs were performed using standardized 12-core random sampling protocol.

Antimicrobial prophylaxis with Ciprofloxacin or other antibiotics, if bacterial resistance to fluoroquinolones was known, was prescribed in all cases prior to the PB.

2.4.2. Collection and processing of blood samples

All blood samples were collected using standard venipuncture technique with an effort to prevent hemolysis. Serum separating tubes for biochemistry were used for the collection of all blood samples. Blood samples were collected immediately before transrectal ultrasound and PB.

Blood samples were collected before DRE or with more than 24 hours elapsed following DRE (30,31).

Blood samples were processed within 3 hours after the collection at Biochemistry Laboratory of Vilnius University Hospital Santaros Klinikos Laboratory Medicine Center or at the Molecular Oncology Laboratory of National Cancer Insitute. All samples were centrifugated for serum separation and frozen at -80∘C (32–34).

They were tested in Biochemistry Laboratory of Vilnius University Hospital Santaros Klinikos Laboratory Medicine Center after 1 to 5 months after blood draw (32,33,35).

Blood samples to measure tPSA, fPSA and [-2]proPSA were tested using the Beckman Coulter Access® 2 Immunoassay Analyzer and Access Hybritech® reagents and calibrators. The quality control procedures were performed on a regular basis to ensure the quality of the tests.

Quantitative determination of [-2]proPSA in serum was performed by using a two-site immunoenzematic “sandwich” Access Hybritech® p2PSA assay. Access Hybritech® p2PSA is intended to be used in combination with Access Hybritech® tPSA and Access Hybritech® fPSA. Hybritech calibration was used for tPSA and fPSA.

PSA and [-2]proPSA derivatives were calculated as follows:

- PSA density (PSAD): PSA / PV;
- tPSA and fPSA ratio (%fPSA): fPSA / tPSA × 100;
- %p2PSA: ([-2]proPSA / fPSA) × 100;
- PHI: ([-2]proPSA / fPSA) × √tPSA ;
- PHID: PHI / PV.

2.4.3. Pathologic examination

PB specimens were evaluated at National Center of Pathology (P. Baublio str. 5, LT – 08406, Vilnius, Lithuania) by specialist pathologists blinded to the blood serum results.

The total number of positive biopsy cores for PC, the percentage of PC leason per core, Gleason grade and score, therefore, present of HPIN were evaluated during PB histological examination.

Pathologic PC stage according to the TNM classification (pTNM), Gleason grade and score, the percentage of PC leason in the prostate and present of HPIN were evaluated at the final RP pathology.

In all histopathological specimens, the Gleason grade and Gleason score were assesed according to 2005 International Society of Urological Pathology (ISUP) (36), and ISUP grade group was assigned according to the 2014 ISUP recommendations (37).

2.4.4. Patients' cohorts

All patients included into the study formed a PB cohort. According to the PB histological report, patients were divided into comparative groups. Subjects in whom PC diagnosis was proven were assigned to PC group, and patients, who were diagnosed with PC, were assigned to benign patients' group. A subgroup of patients with histologically proven HPIN at biopsy was identified.

The RP cohort consisted of patients, who underwent radical surgical treatment due to PC confirmed at biopsy.

2.4.5. The difinition of clinically significant and not clinically significant prostate cancer

2.4.5.1. Prostate biopsy cohort

Two different criteria were used to define clinically significant and not clinically significant PC at PB cohort.

- Epstein's criteria

  - Clinically significant PC was defined as meeting the clinically significant PC definition according to contemporary Epstein's criteria (38–40):

- PSAD ≥ 0.15 ng/mL/g;
- Gleason score ≥ 7;
- ≥ 3 positive cores for PC at biopsy;
- Presence of ≥ 50% of PC per any core.

- Not clinically significant PC was defined as meeting the clinically insignificant PC definition according to contemporary Epstein's criteria:
  - Clinical PC stage T1c;
  - PSAD < 0.15 ng/mL/g;
  - Gleason score ≤ 6;
  - ≤ 2 positive cores for PC at biopsy;
  - Presence of < 50% of PC per any core.

- ISUP grade

  - Clinically significant PC was defined if ISUP grade ≥ 2 had been identified at biopsy.
  - Not clinically significant PC was defined if ISUP grade < 2 had been identified at biopsy.

2.4.5.2. Radical prostatectomy cohort

According to the final RP pathology, clinically significant PC was defined as ISUP grade group ≥ 2 and not clinically significant PC was defined as ISUP grade group < 2 (37,41).

2.5. Statistical analysis

The mean with standard deviation was used to describe continues variables, as well as frequency tabulation with absolute and percentage frequencies were used to describe the distribution of categorical variables.

The Shapiro-Wilk test was used to determine the normality of the continuous variables. Student's t-test was used to compare the

means of two normally distributed independent groups. F-test of equality of variances was used. Mann-Whitney-Wilcoxon test was used for comparisons of non-normally distributed continuous variables.

Pearson's chi-squared test (X^2) was used for comparisons of qualitative variables. The Fisher's exact test was used in case at least one of expected frequencies was < 5.

The correlation analysis using Pearson (Pearson's r) correlation coefficient for normally distributed continuos variables, and Spearman's rank correlation coefficient (Spearman's ρ) to measure rank correlation and correlation among non-normally distributed continous variables was done.

The determination of cut-off values for biomarkers was based on Youden's index. The sensitivity, specificity, PPV and NPV, as well as specificity at fixed 90% sensitivity for investigated biomarkers were estimated.

Univariate and multivariate binary logistic regression models were concluded for the prediction of PC. The multivariate logistic regression models were fitted using forward stepwise approach. Odds ratios (OR) and 95% confidence intervals (CI) were calculated.

The accuracy of the biomarkers and logistic regressions was measured by the area under the receiver operating characteristic (ROC) curves (AUC). DeLong et al.'s method was used to compare the ROC curves (42).

Decision Curve analysis (DCA) was used to determine the net benefit of single biomarkers in guiding clinical decision-making (43).

Statistical analysis was performed using Statistical Analysis System (SAS) version 9.2 (SAS Institute Inc., Carry, NC, USA). P value < 0.05 was defined as statistically significant.

3. RESULTS

3.1. Diagnostic potencial of blood serum biomarkers in predicting prostate cancer in prostate biopsy setting

PC has been diagnosed in 112 (53.3%) out of 210 males enrolled in the study. Clinically significant PC according to Epstein's criteria and ISUP grade ≥ 2 have been identified in 81 (72.3%) and 40 (35.7%) out of 112 patients, respectively. Isolated HPIN at biopsy has been identified in 24 (11.4%) patients. Clinicopathological characteristics of the study cohort are summarized in Table 1.

Overall and clinically significant PC, as well as ISUP < 2 PC, were diagnosed more frequently during first PB than in repeated PB setting (89.3% vs 10.7%, 90.1% vs. 9.9%, 90.0% vs. 10.0%, and 88.9% vs. 11.1%, respectively (all $P < 0.05$; Table 1).

PV has been found to be significantly smaller in patients harboring overall PC, as well as in patients with Epstein's significant and ISUP grade ≥2 PC in comparison to patients in non-PC group (38.55 mL, 36.16 mL, and 37.78 mL vs. 55.02 mL, respectively) or to patients with isolated HPIN at biopsy (38.55 mL, 36.16 mL, and 37.78 mL vs. 52.80 mL, respectively, all $P < 0.05$; Table 1).

tPSA mean value was significantly slightly higher only in patients with Epstein's significant PC in comparison to patients in the non-PC group (4.85 ng/mL vs. 4.11 ng/mL, respectively, $P = 0.004$; Table 1).

PSAD mean value was higher in patients with overall, Epstein's significant, and ISUP grade ≥2 PC in comparison to patients in the non-PC group or with isolated HPIN at biopsy (0.14 ng/mL/cc, 0.16 ng/mL/cc, and 0.13 ng/mL/cc vs. 0.09 ng/ml/cc, respectively, all $P<0.01$; Table 1).

fPSA and %fPSA mean values were significantly lower, so PHI and PHID mean values were higher in patients with overall, Epstein's significant, and ISUP grade ≥2 PC than in patients in the

non-PC group and isolated HPIN at biopsy (0.60 ng/mL, 0.59 ng/mL, and 0.57 ng/mL vs. 0.72 ng/mL and 0.80 ng/mL, so 48.31, 52.26, and 55.62 vs. 35.62 and 38.05, respectively; all $P < 0.05$; Table 1).

%p2PSA mean values were higher in patients with overall PC in comparison to patients in the non-PC group (2.34 vs. 1.83, $P < 0.001$), as well as in patients with Epstein's significant and ISUP ≥ 2 PC in comparison to patients in the non-PC group and isolated HPIN at biopsy (2.44 and 2.62 vs. 1.83 and 1.93, respectively, $P < 0.05$; Table 1).

Table 1. Clinico-pathological characteristics of the study cohort.

				PC cases (N = 112)				
					Epstein's criteria		ISUP grade	
	All cohort	**Benign cases**	**HPIN at biopsy**	**PC cases**	**NCS PC**	**CS PC**	**<2**	**≥2**
Patients, N (%)	210 (100.0)	98 (46.7)	24 (11.4)	112 (53.3)	31 (27.7)	81 (72.3)	72 (64.3)	40 (35.7)
Age, years								
median	62	63	66	62	57	62	60.5	62.5
mean ± SD	63±7.09	63.71±6.90	66.00±6.96	62.42±7.22$^{\#}$	59.74±6.83*$^{\#}$	63.44±7.14	61.64±7.3*$^{\#}$	63.83±6.94
PV, mL								
median	43	50	50	34	47	32	37	33
mean ± SD	46.20±22.47	55.02±25.39	52.80±21.75	38.55±16.13*$^{\#}$	44.81±14.71	36.16±16.1*$^{\#}$	38.99±14.58*$^{\#}$	37.78±18.77*$^{\#}$
Biopsy:								
primary, n (%)	172(81.9)	72 (73.5)	17 (70.8)	100 (89.3)**	27 (87.1)	73 (90.1)**	64 (88.9)**	36 (90)**
repeated, n (%)	38 (18.1)	26 (26.5)	7 (29.2)	12 (10.7)	4 (12.9)	8 (9.9)	8 (11.1)	4 (10)
tPSA, ng/mlL								
median	3.90	3.58	3.81	3.99	3.17	4.39	3.94	4.34
mean ±SD	4.32±1.83	4.11±1.70	4.13±1.55	4.49±1.91	3.55±1.32	4.85±2.00*	4.29±1.69	4.86±2.25
PSAD,								
ng/mL/cc	0.10	0.08	0.08	0.11	0.08	0.13	0.10	0.13
median	0.11±0.07	0.09±0.06	0.09±0.06	0.14±0.08*$^{\#}$	0.09±0.03	0.16±0.08*$^{\#}$	0.13±0.07*$^{\#}$	0.15±0.08*$^{\#}$
mean±SD								
fPSA, ng/mL								
median	0.59	0.63	0.69	0.55	0.58	0.50	0.56	0.50
mean ±SD	0.66±0.33	0.72±0.33	0.80±0.40	0.60±0.32*$^{\#}$	0.63±0.31	0.59±0.32*$^{\#}$	0.62±0.32*	0.57±0.32*$^{\#}$
%fPSA								
median	15.50	17.00	18.00	13.00	18.00	11.00	15.50	10.50
mean ±SD	16.00±6.82	18.10±6.49	19.10±6.54	14.10±6.58*$^{\#}$	18.00±5.99	12.61±6.21*$^{\#}$	15.14±6.89*$^{\#}$	12.23±5.60*$^{\#}$

[-2]proPSA, pg/mL	11.36	11.16	11.13	11.48	11.61	11.37	11.57	11.41
median	12.78±6.96	12.24±5.71	13.61±6.18	13.25±7.88	12.44±5.91	13.56±8.53	12.63±6.36	14.36±10.05
mean ±SD								
%p2PSA								
median	2.02	1.82	2.00	2.21	1.89	2.29	2.10	2.39
mean ±SD	2.10±0.80	1.83±0.62	1.93±0.63	2.34±0.86*	2.08±0.71	2.44±0.89*#	2.18±0.78*	2.62±0.94*#
PHI								
median	40.19	35.03	36.85	46.36	36.4	49.74	42.91	51.62
mean ±SD	42.39±17.80	35.62±12.58	38.05±13.03	48.31±19.55*#	37.97±11.57	52.26±20.56*#	44.24±16.70*	55.62±22.24*#
PHID								
median	0.98	0.65	0.80	1.31	0.85	1.51	1.13	1.59
mean ±SD	1.18±0.80	0.79±0.53	0.88±0.56	1.56±1.07*#	0.99±0.58*	1.772±1.13*#	1.38±0.89*#	1.88±1.28*#

Abbreviations: CS: clinically significant; fPSA: free prostate-specific antigen; HPIN: high grade intraepithelial neoplasia; ISUP: International Society of Urological Pathology; N: number of cases; NCS: not clinically significant; PC: prostate cancer; PSAD: PSA density; PV: prostate volume; PHI: Prostate Health Index; PHID: PHI density; SD: standard deviation; tPSA: total PSA; %fPSA: free to total PSA ratio; %p2PSA: [-2]proPSA to fPSA ratio.

*P < 0.05 for Student's t-test or Wilcoxon singed rank test vs. non-PC cases.

**P < 0.05 for Pearson's Chi-squared test.

# P < 0.05 for Mann-Whitney-Wilcoxon test vs. HPIN.

Significant correlations were revealed between biopsy ISUP grade ≥ 2 and %p2PSA ($\rho = 0.30$, $P < 0.001$), PHI ($\rho = 0.36$, $P < 0.001$), and PHID ($\rho = 0.42$, $P < 0.001$).

Using Youden's index, PHI with cut-off value of 44.49 and PHID with cut-off value of 1.04 for detection of overall PC, 44.47 and 1.06 for Epstein's significant PC, and 44.71 and 1.04 for ISUP grade ≥ 2 PC have outperformed tPSA, PSAD, fPSA, %fPSA, [-2pro]PSA and %p2PSA, and showed the best diagnostic power evaluating the sensitivity, specificity PPV and NPV (Tables 2-4).

Table 2. Sensitivity, specificity, positive and negative predictive value of blood serum biomarkers in predicting overall prostate cancer.

	According to Youden's index				
Biomarker	**Cut-off**	**Sensitivity,% (95% CI)**	**Specificity,% (95% CI)**	**PPV,% (95% CI)**	**NPV,% (95% CI)**
tPSA, ng/mL	4.18	44.6 (35.2-54.3)	62.2 (51.9-71.8)	57.5 (46.4-68.0)	49.6 (40.5-58.8)
PSAD, ng/mL/cc	0.09	70.5 (61.2-78.8)	65.3 (55.0-74.6)	69.9 (60.6-78.2)	66.0 (55.7-75.3)
fPSA, ng/mL	0.45	37.5 (28.5-47.2)	81.6 (72.5-88.7)	70.0 (56.8-81.2)	53.3 (45.0-61.5)
%fPSA	11.41	43.8 (34.4-53.4)	89.8 (82.0-95.0)	83.1 (71.0-91.6)	58.3 (50.0-66.2)
[-2]proPSA, pg/mL	17.69	17.0 (10.5-25.2)	87.8 (79.6-93.5)	61.3 (42.2-78.2)	48.0 (40.5-55.6)

%p2PSA	1.77	77.7 (68.8-85.0)	46.9 (36.8-57.3)	62.6 (54.0-70.6)	64.8 (52.5-75.8)
PHI	44.49	56.3 (46.6-65.6)	83.7 (74.8-90.4)	79.7 (69.2-88.0)	62.6 (53.7-70.9)
PHID	1.04	61.6 (51.9-70.6)	81.6 (72.5-88.7)	79.3 (69.3-87.3)	65.0 (55.9-73.4)

Abbreviations: CI - confidence interval; fPSA: free prostate-specific antigen; NPV: negative predictive value; PPV: positive predictive value; PSAD: PSA density; PHI: Prostate Health Index; PHID: PHI density; tPSA: total PSA; %fPSA: free to total PSA ratio; %fPSA: free to total PSA ratio; %p2PSA: [-2]proPSA to fPSA ratio.

Table 3. Sensitivity, specificity, positive and negative predictive value of blood serum biomarkers in predicting clinically significant prostate cancer according to Epstein's criteria.

	According to Youden's index				
Biomarker	**Cut-off**	**Sensitivity,% (95% CI)**	**Specificity,% (95% CI)**	**PPV,% (95% CI)**	**NPV,% (95% CI)**
tPSA, ng/mL	4.28	51.9 (40.5-63.1)	71.3 (62.7-78.9)	53.2 (41.6-64.5)	70.2 (61.6-77.9)
PSAD, ng/mL/cc	0.09	81.5 (71.3-89.3)	61.2 (52.3-69.7)	56.9 (47.4-66.1)	84.0 (75.1-90.8)
fPSA, ng/mL	0.44	38.3 (27.7-49.7)	82.2 (74.5-88.4)	57.4 (43.2-70.8)	67.9 (60.0-75.2)
%fPSA	12.15-12.90	59.3 (47.8-70.1)	86.8 (79.7-92.1)	73.8 (61.5-84.0)	77.2 (69.6-83.8)
[-2]proPSA, pg/mL	15.19	28.4 (18.9-39.5)	77.5 (69.3-84.4)	44.2 (30.5-58.7)	63.3 (55.3-70.8)

%p2PSA	2.07	69.1 (57.9-78.9)	64.3 (55.4-72.6)	54.9 (44.7-64.8)	76.9 (67.8-84.4)
PHI	44.47	69.1 (57.9-78.9)	81.4 (73.6-87.7)	70.0 (58.7-79.7)	80.8 (72.9-87.2)
PHID	1.06	71.6 (60.5-81.1)	78.3 (70.2-85.1)	67.4 (56.5-77.2)	81.5 (73.5-87.9)

Abbreviations: CI - confidence interval; fPSA: free prostate-specific antigen; NPV: negative predictive value; PPV: positive predictive value; PSAD: PSA density; PHI: Prostate Health Index; PHID: PHI density; tPSA: total PSA; %fPSA: free to total PSA ratio; %fPSA: free to total PSA ratio; %p2PSA: [-2]proPSA to fPSA ratio.

Table 4. Sensitivity, specificity, positive and negative predictive value of blood serum biomarkers in predicting ISUP ≥ 2 grade prostate cancer.

	According to Youden's index				
Biomarker	**Cut-off**	**Sensitivity,% (95% CI)**	**Specificity, % (95% CI)**	**PPV,% (95% CI)**	**NPV,% (95% CI)**
tPSA, ng/mL	4.48	47.5 (31.5-63.9	68.2 (60.7-75.2)	26.0 (16.5-37.6)	84.7 (77.5-90.3)
PSAD, ng/mL/cc	0.13	57.5 (40.9-73.0)	77.6 (70.1-83.7)	37.7 (25.6-51.0)	88.6 (82.4-93.2)
fPSA, ng/mL	0.73	77.5 (61.6-89.2)	34.1 (27.0-41.8)	21.7 (15.2-29.3)	86.6 (76.0-93.7)
%fPSA	11.30-11.69	57.5 (40.9-73.0)	78.8 (71.9-84.7)	39.0 (26.6-52.6)	88.7 (82.6-93.3)
[-2]proPSA, pg/mL	26.11-30.29	7.5 (1.6-20.4)	96.5 (92.5-98.7)	33.3 (7.5-70.1)	81.6 (75.5-86.7)
%p2PSA	1.88	85.0	47.6	27.6	93.1

		(70.2-94.3)	(39.9-55.4)	(20.0-36.4)	(85.6-97.4)
PHI	44.71	75.0 (58.8-87.3)	72.4 (65.0-78.9)	39.0 (28.1-50.8)	92.5 (86.6-96.3)
PHID	1.04	75.0 (58.8-87.3)	66.5 (58.8-73.5)	34.5 (24.6-45.4)	91.9 (85.6-96.0)

Abbreviations: CI - confidence interval; fPSA: free prostate-specific antigen; NPV: negative predictive value; PPV: positive predictive value; PSAD: PSA density; PHI: Prostate Health Index; PHID: PHI density; tPSA: total PSA; %fPSA: free to total PSA ratio; %fPSA: free to total PSA ratio; %p2PSA: [-2]proPSA to fPSA ratio.

At 90% sensitivity for detecting overall PC, PHID with cut-off value of 0.54 had the specificity of 35.7%, which was higher than other biomarkers. At 90% sensitivity for detecting Epstein's significant PC, PSAD with cut-off value of 0.07 ng/mL/cc showed the specificity of 41.9% that was slightly higher than for PHI and PHID (35.7% and 36.4%, respectively). However, at 90% sensitivity for detecting ISUP grade $\geq$ 2 PC, PHI and PHID with cut-off values of 33.2 and 0.63 showed the highest specificity of 34.7% and 34.1%, respectively (Tables 5-7).

At 90% sensitivity for detecting overall PC, PHID may lead to avoid 21.4% of prostate biopsies in comparison to 16.2% for PHI and PSAD, 19% for %p2PSA, 10.5% for tPSA, and less than 7% for the rest of the biomarkers. At 90% sensitivity for detecting ISUP grade $\geq$ 2 PC, PHI and PHID, as well as PSAD for detecting Epstein's significant PC, may lead to avoid 30% of prostate biopsies.

Table 5. Specificity, positive and negative predictive value of blood serum biomarkers at 90% sensitivity in predicting overall prostate cancer.

		90% sensitivity		
Biomarker	**Cut-off**	**Specificity,% (95% CI)**	**PPV,% (95% CI)**	**NPV,% (95% CI)**
tPSA, ng/mL	2.5	11.2 (5.0-17.5)	53.7 (46.6-60.9)	50.0 (29.1-70.9)
PSAD, ng/mL/cc	0.05	24.5 (16.4-34.2)	58.0 (50.3-65.3)	70.6 (52.5-84.9)
fPSA, ng/mL	0.26	3.1 (0.0-6.5)	51.5 (44.5-58.5)	21.4 (0.0-42.9)
%fPSA	7.00	1.0 (0.0-3.0)	50.8 (43.8-57.7)	7.7 (0.0-22.2)
[-2]proPSA, pg/mL	5.04	1.0 (0.0-3.0)	51.0 (44.1-58.0)	8.3 (0.0-24.0)
%p2PSA	1.41	29.6 (20.8-39.7)	59.4 (51.6-66.9)	72.5 (58.7-86.3)
PHI	25.93	23.5 (15.1-31.9)	57.4 (50.1-64.7)	67.6 (51.9-83.4)
PHID	0.54	35.7 (26.3-46.0)	61.8 (53.9-69.3)	77.8 (62.9-88.8)

Abbreviations: CI - confidence interval; fPSA: free prostate-specific antigen; NPV: negative predictive value; PPV: positive predictive value; PSAD: PSA density; PHI: Prostate Health Index; PHID: PHI density; tPSA: total PSA; %fPSA: free to total PSA ratio; %p2PSA: [-2]proPSA to fPSA ratio.

Table 6. Specificity, positive and negative predictive value of blood serum biomarkers at 90% sensitivity in predicting clinically significant prostate cancer according to Epstein's criteria.

		90% sensitivity		
Biomarker	**Cut-off**	**Specificity,% (95% CI)**	**PPV,% (95% CI)**	**NPV,% (95% CI)**
tPSA, ng/mL	2.77	24.0 (16.7-31.4)	42.7 (35.3-50.1)	79.5 (66.8-92.2)
PSAD, ng/mL/cc	0.07	41.9 (33.2-50.9)	49.3 (41.0-57.7)	87.1 (76.2-94.3)
fPSA, ng/mL	0.28	4.7 (1.0-8.3)	36.9 (30.2-43.7)	40.0 (15.2-64.8)
%fPSA	7.00	1.6 (0.0-3.7)	35.5 (28.9-42.2)	15.4 (0.0-35.0)
[-2]proPSA, pg/mL	5.80	5.4 (1.5-9.3)	37.4 (30.6-44.2)	46.7 (21.4-71.9)
%p2PSA	1.54	31.8 (23.8-39.8)	45.3 (37.7-53.0)	83.7 (70.3-92.7)
PHI	31.92	35.7 (27.4-43.9)	46.8 (39.0-54.6)	85.2 (75.7-94.7)
PHID	0.61	36.4 (28.1-45.4)	47.1 (39.0-55.3)	85.5 (73.3-93.5)

Abbreviations: CI - confidence interval; fPSA: free prostate-specific antigen; NPV: negative predictive value; PPV: positive predictive value; PSAD: PSA density; PHI: Prostate Health Index; PHID: PHI density; tPSA: total PSA; %fPSA: free to total PSA ratio; %p2PSA: [-2]proPSA to fPSA ratio.

Table 7. Specificity, positive and negative predictive value of blood serum biomarkers at 90% sensitivity in predicting ISUP ≥ 2 grade prostate cancer.

		90% sensitivity		
Biomarker	**Cut-off**	**Specificity, % (95% CI)**	**PPV,% (95% CI)**	**NPV,% (95% CI)**
tPSA, ng/mL	2.50	10.6 (6.0-15.2)	19.1 (13.5-24.8)	81.8 (65.7-97.4)
PSAD, ng/mL/cc	0.04	5.3 (2.5-9.8)	19.1 (13.9-25.3)	81.8 (48.2-97.7)
fPSA, ng/mL	0.29	6.5 (2.8-10.2)	18.5 (13.0-23.9)	73.3 (51.0-95.7)
%fPSA	7.00	4.7 (1.5-7.9)	17.8 (12.4-23.1)	61.5 (35.1-88.0)
[-2]proPSA, pg/mL	6.21	8.8 (4.6-13.1)	18.8 (13.3-24.4)	78.9 (60.6-97.3)
%p2PSA	1.65	34.1 (27.0-41.2)	24.3 (17.7-32.1)	93.5 (87.4-100.0)
PHI	33.20	34.7 (27.6-41.9)	24.5 (17.54-31.4)	93.7 (87.6-100.0)
PHID	0.63	34.1 (27.0-41.8)	24.3 (17.4-31.2)	93.5 (87.4-98.2)

Abbreviations: CI - confidence interval; fPSA: free prostate-specific antigen; NPV: negative predictive value; PPV: positive predictive value; PSAD: PSA density; PHI: Prostate Health Index; PHID: PHI density; tPSA: total PSA; %fPSA: free to total PSA ratio; %p2PSA: [-2]proPSA to fPSA ratio.

In univariate ROC analysis, PHID with AUC of 0.77 was the most accurate predictor of overall PC significantly outperforming tPSA, fPSA, %fPSA, [-2]proPSA, %p2PSA, and PHI (all $P<0.05$; see Figure 1). PHID was the most accurate predictor of Epstein's significant PC with AUC of 0.80 outperforming tPSA, fPSA, [-2]proPSA, and %p2PSA (all $P<0.05$; see Figure 2). However, PHI was the most accurate predictor of ISUP grade ≥ 2 PC at biopsy with AUC of 0.77 significantly outperforming tPSA and fPSA (all $P<0.05$; see Figure 3).

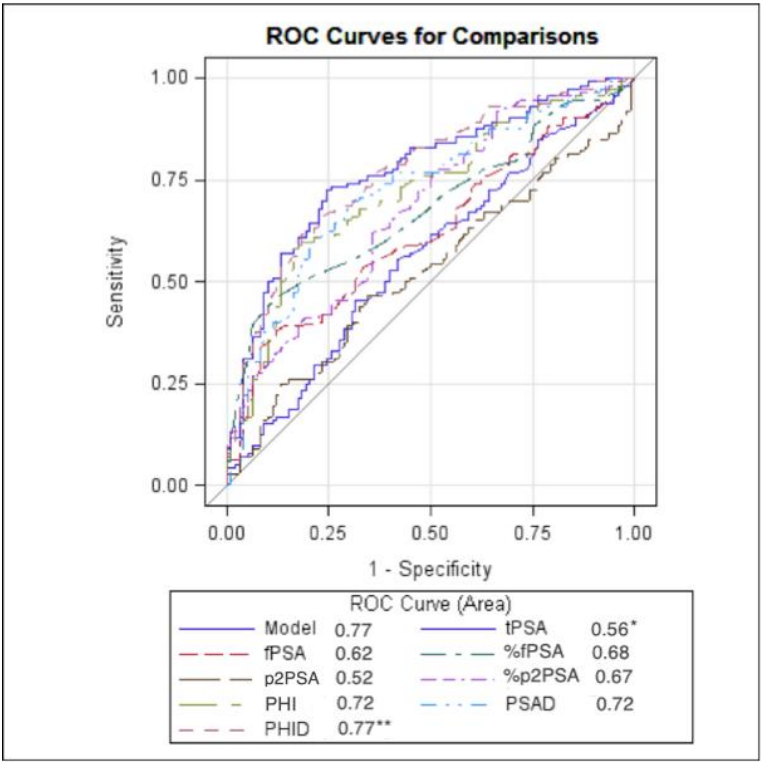

Figure 1. Receiver operating characteristic curves representing the diagnostic ability of blood serum biomarkers in predicting overall prostate cancer.

Abbreviations: ISUP: International Society of Urological Pathology; fPSA: free prostate-specific antigen; PHI: Prostate Health Index; PHID: PHI density; PSAD: PSA density; ROC: receiver operating characteristic; tPSA: total PSA; p2PSA: [-2]proPSA; %fPSA: free to total PSA ratio; %p2PSA: [-2]proPSA to fPSA ratio. All significant differences are marked with an asterisk (*) and (**):
*P < 0.05 for tPSA vs. p2PSA, PHI, %fPSA and PSAD.
**P < 0.05 for PHID vs. tPSA, fPSA, %fPSA, p2PSA, %p2PSA and PHI.

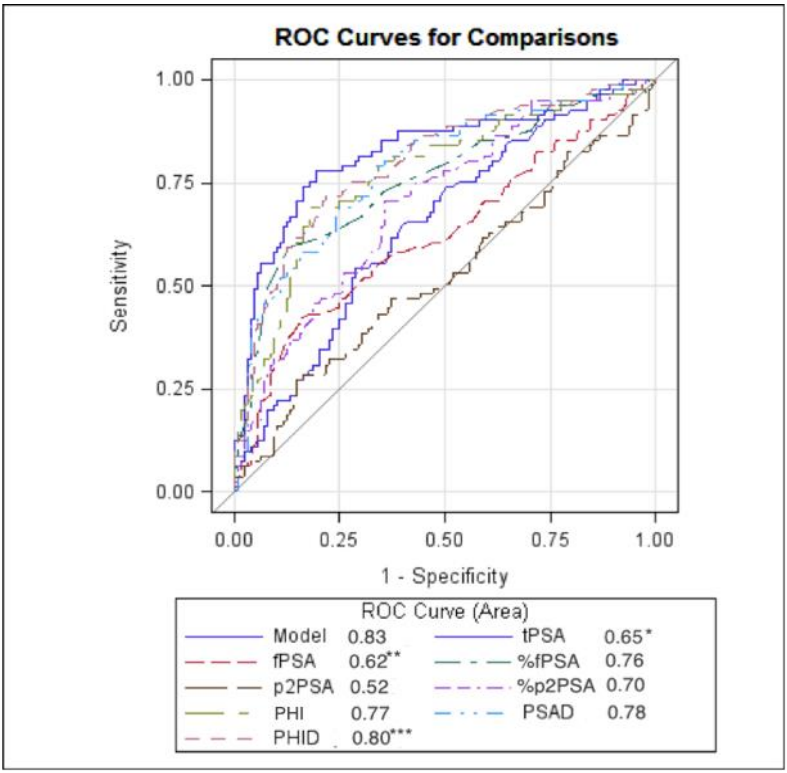

Figure 2. Receiver operating characteristic curves representing the diagnostic ability of blood serum biomarkers in predicting clinically significant prostate cancer according to Epstein's criteria.

Abbreviations: ISUP: International Society of Urological Pathology; fPSA: free prostate-specific antigen; PHI: Prostate Health Index; PHID: PHI density; PSAD: PSA density; ROC: receiver operating characteristic; tPSA: total PSA; p2PSA: [-2]proPSA; %fPSA: free to total PSA ratio; %p2PSA: [-2]proPSA to fPSA ratio. All significant differences are marked with an asterisk (*), (**) and (***):

*P < 0.05 for tPSA vs. %fPSA, p2PSA, PHI, PSAD and PHID.

**P < 0.05 for fPSA vs.%fPSA, PHI, PSAD and PHID.

***P < 0.05 for PHID vs p2PSA and %p2PSA.

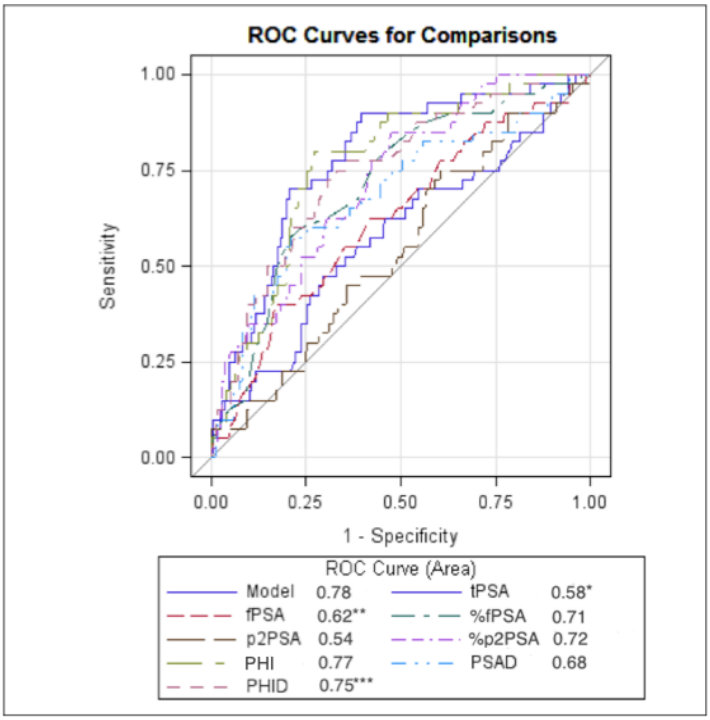

Figure 3. Receiver operating characteristic curves representing the diagnostic ability of blood serum biomarkers in predicting ISUP grade ≥ 2 prostate cancer.

Abbreviations: ISUP: International Society of Urological Pathology; fPSA: free prostate-specific antigen; PHI – Prostate Health Index; PHID - PHI density; PSAD - PSA density; ROC - receiver operating characteristic; tPSA – total PSA; p2PSA: [-2]proPSA; %fPSA – free to total PSA ratio; %p2PSA – [-2]proPSA to fPSA ratio.

All significant differences are marked with an asterisk (*), (**) and (***):

*P < 0.05 for tPSA vs. %fPSA, PHI, PSAD and PHID.

**P < 0.05 for fPSA vs.%fPSA, %p2PSA, PHI, and PHID.

***P < 0.05 for PHID vs p2PSA and PSAD.

In multivariate logistic regression analysis by adding [-2]proPSA and its derivatives one by one to the base logistic regression model, which consisted of repeated biopsy, PV, fPSA, and %fPSA variables, it has been estimated that PHID is the most significant predictor for overall PC (OR 4.34, $P < 0.001$), Epstein's significant PC (OR 3.58, $P < 0.001$), and ISUP grade ≥ 2 PC (OR 2.38, $P < 0.001$). In all multivariate logistic regression model analysis, [-2]proPSA, %p2PSA, PHI, and PHID have achieved an independent predictor status. The only PHI added to the base multivariate logistic regression model significantly improved diagnostic accuracy by 5% in predicting ISUP grade ≥ 2 PC at biopsy (AUC 0.74 and 0.79, respectively, $P = 0.039$).

We performed DCA to determine the net benefit for each biomarker in predicting overall and clinically significant PC. The best net benefit was determined for PHID in predicting overall and Epstein's significant PC and for PHI in predicting ISUP grade ≥ 2 PC at biopsy. At 20% threshold probability, based on PHID, 45 and 26 of 100 biopsied patients would be diagnosed overall and Epstein's significant PC, respectively, therefore, based on PHI, ISUP ≥ 2 PC would be diagnosed in 9 of 100 biopsied males.

3.2. Diagnostic potencial of blood serum biomarkers in predicting clinically significant prostate cancer at the final pathology

Overall**,** 51 patients with confirmed PC at biopsy underwent RP. Clinico-pathological characteristics of patients after RP cohort are summarised in Table 8. Clinically significant PC at the final pathology was diagnosed in 38 (74.5%). Baseline clinical characteristics, such as age and PV, were well balanced among patients with clinically significant and not clinically significant disease (all $P > 0.050$).

Table 8. Clinico-pathological characteristics of patients after radical prostatectomy.

Parameter	**All patients (N=51)**
Age, years	
Mean (SD)	62.40 (5.85)
PV, mL	
Mean (SD)	38.71 (16.03)
Biopsy:	
Primary, N (%)	42 (82.40)
Repeated, N (%)	9 (17.60)
cISUP grade, N (%):	
1	28 (54.90)
2	18 (35.30)
3	2 (3.90)
4	3 (5.90)
5	0 (0.00)
Radical prostatectomy:	
Open, N (%)	44 (86.30)
Laparoscopic, N (%)	7 (13.7)
pISUP grade, N (%):	
1	13 (25.50)
2	32 (62.70)
3	4 (7.80)
4	0 (0.00)
5	2 (3.90)
ISUP upgrading after RP, N (%)	15 (39.47)
pT stage, N (%):	
pT2	34 (66.70)
pT3a	12 (23.50)
pT3b	5 (9.80)

Abbreviations: cISUP: clinical ISUP grading; ISUP: International Society of Urological Pathology; N: number of patients; pISUP: pathological ISUP grading; PV: prostate volume; SD: standard deviation; pT: pathological local tumor staging according to TNM classification.

Mean value of tPSA was 4.77 (± 1.94) ng/mL, fPSA was 0.60 (± 0.26) ng/mL, %fPSA was 12.71 (± 5.83) and PSAD was 0.15 (± 0.08), while mean value of [-2]proPSA was 12.94 (± 7.14) pg/mL, %p2PSA was 2.38 (± 0.79), PHI was 50.55 (± 18.53), and PHID was 1.61 (± 0.99) for all the patients.

Mean value of PHID was significantly higher (1.74 vs. 1.24, P = 0.031) and mean value of %fPSA was significantly lower (11.60 vs. 16.00, P = 0.005) in patients with clinically significant PC at the final pathology, while a tendency for higher values of PHI (53.31 vs. 42.50, P = 0.069) and PSAD (0.16 vs. 0.11, P = 0.079) in these patients were observed (Table 9).

Table 9. Values of estimated serum biomarkers according to pathological ISUP grading.

	Pathological ISUP grading		
Parameter	**ISUP <2**	**ISUP≥2**	**P value**
Patients, N (%)	13 (25.5)	38 (74.5)	-
tPSA, ng/mL			
Mean ± SD	4.09 ± 1.64	5.01 (2.00)	0.136
PSAD			
Mean ± SD	0.11 ± 0.07	0.16 (0.08)	0.079
fPSA, ng/mL			
Mean ± SD	0.60 ± 0.29	0.60 (0.26)	0.298
%fPSA			
Mean ± SD	16.00 ± 4.49	11.60 ± 5.85	0.005
[-2]proPSA, pg/mL			
Mean ± SD	12.83 ± 7.24	12.98 ± 7.20	0.905
%p2PSA			
Mean ± SD	2.09 ± 0.91	2.47 ± 0.74	0.230
PHI			
Mean ± SD	42.50 ± 22.74	53.31 ± 16.30	0.069
PHID			
Mean ± SD	1.24 ± 1.12	1.74 ± 0.92	0.031

Abbreviations: fPSA: free prostate-specific antigen; ISUP: International Society of Urological Pathology; N: number of patients; PV: prostate volume; PHI: Prostate Health Index; PHID: PHI density; PSAD: PSA density; SD: standard deviation; tPSA: total PSA; %fPSA: free to tPSA ratio; %p2PSA: [-2]proPSA to fPSA ratio.

In univariate ROC analysis, PHI, PHID and %fPSA were the most accurate predictors of clinically significant PC at the final pathology with AUC of 0.69, 0.70 and 0.76, respectively (Figure 4). Comparing single components of PHI, PHI and PHID showed the higher predictive power as compared to [-2]proPSA only (AUC: 0.69 vs. 0.51, P = 0.006; and AUC: 0.70 vs. 0.51, P = 0.092, respectively).

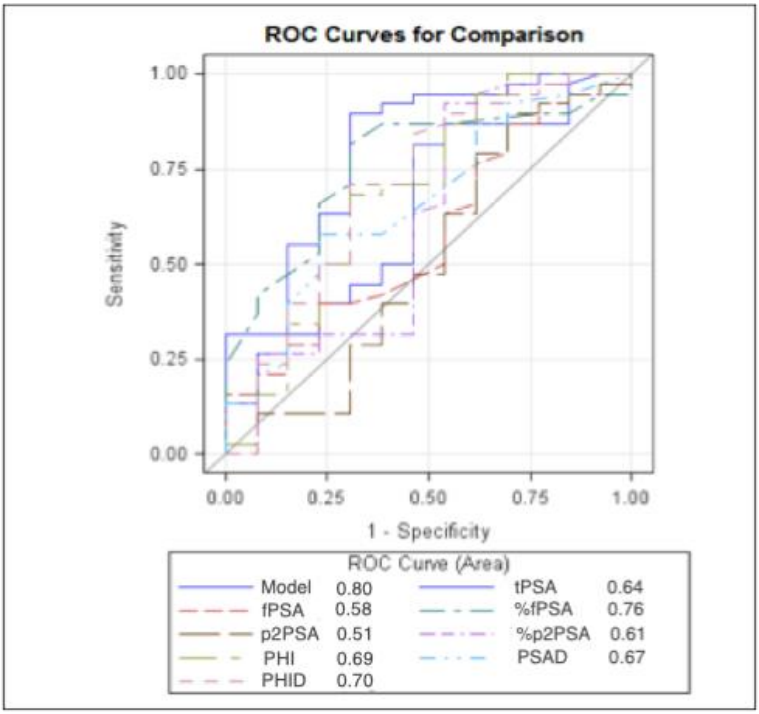

Figure 4. Receiver operating characteristic curves representing diagnostic ability of blood serum biomarkers to predict clinically significant prostate cancer at definitive pathology.

Abbreviations: fPSA: free prostate-specific antigen; PHI: Prostate Health Index; PHID: PHI density; PSAD: prostate-specific antigen density; ROC: receiver operating characteristic; tPSA: total prostate specific antigen; p2PSA: [-2]proPSA; %fPSA: free to tPSA ratio; %p2PSA: [-2]proPSA to fPSA ratio.

We performed DCA to determine the net benefit for each biomarker to predict clinically significant PC at the final RP pathology. The best net benefit at the final pathology was determined for PHI, when at 40% threshold probability 58% of patients after RP would be diagnosed with clinically significant disease.

4. DISCUSSION

In today's clinical practice, there is no universal definition of clinically significant PC. A contemporary Epstein's criteria and 2014 ISUP grading system are the most common used criteria for predicting clinically significant and not clinically singnificant PC (39,44,45). Our study results show that clinically significant PC according to Epstein's criteria have been diagnosed to 72% of patients and PC harboring ISUP grade ≥ 2 to 36% of patients. Therefore, the decision to perform PB based on a single serum biomarker with intent to detect clinically significant disease is still a challenge in urological practice.

PC screening programs based on tPSA only still remains a controversial topic in urological society all over the world and are criticised for its potential harms, such as psychological distress, false-positive results following subsequent PB, as well as over-diagnosis and over-treatment of clinically-insignificant indolent disease, including treatment complications along with negative impact on the quality of male life (8–10). Due to the limited specificity of tPSA, there is a considerable interest in new diagnostic biomarkers for PC that could overcome tPSA limitations and demonstrate improved specificity.

It was found, that precursor forms of PSA constitute the predominant fraction of fPSA in PC serum (46). Histological analyses of prostate specimens have shown that primarily precursor of PSA, called [-2]proPSA, is elevated in peripheral zone, while it was undetectable in transition zone, leading to the consensus that this isoform is more cancer specific than tPSA (47). Subsequently, it was found that [-2]proPSA isoform is providing higher concentration levels in PC patients' blood serum (48). Recently, it has been also revealed that [-2]proPSA could be a marker for PC aggressiveness already several years before diagnosis (16). Consequently, [-2]proPSA derivatives, such as %p2PSA, PHI and PHID, have been

suggested for PC diagnostics with intent to increase the specificity of tPSA. We have also explored the ability of these biomarkers to predict aggressive PC at the final RP pathology.

In fact, our study has demonstrated that %p2PSA and phi are associated with ISUP grade ≥ 2 disease and may be used not only for overall but also for aggressive PC detection, which has been confirmed by other authors as well (19,22,26). It is reported that %p2PSA and phi mean values are significantly higher not only in PC patients in comparison to non-PC patients, but the difference is found between PC patients and patients with isolated HPIN at biopsy (12). It is reported that higher values of PHID have been observed in patients with overall and clincally significant PC in comparison to healthy males (27,28). In our study, isolated HPIN at biopsy have been identified in 11.4% of patients, and as mentioned above, we determined not only %p2PSA and phi as significantly higher but also PHID mean values in patients with overall, Epstein's significant and ISUP grade ≥2 PC in comparison with patients without PC or with isolated HPIN at biopsy (Table 1).

According to our study results, the specificity of 35.7% at 90% sensitivity demonstrated the advantages for PHID at cut-off value of 0.54 in comparison with all other investigated biomarkers for overall PC detection (Table 5). Our results are consistent with previous studies, when PHID at a cut-off of 0.49 and 0.43 at 90.7% and 97.9% sensitivity, respectively, demonstrated the specificity of 30% and 38% for detection of overall PC (27,28).

At 90% sensitivity, to detect Epstein's significant PC, phi with cut-off of 31.92 and PHID with cut-off of 0.61 have shown the specificity of 35.7% and 36.4%, respectively, which was slightly inferior to PSAD with cut-off of 0.07 ng/mL/cc and specificity of 41.9% (Table 6). However, at 90% sensitivity, to detect ISUP grade ≥ 2 PC, phi and PHID with cut-off values of 33.2 and 0.63 have shown the highest specificity of 34.7% and 34.1%, respectively (Table 7). According to the literature, the specificity between 29.7% and 45.2% at 90% sensitivity for phi outperformed the specificity of

tPSA (7.8-26.4%) and %fPSA (28.5%) to detect ISUP grade ≥ 2 PC (19,24,49,50).

At 90% sensitivity, for detecting overall PC, PHID may lead to avoid 21.4% of prostate biopsies in comparison to 16.2% for phi and PSAD, as a result, 19% for %p2PSA. At 90% sensitivity for detecting clinically significant PC, phi and PHID may lead to avoid 30% of prostate biopsies.

On ROC analysis, we have identified PHID as more accurate predictor for overall PC detection in comparison to tPSA, fPSA, %fPSA, [-2]proPSA, %p2PSA, and phi (all $P<0.05$; see Figure 1). What is more important, we came to a conclusion that PHID is the most accurate predictor of Epstein's significant PC with AUC of 0.80 outperforming tPSA, fPSA, [-2]proPSA, and %p2PSA (all $P<0.05$; see Table 2). However, phi was the most accurate predictor of ISUP grade ≥ 2 PC at biopsy with AUC of 0.77 significantly outperforming tPSA and fPSA (all $P<0.05$; see Figure 3). Other authors have reported results that are in agreement with our findings, where PHID significantly outperformed tPSA, fPSA, and %fPSA in prediction for overall PC at biopsy, so PHID and phi had the greatest predictive accuracy for clinically significant prostate cancer at biopsy (28,49).

However, there is no ideal single biomarker and a multivariable approach for improved PC detection to be advocated (17). It was estimated that addition of [-2]proPSA derivatives to multivariate logistic regression models, which consisted of the most common demographic and clinical PC predictors, has improved predictive accuracy for overall PC detection up to 11% and outperformed its independent components (22,26,51,52). Recently, Loeb et al. have come to a conclusion that inclusion of PHI into a multivariate logistic regression model, which consisted of age, previous biopsy, PV, and tPSA, improved AUC from 0.70 to 0.75 to predict ISUP grade ≥ 2 PC in males with negative DRE and PSA between 2 and 10 ng/mL (53). In our study, we have revealed that only phi inclusion into the multivariate logistic regression model, which consisted of

previous biopsy, PV, fPSA, and %fPSA, has improved AUC to predict ISUP grade ≥ 2 PC from 0.74 to 0.79 (P = 0.04).

Summarising the available scientific data, it is concluded that PHI and PHID could help to improve individual risk assessment for early PC, particularly clinically significant PC detection, to reduce unnecessary biopsies, and may help to select patients eligible for active surveillance and play a role in treatment decision-making (54).

According to the literature, just a few studies have investigated the potential of [-2]proPSA and its derivatives to detect clinically significant PC at the final pathology. In a cohort of patients undergoing RP higher values of [-2]proPSA, %p2PSA, as well as PHI and PHID values, advanced disease at the final pathology has been observed pre-operatively in patients with ISUP ≥ 2 PC and locally (16,55–60). Our findings are in line with the literature, where pre-operative value of PHID (1.74 vs. 1.24, P = 0.031; Table 9) was significantly higher in patients harbouring clinically significant PC at the final pathology, while a strong tendency to predict clinically significant disease was observed for PHI (42.50 vs. 53.31, P = 0.069; Table 9).

According to our data, PHID and PHI have demonstrated comparable results to predict clinically significant PC at the final RP pathology (AUC: 0.70 and AUC: 0.69, respectively), while the same AUC value for PHI was reported by Fossati et al. (57). However, %fPSA with AUC of 0.76 remained as a significant predictor in our ROC curve analysis.

Several studies compared diagnostic and prognostic potentials of PHI with other molecular biomarkers, such as prostate cancer antigen 3 (PCA3) and transmembrane protease, serine 2 (TMPRSS:2):v-ets erythroblastosis virus E26 onco-gene homolog (avian) (ERG) gene fusion (T2:ERG), however, there is no similar data available for PHID. Stephan et al. concluded that PHI outperformed the diagnostic accuracy of T2:ERG for PC in PB setting (61). Cantiello et al. published the data on predictive accuracy of PHI and PCA3 to predict adverse pathologic features in males

undergoing RP, where only PHI provided significant predictive accuracy at multivariate analysis for clinically significant and locally advanced PC (56). Joining plasma levels of dysregulated microRNAs with PHI significantly increased prognosis of metastatic PC (62). Combination of PHI with additional serum biomarkers may increase current PC risk stratification tools and should attract more research.

Nevertheless, we should address several limitations of the present study. Firstly, a study cohort partly consisted of patients who underwent repeated PB. Secondly, it was not possible to make a comparative analysis with other commercially available blood biomarkers, including 4K test, PCA3, TMPRSS2:ERG or microRNA, which could be useful tools for detection and prediction of PC. Thirdly, multi-parametric magnetic resonance imaging, widely used nowadays in clinical practice, was not included in our protocol. The small-size RP cohort predisposes a limited statistical significance that precludes strong conclusions. Finally, several specialist pathologists have been involved which could make a bias towards pathological analysis. We do not dedicate the second reference pathologist to assess histological specimens.

In spite of these shortcomings, our study has its strengths. This is a prospective study where all males underwent PB under a standardized protocol. The study cohort consisted of males with tPSA levels within “grey“ zone and negative DRE, representing the most debatable group of population in making decision on PB. It is very important that our statements about the diagnostic power of [-2]proPSA and its derivatives are partially based on the final RP pathology. The study revealed the clinical value of PHI and PHID to predict clinically significant PC at the final pathology, which is crucial not only in primary and in repeat PB setting, but also in decision making on definitive PC therapy.

CONCLUSIONS

1. PC was diagnosed in 53.3% of patients. Clinically significant PC was diagnosed in 35.7% - 72.3% of patients at biopsy, and in 74.5% of patients at final RP pathology.
2. [-2]proPSA derivatives, such as PHI and PHID have demonstrated the best combinations of sensitivity, specificity, PPV and NPV, as well as diagnostic accuracy in predicting overall and clinically significant PC.
3. At 90% sensitivity, for detecting overall and clinically significant PC, PHI and PHID may lead to reduce unnecessary PBs from 21.4% to 30%.
4. PHI significantly improved the diagnostic accuracy of multivariate model consisted of demographic, clinical and other blood serum biomarkers in predicting ISUP grade $\geq$ 2 PC.
5. PHI and PHID have demonstrated the best net benefit in clinical decision making.
6. Isolated HPIN has been identified in 11.4% of patients at biopsy. The diagnostic ability of [-2]proPSA and its derivatives to predict HPIN was not determined.

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LIST OF PUBLICATIONS AND PRESENTATIONS

Publications:

1. Barisiene M, Bakavicius A, Stanciute D, Jurkeviciene J, Zelvys A, Ulys A, Vitkus D, Jankevicius F. Prostate Health Index and Prostate Health Index Density as Diagnostic Tools for Improved Prostate Cancer Detection. Biomed Res Int. 2020 Jul 21;2020:9872146. doi: 10.1155/2020/9872146. PMID: 32775459; PMCID: PMC7396080.
2. Marija Barisienė, Arnas Bakavičius, Diana Stančiūtė, Jolita Jurkevičienė, Arūnas Želvys, Albertas Ulys, Dalius Vitkus, Feliksas Jankevičius. Significance of Prostate Health Index and its density to predict aggressive prostate cancer at final pathology. JBUON;2021:26(3).

Presentations:

1. 5-th Baltic Meeting in conjunction with EAU (Baltic18), 2018 05 25-26 Riga, Latvia. Barisiene M., Stanciute D., Bakavicius A., Jurkeviciene J., Zelvys A., Ulys A., Jankevicius F. Diagnostic accuracy of [-2]proPSA, %p2PSA and Prostate Health Index for prostate cancer detection. Eur Urol Suppl 2018; 17(5);e2184.
2. 6-th Baltic Meeting in conjunction with EAU (Baltic19), 2019 05 24-25 Tallinn, Estonia. Barisiene M., Stanciute D., Jurkeviciene J., Zelvys A., Ulys A., Jankevicius F. Whether p2PSA, %p2PSA and Prostate Health Index improves clinically significant prostate cancer detection in men with total PSA range 2-10 ng/ml. Eur Urol Suppl 2019; 18(3);e2437.

CURRICULUM VITAE

Personal data:

Full name: MARIJA BARISIENĖ
Date of birth: 09-12-1981
Address: Jūratės g. 14-2, Vilnius
Phone: +370 670 04 794
E-mail: marija.barisiene@santa.lt

Education:

1989-2000 High school

2000-2006 Master's degree in Medicine from Vilnius University Faculty of Medicine.

2006-2007 Internship at the Faculty of Medicine in Vilnius University.

2007-2012 Urology Residency at the Faculty of Medicine in Vilnius University.

2015-2020 Doctoral studies (PhD) at the Faculty of Medicine in Vilnius University.

Working experience:

Current position:

01/08/2012-present Urologist at the Center of Urology of Vilnius University Hospital Santaros Klinikos.

Previous positions:

10/2010-06/2011 Physician assistant at the Department of Urology at Vilnius University Emergency Hospital.

08/2011-07/2012 Physician assistant at theDepartment of Urology at Vilnius City Clinical Hospital.

01/08/2012-31/08/2014 Urologist at Department of Urology at Vilnius City Clinical Hospital.

2013-05/10/2020 Urologist at Ginekologijos ir šeimos klinika UAB.

08/10/2014-05/10/2020 Urologist at Centro poliklinika, Vilnius.

Refresher courses:

02/09/2011-09/09/2011 *9-th European Urology Residents Education*

programme (EUREP), Prague, Czech Republic.
07/2012 Austrian-American foundation, OMI - Salzburg Medical Seminars, ESU Master Class in Urology, Salzburg, Austria.
01/04/2017-30/06/2017 Observership at Royal Hallamshire Hospital, Sheffield, United Kingdom, supervised by prof. Ch. Chapple.
Participation in conferences arranged by Lithuanian Association of Urology, European Association of Urology etc.

Good Clinical Practice Training:
17/01/2013 ICH/GCP Foundation training course, ICON Lithuania, Vilnius
05/06/2018 Good Clinical Practice E6 (R2) Refresher Course, Vilnius.

Experience in Biomedical Reserch:
2013 Clinical Study on Bladder Pain Syndrome / Interstitial Cystitis Treatment (Phase II)/ sub-investigator.
2014-2016 Clinical Trial on Overactive Bladder Treatment (Phase III)/ investigator.
2015-2017 Molecular Biomarkers for Prostate Cancer Diagnosis (Phase III)/ investigator.
2016-2017 Treatment of Complicated Urinary Tract Infections, Including Acute Pyelonephritis, in Hospitalized Adults (Phase II/III)/ sub-investigator.
2016-2019 Treatment of Urinary Incontinence in Adults Subjects With Neurogenic Detrusor Over-activity Due to Spinal Cord Injury or Multiple Sclerosis (Phase III)/ investigator.
2020-present Male Overactive Bladder and Concomitant Benign Prostate Hyperplasia Treatment (Phase III)/investigator.
2021- present Treatment of Advanced Prostate Cancer (Phase II)/investigator.

Memberships:
Lithuanian Association of Urology
European Association of Urology

Awards:
1. Berlin Chemie 2nd prize for the presentation *Comparison of three*

surgical approaches for the treatment of female recurrent stress urinary incontinence. EAU Baltic meeting 2014, 2014 05 23-24, Vilnius, Lithuania.

2. Berlin Chemie 2nd prize for the presentation *Complications and its treatment after midurethral sling implantation using retropubic and transobturator route for the treatment of female stress urinary incontinence*. 3rd EAU Baltic meeting 2016, 2016 05 27-28, Tallinn, Estonia.

NOTES

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9 Saulėtekio al., Building III, LT-10222 Vilnius
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