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Original article

Explication of the roles of prostate health index (PHI) and urokinase plasminogen activator (uPA) as diagnostic and predictor tools for prostate cancer in equivocal PSA range of 4–10 ng/mL

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ABSTRACT

Background: Prostate cancer (PCa) is one of the most commonly encountered cancers and the leading cause of death worldwide. Currently used biomarkers accounts difficulties in discriminating benign from malignant cases or predicting outcome, so investigating new biomarkers performance is needed.
Objectives: Assessment of diagnostic and predictor roles of prostate health index (PHI) and urokinase plasminogen activator (uPA) in PCa.
Methods: 194 males with initial tPSA of 4–10 ng/mL were categorized into three groups: PCa, benign prostatic hyperplasia (BPH) and healthy control. Serum levels of tPSA, fPSA, p2PSA, and uPA were performed by ELISA with calculation of PHI as (p2PSA/fPSA) $\times \sqrt{}$PSA.
Results: PHI and uPA were significantly higher in PCa patients relevant to BPH and healthy control ($p \leq 0.001$). Both markers outperformed all assessed biomarkers and showed the highest area under the curve (AUC) in ROC curve analysis. Both were significantly higher in PCa patients with {Gleason score $\geq$ 7, late stages (cT2b,c; T3), LN extension and distant metastasis}relative to their counterparts. Additionally, PHI and uPA and were independent predictors of distant metastasis and Gleason score $\geq$ 7, while PHI was predictor of LN invasion ($\beta = 0.25$, $p = 0.004$).
Conclusion: PHI and uPA would be of potential value in discriminating between PCa, BPH and healthy men in addition, both are promising as independent predictors of adverse pathological features.

1. Introduction

Prostate cancer (PCa) is reported to be the most frequently diagnosed cancer; it accounts for 7.1% of new cases of all cancers and 3.8% of cancer-related deaths worldwide (Bray et al., 2018). About three decades ago, with the approval of Prostate-specific antigen (PSA) as one of the few biomarkers that has been routinely used for detection and monitoring response to therapy, the landscape of PCa screening, diagnosis and active surveillance has been globally changed (Chou et al., 2011; Fenton et al., 2018). However, there are restrictions for using PSA alone for diagnosis and clinical decision-making. First, the test may give false-positive or false-negative results. Furthermore, serum levels in the gray zone (4 ng/mL to 10 ng/mL) may carry discrepancy for interpretation (Crawford et al., 2014). Therefore, total PSA (tPSA)-based testing might lead to over diagnosis and/or overtreatment (Cabarkapa et al., 2016; Printz, 2012).

In fact, PSA serum levels might be affected by prostate manipulation, androgen levels, benign prostatic hyperplasia (BPH) or prostatitis. Also, PSA may be altered by sample handling, laboratory processes or technical standardization issues (Link et al., 2004; Roehrborn et al., 1996). Therefore, researchers and oncologists are studying other biomarkers that could be of significant impact in diagnosis and follow up of PCa patients. A significant

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enhancement of PCa detection and active surveillance utility could be encountered with the emergent use of other PSA precursors, isoforms and derivatives as free PSA fraction (fPSA); [−2] proPSA (p2PSA) and their percentage fractions as %fPSA and %proPSA (Jansen et al., 2010; Sokoll et al., 2008; Tosoian et al., 2012).

A meta-analysis of 66 manuscripts reported the better performance of %fPSA than total PSA (tPSA) (Roddam et al., 2005). However, %proPSA might show better performance than fPSA (Sokoll et al., 2008). Notably, the recently approved prostate health index (PHI) carries a promising diagnostic performance and utility than PSA (N. Fossati et al., 2015; Hsieh et al., 2018; Huang et al., 2014). PHI showed better discriminating capacity between prostate cancerous and non-cancerous patients, thereby lowering the rate of unnecessary trans-rectal prostate biopsies (Lazzeri et al., 2014; Lazzeri, Haese, Abrate, et al., 2013; Lazzeri, Haese, de la Taille, et al., 2013).

Urokinase plasminogen activator (uPA) plays an essential role in the degradation of basement membrane and consequently in cell migration, angiogenesis, invasion and progression of malignancies. Elevated levels of uPA has been associated with aggressiveness and adverse outcomes in many cancers, including PCa (Al-Janabi et al., 2014; Duffy, 2002; Su et al., 2016). Increased expression and elevated serum levels of uPA and/or their binding receptors were highly associated with extension of PCa beyond the capsule and further metastasizing potential (Kumano et al., 2009; Miyake et al., 1999; Van Veldhuizen et al., 1996). In spite of the development of novel prediction tools that may help oncologists in the biopsy decision track, no single biomarker could precisely discriminate and predict the result of the initial prostate biopsy (Chun et al., 2010). Thus, in this study we aimed to assess the utility of PHI and uPA as a potential diagnostic, prognostic and a predictor tool for PCa in the area of grey zone decision between 4 and 10 ng/mL of tPSA in comparison with other biomarkers for PCa detection, invasion, metastasis, and progression. To our knowledge, our study would be the first in Saudi Arabia to investigate both markers as a new tool for diagnosis and prediction of adverse pathological features of PCa.

2. Methods

2.1. Study populations

The study population was enrolled during the interval from March 2015 to September 2018, it included a cohort of 194 males with initial tPSA of 4–10 ng/mL, who were initially underwent prostate biopsy and accordingly categorized into three age and BMI matched groups. The first group being the PCa group: 71 male patients' histopathologically confirmed and samples were collected before any surgical, hormonal, or radiotherapeutics' interventions were made. The second group is the benign prostatic hyperplasia (BPH) group: 69 males who were confirmed by histopathological examination to be benign hyperplasia of the prostate with exclusion of cases of prostatitis or acute urinary tract infection. The final group is the control group: 54 males with no past history or family history of malignancies.

Exclusion criteria for all were; past history of any urogenital cancers, acute or chronic prostatitis, untreated urinary tract infection within the past three months, chronic kidney disease, previous endoscopic surgery, prostate biopsy or usage of 5 alpha-reductase inhibitors as dutasteride or finasteride, which could affect levels of measured biomarkers.

All participants in this study were informed about the aim of our study, they signed consent for agreement to participate in this study with maintenance of their anonymity in complies with The Code of Ethics of the World Medical Association (Declaration of Helsinki). The Ethics Review Board for Human Studies of Faculty of Medicine, Umm Al-Qura University approved this study; protocol number was (HAPO-02-K-012-2015-01-103). For all participants, inclusive clinical data forms were used to collect all medical information. Comprehensive history including lifestyle behaviors, current, past medical history, family, operative and drug history was obtained. Questionnaires, data, and specimens were obtained from patients prior to any surgical, hormonal and radiotherapy intervention and/or any other therapy modality, therefore, any influence of treatment was unlikely.

2.2. Clinical examination and blood sampling

Body mass index (BMI) was reported as being one of the confounders for prostate cancer detection; higher BMI is associated with lower PSA levels in men over fifty years of age (Seo et al., 2017). It was previously reported that tPSA levels are likely to decrease with the increase of BMI (Freedland et al., 2008), thus, height in meters, weight in kilograms were measured to calculate BMI as weight divided by square of height and all studied groups were matched for age and BMI. Detailed clinicopathological and surgical data for PCa group included but was not limited to TNM stage, Gleason score, LN, histological grade safety margin, seminal vesicles, and perineum invasion if available. From each participant five mL of blood was drawn with respect to evading any prostatic manipulations that might cause sequential alteration of any of the assessed biomarkers.

Five mL blood sample was drawn by vacutainer technique, then after a maximum of two hours, samples were centrifuged at 2000 g for 4 min to overcome the low stability of p2PSA at room temperature (Semjonow et al., 2010). Samples were kept at −80 °C until further analysis.

2.3. Laboratory measurements

All assessed parameters: tPSA, fPSA, p2PSA, and UPA were performed using Enzyme-Linked Immunosorbent Assay (ELISA) technique. Kits for measurement of tPSA (Cat NO.:SL1727Hu); fPSA (Cat NO.:SL0732Hu), and uPA (Cat NO.:SL1796Hu) were brought from Sunlong Biotech Co. Ltd, Zhejiang, China. While kits for p2PSA (Cat NO.:AE98075Hu) were brought from Shanghai Lianshuo Biotechnology (AMEKO) Co., Ltd. Shanghai; China. All samples were tested in duplicate and evaluated blindly to the diagnostic information. Entire procedures were strictly followed according to the manufacturer's instructions and quality control measurements were within the ranges recommended by the manufacturers. Calculation of %fPSA was as a proportion of fPSA/tPSA x100 and %p2PSA as ([p2PSA pg/mL]/[fPSA ng/mL × 1000]) x100. PHI was calculated according to the formula (p2PSA/fPSA) × root square of PSA.

3. Statistical analysis

Statistical analysis was performed using IBM® Statistical Package for the Social Sciences (SPSS) software, version 20 (Microsoft Corporation, Redmond, WA, USA). Both Kolmogorov-Smirnov and Shapiro-Wilk were used to assess the normality of variables' distribution. Data have been presented as the means ± standard deviation (SD). The *t*-test and Mann-Whitney test were used for comparison of two groups while ANOVA post hoc test and Kruskal-Wallis test were used for comparison of more than two groups for parametric and non-parametric data respectively. We used Spearman rank correlation analysis for the determination of associations between clinicopathological parameters and the assessed biomarkers. The analysis was commenced through

multivariable regression model for evaluation of independent predictors of adverse clinicopathological features of the assessed biomarkers levels in patients with PCa. P values < 0.05 were considered significant.

4. Results

One hundred and ninety-four men were included in the current study according to inclusion criteria. Their serum levels of tPSA for all cohort were within the range of 4–10 ng/mL i.e. equivocal or gray zone; (mean ± SD) 6.76 ± 1.27, respectively. PCa was diagnosed and confirmed in 71(36.6%) patients, benign prostatic hyperplasia in 69 (35.6%) and 54 (27.8%) were found to be healthy normal subjects. Demographic and clinicopathological features of the study cohort are listed in Table 1. Age of the study cohort was non-significantly different among PCa; BPH and normal control groups ($p = 0.256$). BMI were non-significantly different as well ($p = 0.768$). Serum uPA, fPSA, %fPSA, P2PSA, and PHI levels showed highly significant difference between three studied groups ($p \leq 0.001$). Comparing patients diagnosed as PCa with those of BPH; mean ± SD of uPA serum levels and PHI serum levels were significantly higher in PCa group ($p \leq 0.001$), while PSA, fPSA, %fPSA and %p2PSA were not significantly different ($p = 0.781$, 0.434, 0.162, 0.055, respectively). Interestingly, when comparing PCa to normal control, %p2PSA, levels were significantly higher ($p = 0.004$). As illustrated, there was a highly significant difference in serum levels of uPA (Fig. 1 A) and PHI (Fig. 1 B) in between the three investigated groups. Serum levels of uPA showed gradual tendency to be significantly increased from normal to BPH to PCa group ($p \leq 0.001$). PHI showed also highest levels in PCa and higher levels in BPH in comparison to normal control ($p \leq 0.001$).

To study and compare the performance of investigated biomarkers in all groups, ROC curve was applied. ROC analyses of uPA, PSA, fPSA, %fPSA, P2PSA, %P2PSA and PHI levels between the PCa and BPH patients are shown in Fig. 2A, and ROC analysis between PCa and normal control men are shown in Fig. 2 B. For discrimination between PCa and normal men PHI and uPA showed the best performance (AUC = 0.887; 95% CI = 0.825–0.948, and AUC = 0.843; 95%CI = 0.757–0.930, respectively). At cut off value of 0.65 ng/mL, sensitivity and specificity of uPA were 95.8% and 79.6%, respectively; 95% CI, 0.701–0.856). On the other hand, the best cut off point for PHI was 33.14, which maximized the sum of sensitivity and specificity (83.1% and 79.7%, respectively). Interestingly, p2PSA achieved better performance than PHI in differentiating between PCa and BPH patients (AUC = 0.733; 95% CI = 0.644–0.822, AUC = 0.639; 95% CI = 0.546–0.733, respectively). The cut off for p2PSA was 15.47 pg/mL (sensitivity = 69%; specificity = 79.9%) whilst, PHI cut off was 46.04 at selected sensitivity of 60.6% and specificity of 65.2%. Whereas, the best cut-off for serum levels of uPA was 0.72 ng/mL that showed sensitivity and specificity of 77.5% and 69.6%. Table 2 shows a comparison of all studied biomarkers as independent predictors of prostate cancer in normal men and patients with prostatic hyperplasia. Among patients with prostatic hyperplasia, uPA was found to be of highest AUC (0.779, $p \leq 0.001$), while among normal men PHI shows AUC equal to 0.887 and $p \leq 0.001$.

Table 3 represents the correlation of serum levels of all investigated biomarkers with the clinicopathological parameters of PCa patients. Serum levels of PHI were significantly higher in patients with Gleason score ≥ 7 than those with < 7 ($p \leq 0.001$), PSA and uPA levels were significantly higher ($p = 0.041$, $p = 0.001$ respectively). Whereas, levels of fPSA and %fPSA were significantly lower in Gleason score ≥ 7 than < 7 ($p \leq 0.001$). Regarding Clinical stages, a highly significant lower level of fPSA and %fPSA were demonstrated in late stages (cT2b,c; T3) in comparison to early clinical stages (cT1; cT2a) $p \leq 0.001$. On the other hand, levels of PHI were significantly very high ($p \leq 0.001$), and uPA were high ($p = 0.012$) in late stages. PCa patients with positive LN extension showed highly significant increased levels of PHI ($p \leq 0.001$) and significantly lower levels of fPSA and %fPSA than patients with no LN extension ($p \leq 0.001$, $p = 0.001$respectively). In the same manner, higher significant levels of PHI and uPA were found in patients with distant metastasis than those with no metastasis ($p \leq 0.001$). Patients who underwent radical prostatectomy and had positive invasion of surgical margin and seminal vesicles were found to have significantly higher levels of PHI than their negative counterparts ($p = 0.017$, $p = 0.040$ respectively). Fig. 3 illustrates the significant difference in levels of PHI and uPA in PCa patients with Gleason score ≥ 7 vs < 7, and in patients with positive distant metastasis vs patients with no metastasis and in patients diagnosed with early vs late clinical stages ($p \leq 0.001$).

As shown in Table 4, multiple regression analyses for studied parameters besides age and BMI for prediction of distant metastasis depicted that uPA, PHI, and P2PSA were significantly associated with distant metastasis. Among all studied parameters, levels of PHI, uPA and P2PSA were found to be independent predictors for metastasis, Beta Coefficient (β) = 0.28, $p \leq 0.001$; $\beta = 0.856$, $p = 0.004$ and $\beta = 0.144$; $p = 0.002$, respectively.

In Table 5, multiple regression analysis of age, BMI, serum levels of uPA, PSA, fPSA, %fPSA, p2PSA, %p2PSA, and PHI, as potential predictors of LN involvement, revealed that only PHI levels were an independent determinant for LN invasion, $\beta = 0.25$, $p = 0.004$. Linear regression analysis of age, BMI, serum levels of uPA, tPSA, fPSA, %fPSA, p2PSA, %p2PSA and PHI as potential predictors of Gleason score revealed that PHI and uPA levels were independent determinant for Gleason score, $\beta = 0.27$, $p \leq 0.001$ and $\beta = 0.761$, $p = 0.02$ respectively as depicted in Table 6.

5. Discussion

In the current study, at the grey zone of tPSA levels; 4–10 ng/mL, PHI and uPA showed higher significant levels in PCa patients relative to BPH and to normal control groups; their levels were gradually increasing from normal subjects to BPH to PCa patients. Moreover, PHI showed the best characteristic performance among studied biomarkers in discrimination between PCa and normal subjects with AUC of 0.887, sensitivity and specificity of 83.1% and 79.7% respectively at cut off value of 33.14. On the other hand, uPA showed better performance than other PSA derivatives (fPSA, %fPSA, p2PSA, and %p2PSA) at cut off value of 0.65 ng/mL with sensitivity of 95.8% and specificity of 79.6%.

In accordance with our results, several studies showed outperformance of PHI when compared with other PSA derivatives; a retrospective study in China comprising 230 males with tPSA level of 4–10 ng/mL and negative DRE. PHI had AUC of 0.781; sensitivity was 90% while specificity was 49.76% at cut off value of 26.54 (Ng et al., 2014). A prospective study in Taiwan furtherly validates our results; *Tan* and his colleagues assessed PHI in 157 men with tPSA level also in grey zone (4–10 ng/mL). They reported that AUC was 0.793; sensitivity was 90% and specificity was 58.27% at cut off value of 26.75 (Tan et al., 2017). Recently, in another prospective study among 121 Taiwanese males with tPSA levels between 4.3 and 7.6 ng/ml, PHI showed AUC of 0.772; at 90% sensitivity, specificity was 27.27% at cut off 21.62 (Cheng et al., 2019). The range of AUC from three previous meta-analysis studies was 0.69–0.781 (Bruzzese et al., 2014; X. Filella and Gimenez, 2013; Wang et al., 2014). National Comprehensive Cancer Network (NCCN) addressed the PHI score of more than 35 to be a high predictor of PCa (Xavier Filella and Foj, 2018). Additionally, in a multicenter study PHI of 55 or more was found to be related to

Table 1
Clinicopathological features and results of studied parameters in all groups.

Character	Healthy control group (HC; n = 54)	Benign Prostate Hyperplasia (BPH; n = 69)	Prostate Cancer (PCa; n = 71)	P value
Age (years)				0.256
Mean ± SD	60.75 ± 3.47	61.89 ± 4.36	61.55 ± 4.28	
Median (Range)	60.40 (55.5–68.0)	63.0 (53.0–72.20)	62.4 (53.0–72.0)	
BMI				0.768
Mean ± SD	26.86 ± 2.07	26.68 ± 1.96	27.16 ± 2.11	
Median (Range)	26.81 (23.81–33.2)	26.0 (24.5–31.4)	26.8 (25.1–32.2)	
uPA (ng/mL)				≤0.001**
Mean ± SD	0.46 ± 0.37	0.68 ± 0.2	0.83 ± 0.13	
Median (Range)	0.42 (0.01–1.8)	0.66 (0.4–1.7)	0.88 (0.54–1.1)	
PSA (ng/mL)				0.954
Mean ± SD	6.6 ± 1.1	6.7 ± 1.2	6.8 ± 1.34	
Median (Range)	6.4 (4.67–9.45)	6.75(4.01–9.65)	6.67(4.12–9.82)	
fPSA (ng/mL)				≤0.001**
Mean ± SD	1.5 ± 0.61	0.95 ± 0.35	0.9 ± 0.32	
Median (Range)	1.56 (0.48–2.55)	0.89 (0.52–2.54)	0.87 (0.43–2.23)	
				≤0.001**
%fPSA	0.23 ± 0.09	0.15 ± 0.07	0.14 ± 0.06	
Mean ± SD	0.22 (0.08–0.52)	0.13 (0.08–0.4)	0.12 (0.05–0.45)	
Median (Range)				
P2PSA (pg/mL)				≤0.001**
Mean ± SD	13.97 ± 2.6	14.46 ± 1.3	16.15 ± 2.5)	
Median (Range)	13.43 (8.98–19.78)	14.7(11.34–17)	16.97(10.78–22.51)	
%P2PSA				≤0.001**
Mean ± SD	2.13 ± 0.59	2.23 ± 0.44	2.47 ± 0.68	
Median (Range)	2.07(1.09–4.0)	2.21(1.43–3.42)	2.42(1.35–4.7)	
PHI				≤0.001**
Mean ± SD	27.52 ± 13.85	42.51 ± 11.12	51.56 ± 17.69	
Median (Range)	24 (13.18–94.25)	44.86(13.16)	47.67(16–96.18)	
Biopsy gleason score categories n (%)				
<7			17 (23.9%)	
≥7	NA	NA	54 (76.05)	
Clinical Stage n (%)				
Early: cT1; cT2a	NA	NA	25 (35.21%)	
Late: cT2b,c; T3			46 (64.78%)	
LN extension n (%)	NA	NA		
+ve			47 (66.21%)	
−ve			24(33.8%	
Metastasis n (%)				
M0	NA	NA	8 (11.27%)	
M1			63(88.73%)	
[RP] **Seminal vesicle involvement[&], n (%)**				
+ve			37(58.73%)	
−ve	NA	NA	26(41.26%)	
[RP] **Surgical margin[&], n (%)**				
R0	NA	NA	35(55.56%)	
R1			28(44.44%)	

BMI, body mass index; BPH, benign prostate hyperplasia; %fPSA, percentage of free PSA to total PSA; p2PSA, [-2]proPSA; %p2PSA, percentage of [-2]proPSA to free PSA; PCa, prostate cancer; PHI, Prostate Health Index; PSA, prostate-specific antigen, [RP], Radical prostatectomy, ** = p value < 0.001.

likelihood percentage of 52.1% for PCa (Catalona et al., 2011). However, there was no consensus about the exact AUC or cut off value of PHI for predicting PCa. This may be due to the different study designs or due to the determinant of diagnostic accuracy in each, which was considered the highest sensitivity values at 90% or in other studies by choosing the best combination of sensitivity and specificity as was considered in the current study. In concordance, a multi-institutional study, "European Randomized Study of Screening of Prostate Cancer" (ERSPC-PHI), which comprised one thousand thirty three patients and incorporated PHI score into the risk calculation tool for PCa that lead to increase AUC by 0.06 in case of prediction of significant PCa with added benefit at around seventeen percentage less risk threshold (Foley et al., 2016).

In discriminating between PCa versus BPH, p2PSA achieved better performance than PHI (AUC = 0.733, 0.639 respectively) in a study conducted by *Lezzeri* and his colleagues who reported the higher significant levels of PHI and %p2PSA in PCa patients in comparison to chronic histologic prostatic inflammation (CHPI) and BPH as well. It was concluded that p2PSA, %p2PSA, and PHI values could distinguish between PCa and other non-malignant conditions as CHPI or BPH. Thus, they could be of great benefit for decision making to avoid unnecessary biopsies (Lazzeri et al., 2014).

In concordance with our findings regarding uPA, mean serum levels of uPA were observed to be higher in PCa patients than in healthy men with no cancer and BPH (Miyake et al., 1999; Shariat et al., 2007). As previously reported, uPA is a serine protease that binds to uPA receptor (uPAR) to play a key role in basement membrane breakdown, tumor progression, and invasion. Hence the increase in their expression was detected in several malignancies such as; breast (Foekens et al., 2000), ovarian (Hoffmann et al., 1999), colorectal (Herszenyi et al., 2008), and lung cancer (Salden et al., 2000). Moreover, uPA elevation or overexpression has been frequently linked to poor prognosis (Mahmood et al., 2018; Su et al., 2016). In 153 patients, underwent radical prostatectomy for organ-confined prostate cancer, overexpression of uPA was strongly associated with poor prognosis, and predict biochemical recurrence (Kumano et al., 2009). Previously,

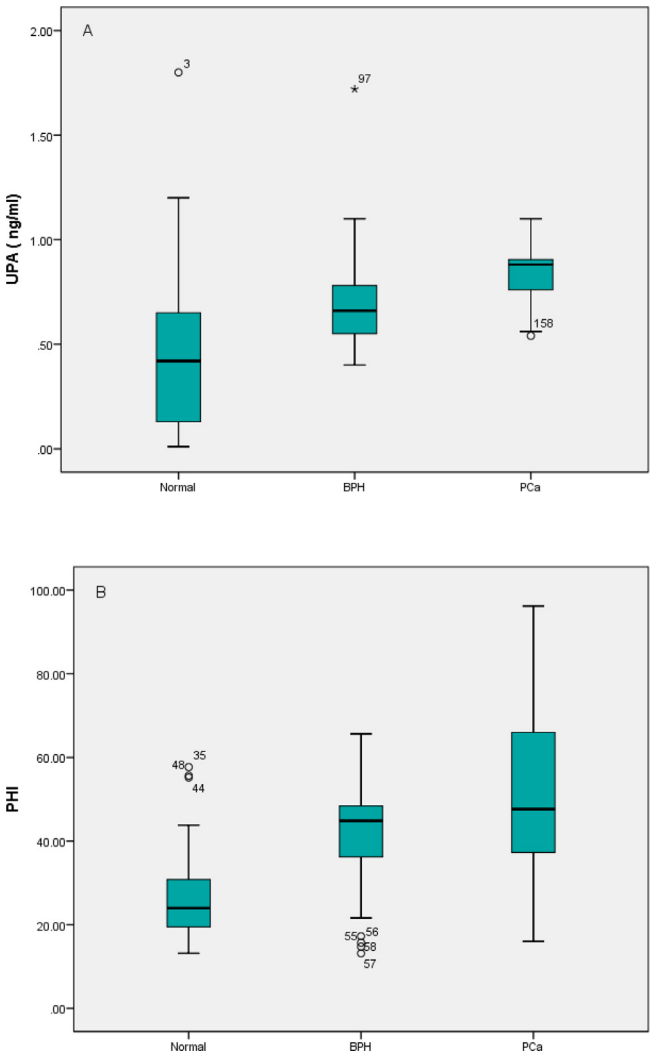

Fig. 1. The box-and-whisker plots of (A) uPA and (B) PHI relative to studied groups: healthy normal, BPH and PCa groups. Circles represent the outliers with their values; asterisks represent the extremes of outliers'. The green box illustrated the interquartile range. The bold line represents the median.

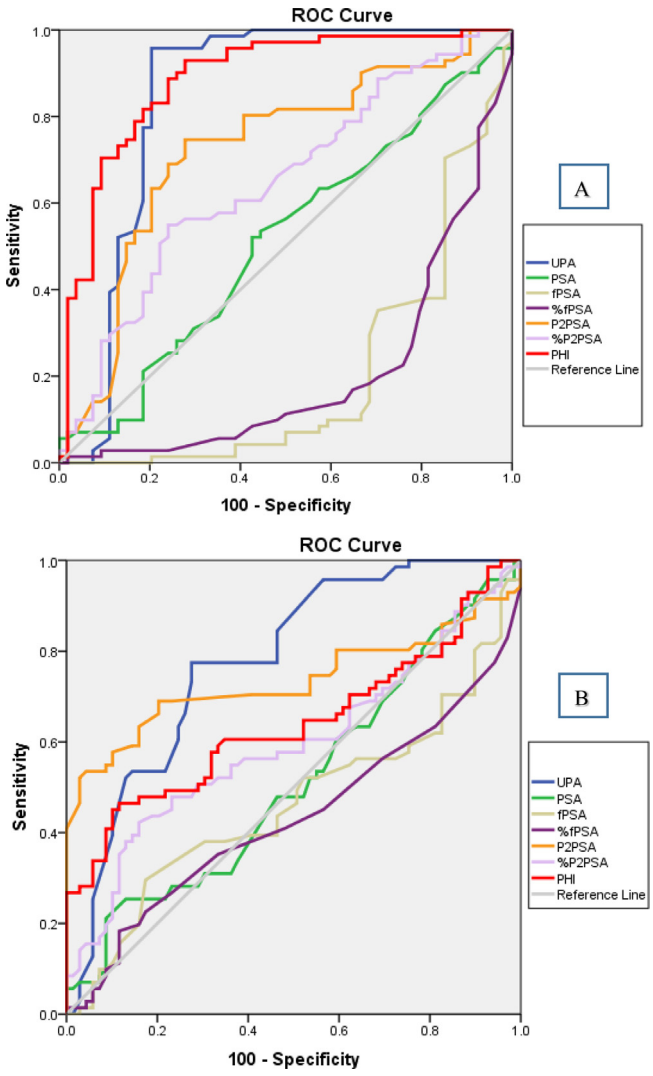

Fig. 2. Receiver operating characteristic (ROC) curves analysis of PSA derivatives and uPA. (A) Represents a comparison of PCa vs BPH, (B) represents comparison of PCa vs healthy control men.

Crowley et al. (1993) reported that a competitive analogue of uPA that inhibits binding of uPA with its receptor in vivo consequently inhibits metastasis in prostate cancer. Recently, serum uPA were found to be significantly elevated in breast cancer patients compared to non-malignant counterparts (Banys-Paluchowski et al., 2019).

In this study, the association of PHI and uPA of clinicopathological features and their potential role for prediction of aggressive and advanced PCa has been assed. Serum levels of uPA and PHI were found to be significantly higher in PCa patients having pathological indicators and features of poor prognosis, late stages, more extension, and aggressiveness. Both of PHI scores and uPA levels were found to be higher in patients with Gleason score $\geq$ 7, advanced clinical stages (cT2b, c; T3), distant metastasis ($p \leq 0.001$), positive LN, seminal vesicles and surgical margin extension ($p < 0.01$) when compared to their counterparts for each pathological determinant. Being the main proteinase system, it is not surprising to link the elevated levels uPA or its binding receptors (uPAR) with poor prognosis in several malignancies (Andreasen et al., 2000; Berger, 2002; Shi and Stack, 2007) as well as in PCa (Miyake et al., 1999; Shariat et al., 2007; Sheng, 2001). Shariat et al. (2007) evaluated the preoperative serum levels of uPA and uPAR in 429 patients with clinically localized PCa before radical prostatectomy. In agreement with our findings regarding uPA, they reported the significantly higher levels of both markers in patients with higher Gleason sum, LN, capsular and seminal vesicle invasion. Highly significant values of uPA were found in bone metastasizing PCa patients than with patients with local LN metastasis or confined tumors (Shariat et al., 2007).

In several studies, PHI had a significant positive association with biopsy Gleason score, in 892 patients and 646 patients with tPSA 2–10 ng/mL, respectively (Catalona et al., 2011; Lazzeri, Haese, de la Taille, et al., 2013) and in 1362 patients with initial tPSA 1.6–8.0 ng/mL (Stephan et al., 2013). In a prospective, study including 489 patients at five European Urology centers, the median of PHI was 64.9 in PCa patients with advanced pathologic Gleason $\geq$ 7 and stage T3 , which was significantly higher than that in other PCa patients with lower stage and pathologic Gleason score, who had median PHI of 42.9 (Nicola Fossati et al., 2015).

In the current study, multiple regression analysis indicated that PHI ($p \leq 0.001$), uPA ($p \leq 0.001$) and P2PSA ($p = 0.002$) were independent predictors of distant metastasis. Both of PHI ($p \leq 0.001$) and uPA ($p = 0.020$) were independent predictors of Gleason score, while only PHI ($p = 0.004$) was an independent predictor of LN

Table 2
Comparison of AUC for PSA derivatives and uPA as predictors for prostate cancer vs benign prostatic hyperplasia and vs healthy control men.

Parameter	PCa vs healthy control				PCa vs BPH			
	AUC	Asymptotic Significant	Asymptotic 95% Confidence Interval		AUC	Asymptotic Significant	Asymptotic 95% Confidence Interval	
			Lower	Upper			Lower	Upper
UPA	0.843	0.000*	0.757	0.930	0.779	0.000*	0.701	0.856
PSA	0.513	0.809	0.410	0.616	0.514	0.782	0.417	0.610
fPSA	0.202	0.000*	0.119	0.285	0.462	0.435	0.364	0.559
%fPSA	0.204	0.000*	0.123	0.286	0.432	0.164	0.336	0.528
P2PSA	0.724	0.000*	0.631	0.817	0.733	0.000*	0.644	0.822
%P2PSA	0.649	0.004*	0.552	0.746	0.594	0.055	0.499	0.689
PHI	0.887	0.000*	0.825	0.948	0.639	0.004*	0.546	0.733

. PHI: Prostate Health Index; PSA: prostate specific antigen; tPSA: total PSA; fPSA: free PSA; %fPSAZpercentage of free to total PSA; p2PSA: [-2]pro PSA; %p2PSA: percentage of p2PSA to fPSA ratio.

Table 3
Serum levels of PSA derivatives and uPA in relation to clinicopathological features of prostate cancer.

Mean Rank	UPA	PSA	fPSA	%fPSA	P2PSA	%P2PSA	PHI
Gleason Score	29.44	32.08	45.91	45.69	31.86	35.27	23.73
<7	46.69	42.39	19.85	20.20	42.74	37.19	56.00
≥7							
	P = 0.001*	P = 0.041*	P ≤ 0.001**	P ≤ 0.00**	P = 0.031*	P = 0.705	P ≤ 0.001**
Clinical Stage							
Early: cT1; cT2a	30.26	33.20	46.42	45.49	32.97	35.92	24.68
Late: cT2b,c; T3	42.61	39.23	24.00	25.08	39.48	36.09	49.03
	P = 0.012*	P = 0.219	P ≤ 0.001**	P ≤ 0.001**	P = 0.184	P = 0.972	P ≤ 0.001**
LN extension							
−ve	33.76	35.68	42.97	41.68	36.77	35.78	28.91
+ve	40.40	36.63	22.35	24.88	34.50	36.44	49.88
	P = 0.198	P = 0.855	P ≤ 0.001**	P = 0.001*	P = 0.661	P = 0.898	P ≤ 0.001**
Metastasis							
M0	32.60	34.83	39.37	39.49	36.11	36.83	32.21
M1	62.81	45.25	9.44	8.50	35.13	29.44	65.88
	P ≤ 0.00**	P = 0.178	P ≤ 0.001**	P ≤ 0.001**	P = 0.899	P = 0.340	P ≤ 0.001**
[RP] **Seminal vesicle**							
−ve	31.34	30.91	36.61	36.54	33.36	34.28	28.03
+ve	32.94	33.56	25.44	25.54	30.06	28.75	37.65
	P = 0.731	P = 0.571	P = 0.017	P = 0.019	P = 0.480	P = 0.238	P = 0.040*
[RP] **Surgical margin**							
R0	27.50	28.78	34.79	36.00	29.91	32.76	26.93
R1	37.28	35.78	28.72	27.31	34.45	31.10	37.95
	P = 0.034*	P = 0.130	P = 0.190	P = 0.060	P = 0.327	P = 0.720	P = 0.017*

. PHI: Prostate Health Index; PSA: prostate specific antigen; tPSA: total PSA; fPSA: free PSA; %fPSAZpercentage of free to total PSA; p2PSA: [-2]pro PSA; %p2PSA:Zpercentage of p2PSA to fPSA ratio; PSAD: PSA density; AUC: area under the receiver operating characteristic curve; OR: odds ratio; CI: confidence interval.

involvement. Several retrospective studies investigated the potential role of PHI for prediction of PCa (Cheng et al., 2019; Lazzeri, Haese, Abrate, et al., 2013; Stephan et al., 2013), advanced pathological features, aggressiveness of PCa (Chiu et al., 2016; N. Fossati et al., 2015), or pathological reclassification after one year at active surveillance (Hirama et al., 2014; Tosoian et al., 2012). Of annotation, PHI was reported to be the most precise independent predictor for pathologic Gleason score ≥ 7 and pathologic stage T3 in patients who underwent radical prostatectomy (Nicola Fossati et al., 2015). Likewise, the percentage of aggressive PCa was significantly increased linearly with the higher PHI levels (Cheng et al., 2019). Furthermore, Roobol et al. (2015) reported accuracy of 0.69 in prediction of significant PCa (patients with pathological Gleason score ≥ 7 or pathological stage T3) and accuracy of 0.75 in prediction of all PCa cases, with a PHI based monogram, adding PHI to the ERSPC calculator.

Prognostic and predictive roles of uPA in PCa have been previously investigated in clinical contexts (Gupta et al., 2009; Shariat et al., 2007) and in other *in vitro* studies (Dong et al., 2008). In prostate tissue samples from 62 patients with BPH and PCa, the mean tissue level of uPA to plasmin activator inhibitor-1 (PAI-1) in PCa samples was found to be significantly higher than in BPH samples. Therefore, the ratio of uPA /PAI-1 was considered a promising marker to discriminate between BPH and PCa (Bohm et al., 2013). In immunohistochemicall studies of prostatic tissue after RP, overexpression of uPA was significantly correlated with advanced pathological features as higher Gleason sum, LN extension, distant metastasis (Cozzi et al., 2006; Kirchheimer et al., 1985) and associated with aggressive PCa with high risk for recurrence (Gupta et al., 2009). Moreover, uPA showed an association with bone metastasis, so it may be a marker for progressive PCa and adverse outcomes (Dong et al., 2008). In the same framework

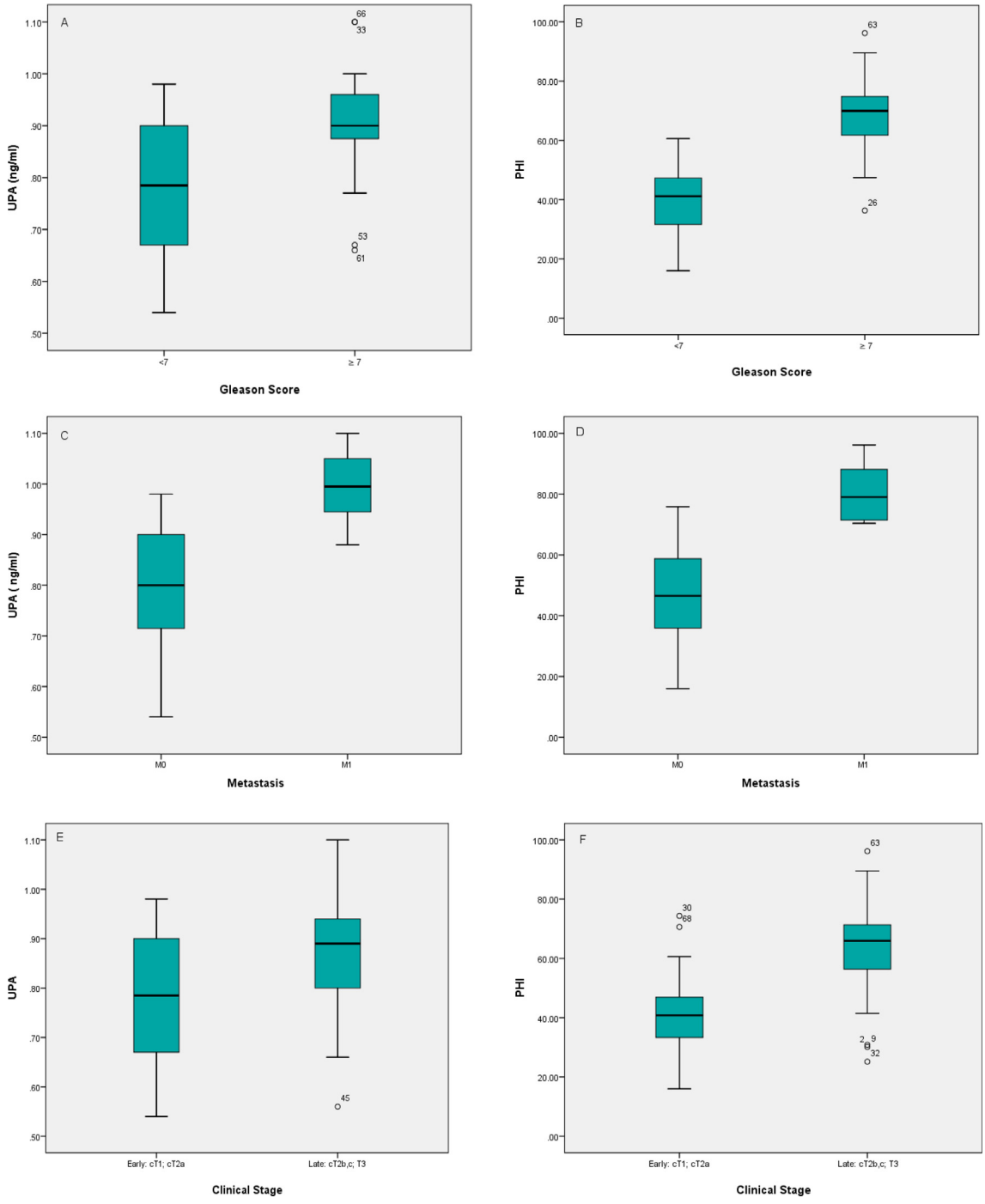

Fig. 3. The box-and-whisker plots of (A-F) UPA and PHI relative to Gleason score (A&B), metastasis (C&D) and clinical stage (E & F). Circles represent the outliers with their values; asterisks represent the extremes of outliers'. The green box illustrated the interquartile range. The bold line represents the median.

of our findings, preoperative serum levels of uPA were found to be an independent predictor of metastases and biochemical recurrence (Shariat et al., 2007).

Linking uPA with progression and metastesis of malignancies has been rationalized that once uPA binds to uPAR, it catalyzes many cellular events such as activation of plasminogen into plasmin, which activates other growth factors and metalloproteses (MMP) (Duffy, 2002). In addition, uPA has a pivotal role in angiogenesis and metastesis (Rabbani and Mazar, 2001).

There are some limitations in our study due to smaller number availabe for analysis. Future studies with larger number of patients can achive stronger conclusions. Furtherly, longer follow-up of PCa patients may reveal more useful details for better correlations.

6. Conclusion

In summary, our results demonstrated significantly higher serum levels of both PHI and uPA in patients with PCa relative to BPH and healthy control men. Furthermore, both markers outperformed other PSA derivatives (PSA, fPSA, %fPSA, P2PSA, %P2PSA) and both showed higher AUC for discrimination between PCa versus healthy controls and PCa versus BPH. In PCa patients, serum levels of uPA and PHI were significantly higher in adverse

Table 4
Multiple regression analysis of age, BMI, serum levels of PSA derivatives and uPA as independent predictors of Metastasis.

Variable	β	*P*	CI
Age	0.000	0.978	−0.012 −0.013
BMI	−0.020	0.122	−0.046 −0.006
uPA	0.856	≤0.001**	0.392–1.321
PSA	0.015	0.811	−0.108 − 0.137
fPSA	1.698	0.010	0.425–2.972
%fPSA	−3.866	0.199	−9.824–2.091
P2PSA	−0.144	0.002*	−0.232 −0.055
%P2PSA	0.404	0.085	−0.057–0.865
PHI	0.028	≤0.001**	0.019–0.036

. PHI: Prostate Health Index; PSA: prostate specific antigen; tPSA: total PSA; fPSA: free PSA; %fPSAZpercentage of free to total PSA; p2PSA: [-2]pro PSA; %p2PSA: Zpercentage of p2PSA to fPSA ratio; PSAD: PSA density; OR: odds ratio; CI: confidence interval.

Table 5
Multiple Linear regression analysis of age, BMI, serum levels of PSA derivatives and UPA as independent predictors of LN involvement.

Variable	β	*P*	CI
Age	0.002	0.840	−0.022–0.027
BMI	−0.030	0.230	−0.080– 0.020
uPA	0.042	0.927	−0.866–0.949
PSA	−0.121	0.316	−0.359–0.118
fPSA	1.251	0.318	−1.235–3.737
%fPSA	−4.344	0.458	−15.973–7.285
P2PSA	−0.083	0.339	−0.257–0.090
%P2PSA	0.155	0.732	−0.746–1.055
PHI	0.025	0.004*	0.009–0.042

. PHI: Prostate Health Index; PSA: prostate specific antigen; tPSA: total PSA; fPSA: free PSA; %fPSAZpercentage of free to total PSA; p2PSA: [-2]pro PSA; %p2PSA: Zpercentage of p2PSA to fPSA ratio; PSAD: PSA density; OR: odds ratio; CI: confidence interval; Ref: reference.

Table 6
Multiple Linear regression analysis of age, BMI, serum levels of PSA derivatives and UPA as independent predictors of Gleason score.

Variable	β	*P*	CI
Age	0.015	0.077	−0.002–0.033
BMI	0.007	0.699	−0.028–0.042
uPA	0.761	0.020*	0.126–1.396
PSA	−0.158	0.063	−0.326–0.009
fPSA	0.026	0.976	−1.714–1.766
%fPSA	3.225	0.431	−4.914–11.364
P2PSA	0.093	0.131	−0.028–0.214
%P2PSA	−0.566	0.078	−1.196–064
PHI	0.027	≤0.001**	0.016–0.39

pathological features (Gleason score ≥ 7, cT2b, c; T3; LN invasion, surgical margin; seminal vesicles extension and distant metastasis). PHI was found to be an independent predictor of Gleason score ≥ 7, LN extension and distant metastasis, while uPA was a predictor of Gleason score ≥ 7 and distant metastasis. These findings highlight the promising value of PHI and uPA in discriminating between PCa, BPH and healthy men in addition to their role in prediction of adverse pathological features. Future studies are needed for better delineation of the prospective roles of both biomarkers.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Contribution of authors

Anmar M. Nassir was involved in the design of the study, selection and clinical examination of patients. **Hala F. M. Kamel** was involved in design and methodology of the study. Both have contributed equally to the attainment, statistical analysis and interpretation of data; drafting and critically revising the article, and final approval of the manuscript.

References

Al-Janabi, O., Taubert, H., Lohse-Fischer, A., Frohner, M., Wach, S., Stohr, R.... Fussel, S., 2014. Association of tissue mRNA and serum antigen levels of members of the urokinase-type plasminogen activator system with clinical and prognostic parameters in prostate cancer 972587 Biomed. Res. Int. 2014. https://doi.org/10.1155/2014/972587.

Andreasen, P.A., Egelund, R., Petersen, H.H., 2000. The plasminogen activation system in tumor growth, invasion, and metastasis. Cell. Mol. Life Sci. 57 (1), 25–40. https://doi.org/10.1007/s000180050497.

Banys-Paluchowski, M., Witzel, I., Aktas, B., Fasching, P.A., Hartkopf, A., Janni, W.... Muller, V., 2019. The prognostic relevance of urokinase-type plasminogen activator (uPA) in the blood of patients with metastatic breast cancer. Sci. Rep. 9 (1), 2318. https://doi.org/10.1038/s41598-018-37259-2.

Berger, D.H., 2002. Plasmin/plasminogen system in colorectal cancer. World J. Surg. 26 (7), 767–771. https://doi.org/10.1007/s00268-002-4050-8.

Bohm, L., Serafin, A., Akudugu, J., Fernandez, P., van der Merwe, A., Aziz, N.A., 2013. uPA/PAI-1 ratios distinguish benign prostatic hyperplasia and prostate cancer. J. Cancer Res. Clin. Oncol. 139 (7), 1221–1228. https://doi.org/10.1007/s00432-013-1428-y.

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R.L., Torre, L.A., Jemal, A., 2018. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J. Clin. 68 (6), 394–424. https://doi.org/10.3322/caac.21492.

Bruzzese, D., Mazzarella, C., Ferro, M., Perdona, S., Chiodini, P., Perruolo, G., Terracciano, D., 2014. Prostate health index vs percent free prostate-specific antigen for prostate cancer detection in men with "gray" prostate-specific antigen levels at first biopsy: systematic review and meta-analysis. Transl. Res. 164 (6), 444–451. https://doi.org/10.1016/j.trsl.2014.06.006.

Cabarkapa, S., Perera, M., McGrath, S., Lawrentschuk, N., 2016. Prostate cancer screening with prostate-specific antigen: A guide to the guidelines. Prostate Int. 4 (4), 125–129. https://doi.org/10.1016/j.prnil.2016.09.002.

Catalona, W.J., Partin, A.W., Sanda, M.G., Wei, J.T., Klee, G.G., Bangma, C.H.... Mizrahi, I.A., 2011. A multicenter study of [-2]pro-prostate specific antigen combined with prostate specific antigen and free prostate specific antigen for prostate cancer detection in the 2.0 to 10.0 ng/ml prostate specific antigen range. J. Urol. 185 (5), 1650–1655. https://doi.org/10.1016/j.juro.2010.12.032.

Cheng, Y.T., Chiang, C.H., Pu, Y.S., Liu, S.P., Lu, Y.C., Chang, Y.K.... Huang, C.Y., 2019. The application of p2PSA% and prostate health index in prostate cancer detection: A prospective cohort in a Tertiary Medical Center. J. Formos. Med. Assoc. 118 (1 Pt 2), 260–267. https://doi.org/10.1016/j.jfma.2018.05.001.

Chiu, P.K., Lai, F.M., Teoh, J.Y., Lee, W.M., Yee, C.H., Chan, E.S.... Ng, C.F., 2016. Prostate health index and %p2PSA predict aggressive prostate cancer pathology in Chinese patients undergoing radical prostatectomy. Ann. Surg. Oncol. 23 (8), 2707–2714. https://doi.org/10.1245/s10434-016-5183-6.

Chou, R., Croswell, J.M., Dana, T., Bougatsos, C., Blazina, I., Fu, R.... Lin, K., 2011. Screening for prostate cancer: a review of the evidence for the U.S. Preventive Services Task Force. Ann. Intern. Med. 155 (11), 762–771. https://doi.org/10.7326/0003-4819-155-11-201112060-00375.

Chun, F.K., Epstein, J.I., Ficarra, V., Freedland, S.J., Montironi, R., Montorsi, F.... Scattoni, V., 2010. Optimizing performance and interpretation of prostate biopsy: a critical analysis of the literature. Eur. Urol. 58 (6), 851–864. https://doi.org/10.1016/j.eururo.2010.08.041.

Cozzi, P.J., Wang, J., Delprado, W., Madigan, M.C., Fairy, S., Russell, P.J., Li, Y., 2006. Evaluation of urokinase plasminogen activator and its receptor in different grades of human prostate cancer. Hum. Pathol. 37 (11), 1442–1451. https://doi.org/10.1016/j.humpath.2006.05.002.

Crawford, E.D., Ventii, K., Shore, N.D., 2014. New biomarkers in prostate cancer. Oncology (Williston Park) 28 (2), 135–142.

Crowley, C.W., Cohen, R.L., Lucas, B.K., Liu, G., Shuman, M.A., Levinson, A.D., 1993. Prevention of metastasis by inhibition of the urokinase receptor. Proc. Natl. Acad. Sci. U. S. A. 90 (11), 5021–5025.

Dong, Z., Saliganan, A.D., Meng, H., Nabha, S.M., Sabbota, A.L., Sheng, S.... Cher, M.L., 2008. Prostate cancer cell-derived urokinase-type plasminogen activator contributes to intraosseous tumor growth and bone turnover. Neoplasia 10 (5), 439–449.

Duffy, M. J. (2002). Urokinase-type plasminogen activator: a potent marker of metastatic potential in human cancers. Biochem. Soc. Trans., 30(2), 207-210. doi: 10.1042/

Fenton, J.J., Weyrich, M.S., Durbin, S., Liu, Y., Bang, H., Melnikow, J., 2018. Prostate-specific antigen-based screening for prostate cancer: evidence report and systematic review for the US preventive services task force. JAMA 319 (18), 1914–1931. https://doi.org/10.1001/jama.2018.3712.

Filella, X., Foj, L., 2018. Novel biomarkers for prostate cancer detection and prognosis. In: Schatten, H. (Ed.), Cell & Molecular Biology of Prostate Cancer: Updates, Insights and New Frontiers. Springer International Publishing, Cham, pp. 15–39.

Filella, X., Gimenez, N., 2013. Evaluation of [-2] proPSA and Prostate Health Index (phi) for the detection of prostate cancer: a systematic review and meta-analysis. Clin. Chem. Lab. Med. 51 (4), 729–739. https://doi.org/10.1515/cclm-2012-0410.

Foekens, J.A., Peters, H.A., Look, M.P., Portengen, H., Schmitt, M., Kramer, M.D.... Klijn, J.G., 2000. The urokinase system of plasminogen activation and prognosis in 2780 breast cancer patients. Cancer Res. 60 (3), 636–643.

Foley, R.W., Maweni, R.M., Gorman, L., Murphy, K., Lundon, D.J., Durkan, G.... William Watson, R., 2016. European Randomised Study of Screening for Prostate Cancer (ERSPC) risk calculators significantly outperform the Prostate Cancer Prevention Trial (PCPT) 2.0 in the prediction of prostate cancer: a multi-institutional study. BJU Int. 118 (5), 706–713. https://doi.org/10.1111/bju.13437.

Fossati, N., Buffi, N.M., Haese, A., Stephan, C., Larcher, A., McNicholas, T.... Lazzeri, M., 2015a. Preoperative prostate-specific antigen isoform p2PSA and its derivatives, %p2PSA and prostate health index, predict pathologic outcomes in patients undergoing radical prostatectomy for prostate cancer: results from a multicentric European prospective study. Eur. Urol. 68 (1), 132–138. https://doi.org/10.1016/j.eururo.2014.07.034.

Fossati, N., Lazzeri, M., Haese, A., McNicholas, T., de la Taille, A., Buffi, N.M.... Guazzoni, G., 2015b. Clinical performance of serum isoform [-2]proPSA (p2PSA), and its derivatives %p2PSA and the Prostate Health Index, in men aged <60 years: results from a multicentric European study. BJU Int. 115 (6), 913–920. https://doi.org/10.1111/bju.12718.

Freedland, S.J., Wen, J., Wuerstle, M., Shah, A., Lai, D., Moalej, B.... Aronson, W.J., 2008. Obesity is a significant risk factor for prostate cancer at the time of biopsy. Urology 72 (5), 1102–1105. https://doi.org/10.1016/j.urology.2008.05.044.

Gupta, A., Lotan, Y., Ashfaq, R., Roehrborn, C.G., Raj, G.V., Aragaki, C.C.... Shariat, S.F., 2009. Predictive value of the differential expression of the urokinase plasminogen activation axis in radical prostatectomy patients. Eur. Urol. 55 (5), 1124–1133. https://doi.org/10.1016/j.eururo.2008.06.054.

Herszenyi, L., Farinati, F., Cardin, R., Istvan, G., Molnar, L.D., Hritz, I.... Tulassay, Z., 2008. Tumor marker utility and prognostic relevance of cathepsin B, cathepsin L, urokinase-type plasminogen activator, plasminogen activator inhibitor type-1, CEA and CA 19–9 in colorectal cancer. BMC Cancer 8, 194. https://doi.org/10.1186/1471-2407-8-194.

Hirama, H., Sugimoto, M., Ito, K., Shiraishi, T., Kakehi, Y., 2014. The impact of baseline [-2]proPSA-related indices on the prediction of pathological reclassification at 1 year during active surveillance for low-risk prostate cancer: the Japanese multicenter study cohort. J. Cancer Res. Clin. Oncol. 140 (2), 257–263. https://doi.org/10.1007/s00432-013-1566-2.

Hoffmann, G., Pollow, K., Weikel, W., Strittmatter, H.J., Bach, J., Schaffrath, M.... Pollow, B., 1999. Urokinase and plasminogen activator-inhibitor (PAI-1) status in primary ovarian carcinomas and ovarian metastases compared to benign ovarian tumors as a function of histopathological parameters. Clin. Chem. Lab. Med. 37 (1), 47–54. https://doi.org/10.1515/CCLM.1999.007.

Hsieh, P.F., Chang, C.H., Yang, C.R., Huang, C.P., Chen, W.C., Yeh, C.C.... Wu, H.C., 2018. Prostate Health Index (PHI) improves prostate cancer detection at initial biopsy in Taiwanese men with PSA 4–10 ng/mL. Kaohsiung J. Med. Sci. 34 (8), 461–466. https://doi.org/10.1016/j.kjms.2018.02.007.

Huang, Y.Q., Sun, T., Zhong, W.D., Wu, C.L., 2014. Clinical performance of serum [-2] proPSA derivatives, %p2PSA and PHI, in the detection and management of prostate cancer. Am. J. Clin. Exp. Urol. 2 (4), 343–350.

Jansen, F.H., van Schaik, R.H., Kurstjens, J., Horninger, W., Klocker, H., Bektic, J.... Bartsch, G., 2010. Prostate-specific antigen (PSA) isoform p2PSA in combination with total PSA and free PSA improves diagnostic accuracy in prostate cancer detection. Eur. Urol. 57 (6), 921–927. https://doi.org/10.1016/j.eururo.2010.02.003.

Kirchheimer, J.C., Pfluger, H., Ritschl, P., Hienert, G., Binder, B.R., 1985. Plasminogen activator activity in bone metastases of prostatic carcinomas as compared to primary tumors. Invasion Metastasis 5 (6), 344–355.

Kumano, M., Miyake, H., Muramaki, M., Furukawa, J., Takenaka, A., Fujisawa, M., 2009. Expression of urokinase-type plasminogen activator system in prostate cancer: correlation with clinicopathological outcomes in patients undergoing radical prostatectomy. Urol. Oncol. 27 (2), 180–186. https://doi.org/10.1016/j.urolonc.2008.01.012.

Lazzeri, M., Abrate, A., Lughezzani, G., Gadda, G.M., Freschi, M., Mistretta, F.... Guazzoni, G., 2014. Relationship of chronic histologic prostatic inflammation in biopsy specimens with serum isoform [-2]proPSA (p2PSA), %p2PSA, and prostate health index in men with a total prostate-specific antigen of 4–10 ng/ml and normal digital rectal examination. Urology 83 (3), 606–612. https://doi.org/10.1016/j.urology.2013.10.016.

Lazzeri, M., Haese, A., Abrate, A., de la Taille, A., Redorta, J.P., McNicholas, T.... Guazzoni, G., 2013a. Clinical performance of serum prostate-specific antigen isoform [-2]proPSA (p2PSA) and its derivatives, %p2PSA and the prostate health index (PHI), in men with a family history of prostate cancer: results from a multicentre European study, the PROMEtheuS project. BJU Int. 112 (3), 313–321. https://doi.org/10.1111/bju.12217.

Lazzeri, M., Haese, A., de la Taille, A., Palou Redorta, J., McNicholas, T., Lughezzani, G.... Guazzoni, G., 2013b. Serum isoform [-2]proPSA derivatives significantly improve prediction of prostate cancer at initial biopsy in a total PSA range of 2–10 ng/ml: a multicentric European study. Eur. Urol. 63 (6), 986–994. https://doi.org/10.1016/j.eururo.2013.01.011.

Link, R.E., Shariat, S.F., Nguyen, C.V., Farr, A., Weinberg, A.D., Morton, R.A.... Slawin, K.M., 2004. Variation in prostate specific antigen results from 2 different assay platforms: clinical impact on 2304 patients undergoing prostate cancer screening. J. Urol. 171 (6 Pt 1), 2234–2238.

Mahmood, N., Mihalcioiu, C., Rabbani, S.A., 2018. Multifaceted role of the urokinase-type plasminogen activator (uPA) and its receptor (uPAR): diagnostic, prognostic, and therapeutic applications. Frontiers Oncology 8 (24). https://doi.org/10.3389/fonc.2018.00024.

Miyake, H., Hara, I., Yamanaka, K., Gohji, K., Arakawa, S., Kamidono, S., 1999. Elevation of serum levels of urokinase-type plasminogen activator and its receptor is associated with disease progression and prognosis in patients with prostate cancer. Prostate 39 (2), 123–129.

Ng, C.F., Chiu, P.K., Lam, N.Y., Lam, H.C., Lee, K.W., Hou, S.S., 2014. The Prostate Health Index in predicting initial prostate biopsy outcomes in Asian men with prostate-specific antigen levels of 4–10 ng/mL. Int. Urol. Nephrol. 46 (4), 711–717. https://doi.org/10.1007/s11255-013-0582-0.

Printz, C., 2012. To screen or not to screen: US Preventive Services Task Force's draft guidelines add to prostate cancer screening debate. Cancer 118 (8), 1959–1961. https://doi.org/10.1002/cncr.27559.

Rabbani, S.A., Mazar, A.P., 2001. The role of the plasminogen activation system in angiogenesis and metastasis. Surg. Oncol. Clin. N. Am., 10(2), 393-415, x.

Roddam, A.W., Duffy, M.J., Hamdy, F.C., Ward, A.M., Patnick, J., Price, C.P., ... Programme, N.H.S.P.C.R.M., 2005. Use of prostate-specific antigen (PSA) isoforms for the detection of prostate cancer in men with a PSA level of 2-10 ng/ml: systematic review and meta-analysis. Eur. Urol., 48(3), 386–399; discussion 398–389. doi: 10.1016/j.eururo.2005.04.015.

Roehrborn, C.G., Pickens, G.J., Carmody 3rd., T., 1996. Variability of repeated serum prostate-specific antigen (PSA) measurements within less than 90 days in a well-defined patient population. Urology 47 (1), 59–66.

Roobol, M.J., Vedder, M.M., Nieboer, D., Houlgatte, A., Vincendeau, S., Lazzeri, M.... Steyerberg, E.W., 2015. Comparison of two prostate cancer risk calculators that include the prostate health index. Eur. Urol. Focus 1 (2), 185–190. https://doi.org/10.1016/j.euf.2015.06.004.

Salden, M., Splinter, T.A., Peters, H.A., Look, M.P., Timmermans, M., van Meerbeeck, J. P., Foekens, J.A., 2000. The urokinase-type plasminogen activator system in resected non-small-cell lung cancer. Rotterdam Oncology Thoracic Study Group. Ann. Oncol. 11 (3), 327–332.

Semjonow, A., Kopke, T., Eltze, E., Pepping-Schefers, B., Burgel, H., Darte, C., 2010. Pre-analytical in-vitro stability of [-2]proPSA in blood and serum. Clin. Biochem. 43 (10–11), 926–928. https://doi.org/10.1016/j.clinbiochem.2010.04.062.

Seo, D.H., Yoon, S., Choi, J.H., Do, J., Lee, S.W., Lee, C.... Hyun, J.S., 2017. The correlation between body mass index and routine parameters in men over fifty. World J. Mens Health 35 (3), 178–185. https://doi.org/10.5534/wjmh.16032.

Shariat, S.F., Roehrborn, C.G., McConnell, J.D., Park, S., Alam, N., Wheeler, T.M., Slawin, K.M., 2007. Association of the circulating levels of the urokinase system of plasminogen activation with the presence of prostate cancer and invasion, progression, and metastasis. J. Clin. Oncol. 25 (4), 349–355. https://doi.org/10.1200/JCO.2006.05.6853.

Sheng, S., 2001. The urokinase-type plasminogen activator system in prostate cancer metastasis. Cancer Metastasis Rev. 20 (3–4), 287–296.

Shi, Z., Stack, M.S., 2007. Urinary-type plasminogen activator (uPA) and its receptor (uPAR) in squamous cell carcinoma of the oral cavity. Biochem. J., 407(2), 153–159. doi: 10.1042/BJ20071037.

Sokoll, L.J., Wang, Y., Feng, Z., Kagan, J., Partin, A.W., Sanda, M.G., ... Chan, D.W., 2008. [-2]proenzyme prostate specific antigen for prostate cancer detection: a national cancer institute early detection research network validation study. J. Urol., 180(2), 539–543; discussion 543. doi: 10.1016/j.juro.2008.04.015.

Stephan, C., Vincendeau, S., Houlgatte, A., Cammann, H., Jung, K., Semjonow, A., 2013. Multicenter evaluation of [-2]proprostate-specific antigen and the prostate health index for detecting prostate cancer. Clin. Chem. 59 (1), 306–314. https://doi.org/10.1373/clinchem.2012.195784.

Su, S.C., Lin, C.W., Yang, W.E., Fan, W.L., Yang, S.F., 2016. The urokinase-type plasminogen activator (uPA) system as a biomarker and therapeutic target in

human malignancies. Expert Opin. Ther. Targets 20 (5), 551–566. https://doi.org/10.1517/14728222.2016.1113260.

Tan, L.G., Tan, Y.K., Tai, B.C., Tan, K.M., Gauhar, V., Tiong, H.Y., Chiong, E., 2017. Prospective validation of %p2PSA and the Prostate Health Index, in prostate cancer detection in initial prostate biopsies of Asian men, with total PSA 4–10 ng ml-1. Asian J. Androl. 19 (3), 286–290. https://doi.org/10.4103/1008-682X.168687.

Tosoian, J.J., Loeb, S., Feng, Z., Isharwal, S., Landis, P., Elliot, D.J., Sokoll, L.J., 2012. Association of [-2]proPSA with biopsy reclassification during active surveillance for prostate cancer. J. Urol. 188 (4), 1131–1136. https://doi.org/10.1016/j.juro.2012.06.009.

Van Veldhuizen, P.J., Sadasivan, R., Cherian, R., Wyatt, A., 1996. Urokinase-type plasminogen activator expression in human prostate carcinomas. Am. J. Med. Sci. 312 (1), 8–11.

Wang, W., Wang, M., Wang, L., Adams, T.S., Tian, Y., Xu, J., 2014. Diagnostic ability of %p2PSA and prostate health index for aggressive prostate cancer: a meta-analysis. Sci. Rep. 4, 5012. https://doi.org/10.1038/srep05012.