

Review Article

Clinical performance of serum [-2]proPSA derivatives, %p2PSA and PHI, in the detection and management of prostate cancer

Ya-Qiang Huang^{1,3}, Tong Sun², Wei-De Zhong³, Chin-Lee Wu^{1,4}

¹Department of Urology, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114, USA;

²Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02215,

USA; ³Department of Urology, Guangzhou First People's Hospital, Guangzhou, Guangdong 510180, China; ⁴De-

partment of Pathology, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114, USA

Received October 17, 2014; December 9, 2014; Epub December 25, 2014; Published December 31, 2014

Abstract: Prostate-specific antigen (PSA) has been widely used as a serum marker for prostate cancer (PCa) screening or progression monitoring, which dramatically increased rate of early detection while significantly reduced PCa-specific mortality. However, a number of limitations of PSA have been noticed. Low specificity of PSA may lead to overtreatment in men who presenting with a total PSA (tPSA) level of < 10 ng/mL. As a type of free PSA (fPSA), [-2] proPSA is differentially expressed in peripheral zone of prostate gland and found to be elevated in serum of men with PCa. Two p2PSA-based derivatives, prostate health index (PHI) and %p2PSA, which were defined as $[(p2PSA/fPSA) \times \sqrt{tPSA}]$ and $[(p2PSA/fPSA) \times 100]$ respectively, have been suggested to be increased in PCa and can better distinguish PCa from benign prostatic diseases than tPSA or fPSA. We performed a systematic review of the available scientific evidences to evaluate the potentials of %p2PSA and PHI in clinical application. Mounting evidences suggested that both %p2PSA and PHI possess higher area under the ROC curve (AUC) and better specificity at a high sensitivity for PCa detection when compare with tPSA and %fPSA. It indicated that measurements of %p2PSA and PHI significantly improved the accuracy of PCa detection and diminished unnecessary biopsies. Furthermore, elevations of %p2PSA and PHI are related to more aggressive diseases. %p2PSA and PHI might be helpful in reducing overtreatment on indolent cases or assessing the progression of PCa in men who undergo active surveillance. Further studies are needed before being applied in routine clinical practice.

Keywords: PSA, prostate cancer, [-2]proPSA, %p2PSA, PHI, biopsy

Introduction

Prostate cancer (PCa) is the most common cancer and the second leading cause of cancer-related death in American men. Radical prostatectomy is considered as the first-line option for clinically localized and locally advanced PCa [1]. Prostate-specific antigen (PSA) is the most widely used serum marker for early PCa detection [2]. Use of PSA in PCa screening has revolutionized the clinical practice of PCa Detection. Large randomized clinical trials showed that PSA use lead to a significant reduction in PCa-specific mortality [3-5].

Despite outstanding performance of PSA in screening for PCa, a number of limitations

emerged, including low specificity in determining the presence of PCa and inability to discriminate between clinically significant prostate cancer (Gleason \geq 7) and indolent cancer. It is especially prominent in so-called "diagnostic grey zone", referring that with total PSA (tPSA) < 10 ng/ml, patients frequently bear both benign and malignant prostatic conditions [6, 7]. It was reported that the specificity of PSA is only 12.8% if use a cut-off of 4 ug/L [8], leading to a large number of false-positive diagnoses and up to 75% of unnecessary prostate biopsies [9]. Overdetection and subsequent overtreatment of indolent PCa was estimated as > 50% in the European screening program [10]. Several PSA derivatives, including free PSA (fPSA), percent-

Clinical significance of %p2PSA and PHI in PCa

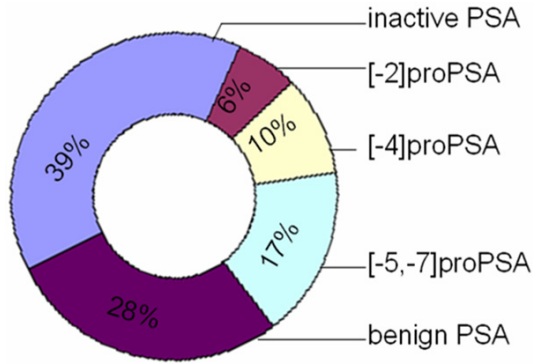


Figure 1. Composition of free PSA in the serum of prostate cancer patient. Free PSA includes inactive PSA (iPSA), benign PSA (BPSA) and precursor of PSA (proPSA). The compositions of proPSA are [-2]proPSA, [-4]proPSA, [-5]proPSA as well as [-7]proPSA. Their percentages in the serum of PCa patient were showed in the figure.

age of free to total PSA ratio (%fPSA), PSA density (PSAD) and PSA velocity (PSAV), have made a major improvement in patient selection for prostate biopsy. However, our capability to determine the presence or aggressiveness of PCa prior to prostate biopsy remains limited [11, 12]. Significant efforts have been made to explore new serum markers that can overcome PSA limitations.

Precursor of PSA (proPSA) is one of the identified forms of fPSA, which being considered as one of promising PCa serum marker candidates [13, 14]. Four different proPSA isoforms exist in serum, named as [-2]proPSA, [-4]proPSA, [-5]proPSA and [-7]proPSA by the length of the pro-leader peptide sequences is of seven, five, four or two amino acids (**Figure 1**) [5, 15, 16]. In histological analyses, proPSA was differentially expressed in the peripheral zone while undetectable in transition zone in most prostate specimen, leading to the conclusion that proPSA appears to be a more cancer specific form of PSA [13, 17]. Amino acid sequencing of whole purified PSA isolated from prostate tissues showed that the proPSA in peripheral zone cancer consisted mainly of [-2]proPSA (p2PSA) rather than other proPSA [13]. Furthermore, serum level of p2PSA was found to be high in men with PCa when compare with men without PCa [18]. p2PSA is a stable isoform of proPSA in serum as a result of the presence of only a two- amino acid propeptide, hK2 is unable to activate p2PSA into mature PSA [15]. Therefore, serum p2PSA emerged as a

promising marker for PCa detection. Afterward, the p2PSA-based Beckman Coulter Prostate Health Index (PHI), defined as $(p2PSA/fPSA) \times \sqrt{PSA}$, and %p2PSA, defined as $[(p2PSA/fPSA) \times 100]$ were developed as the important derivatives of p2PSA [5]. Preliminary investigations showed that p2PSA, %p2PSA, and PHI are higher in PCa than benign prostate conditions and their use significantly improved cancer detection compared to tPSA and %fPSA [11, 12, 19].

In this review, we discussed the clinical performances of serum p2PSA derivatives, %p2PSA and PHI, in PCa detection and management. We focused on their application in diagnosis and following up of active surveillance.

Roles of %p2PSA and PHI in the detection of prostate cancer

Improving the detection of prostate cancer

Despite unsatisfying accuracy of currently available markers such as tPSA, %fPSA, and PSAD in PCa detection, p2PSA and its derivatives have shown outstanding potentials to increase diagnostic accuracy [11]. Accumulating studies have been carried out to evaluate the performance of p2PSA and its derivatives as standard serum markers. To compare the capacity of p2PSA and its derivatives with other predictors in determining the presence of PCa at initial biopsy in patients with tPSA 2.0-10.0 ng/ml, Guazzoni G et al. conducted a prospective study involved 268 consecutive men who had negative digital rectal examination (DRE) and were scheduled for prostate biopsy. Among the subjects, 107 patients were diagnosed with PCa, the values of p2PSA, %p2PSA and PHI were substantially higher in patients with PCa than patients without PCa. In univariate accuracy analysis, PHI and %p2PSA were the most accurate predictors of PCa (AUC: 75.6% and 75.7%, respectively), followed by patient age (63%), PSAD (61%), %fPSA (58%), and tPSA (53%). In multivariate accuracy analysis, both PHI and %p2PSA significantly increased the accuracy of established diagnostic model including tPSA, %fPSA, and PSAD by 11% and 10% respectively [12]. In another multicenter study which recruited 756 patients, the highest PCa predictive value was likewise achieved by PHI and %p2PSA with significantly higher AUC. At 90% sensitivity, the specificities of PHI and

Clinical significance of %p2PSA and PHI in PCa

Table 1. The areas under the ROC curve (AUC) of predictors in predicting prostate cancer

Reference	No of patients	Study design	tPSA range (ng/ml)	Median AUC of predictors					
				tPSA	fPSA	%fPSA	p2PSA	%p2 PSA	PHI
[5]	74	prospective	2.5-10	0.50	-	0.68	-	0.76	0.77
[11]	756	retrospective	2-10	0.58	0.58	0.67	0.61	0.71	0.75
[12]	268	prospective	2-10	0.53	0.58	0.58	0.59	0.76	0.76
[20]	1362	retrospective	1.6-8.0	0.56	-	0.60	0.63	0.72	0.73
[21]	892	Prospective	2-10	0.52	0.61	0.65	0.56	-	0.70
[22]	636	prospective	abnormal	0.81	-	0.65	-	0.72	0.88
[23]	160	prospective	abnormal	0.51	0.59	0.61	0.57	0.68	0.71
[24]	222	prospective	abnormal	0.52	0.59	0.60	0.56	0.72	0.67
[28]	230	retrospective	4-10	0.55	0.54	0.57	0.65	0.77	0.78

The “-” means there is no detail data available, the “abnormal” means overall increased PSA.

Table 2. The specificity and cut-off point of predictors at 90% sensitivity for PCa detection

reference	tPSA		%fPSA		%p2PSA		PHI	
	specificity	Cut-off \geq	specificity	Cut-off \leq	specificity	Cut-off \geq	specificity	Cut-off \geq
[8]	12.8	4.00	17.9	0.26	16.2	1.21	27.4	31.90
[11]	16.5	-	22.2	-	31.8	-	34.7	-
[12]	5.1	8.90	20.0	0.29	38.8	2.18	42.9	48.50
[23]	6.0	3.60	9.0	0.32	13.0	1.04	25.0	22.80
[24]	8.6	3.23	17.9	0.24	40.4	1.23	25.2	28.80
[27]	20.4	3.66	22.0	0.23	25.3	1.06	33.3	24.9
[28]	17.2	5.25	11.0	0.28	42.1	0.99	49.7	26.54

The “-” means there is no detail data available.

%p2PSA were 31% and 33%, compared to 10% and 11% for tPSA as well as %f PSA respectively. Multivariate analysis also revealed a significant increase in PCa predictive value after adding of p2PSA and PHI into a model consisting of tPSA and fPSA (increase in AUC from 0.675 to 0.755 and from 0.581 to 0.697, respectively) [11]. These findings were consistent with the first study which evaluated the predictive ability of p2PSA and PHI in a prospective prostate cancer screening with a total PSA of 2.5 to 10 ng/ml and a negative DRE by Le BV et al.[5], and were confirmed by two recent larger size prospective multicenter study [20, 21]. These studies demonstrated that PHI and %p2PSA are superior to tPSA or %fPSA in detecting PCa at tPSA less than 10 $\mu\text{g/L}$. The similar results for PHI and %p2PSA were gotten in other prospective studies that used the overall abnormal increased tPSA range [8, 22, 23].

Besides the setting of initial biopsy, the potentials of %p2PSA and PHI in the diagnosis of prostate cancer were also tested in repeat

prostate biopsy. In a study with 222 men scheduled for re-biopsy, PCa was diagnosed in 71 subjects (31.9%). %p2PSA and PHI were significantly higher in the PCa group and better performed than tPSA, f PSA, % f PSA and p2PSA in predicting presence of PCa. There is no statistical significant difference between %p2PSA and PHI ($p = 0.094$). Multivariable logistic regression models showed %p2PSA and PHI achieved independent predictor status, and significantly increased the accuracy of multivariable models including tPSA, prostate volume, %fPSA and PSAD by 8% and 11% [24]. Later, Scattoni et al. evaluated diagnostic performance of PHI and other markers in initial or repeat prostate biopsy, results demonstrated PHI was the most accurate one in both initial setting and repeat setting [25].

In general, studies to date indicated that both PHI and %p2PSA are higher in PCa patients and significantly improve the predictive value of the currently used tests (tPSA, fPSA, %fPSA). Summary of studies for evaluating the ability of

Clinical significance of %p2PSA and PHI in PCa

PSA isoforms in PCa detection was indicated by the area under the ROC curve (AUC) and showed in **Table 1**, both PHI and %p2PSA achieved higher AUC than other markers in all summarized studies.

Increasing specificity and reducing unnecessary biopsy

One of the most important characteristics of an optimal marker for PCa screening is maintaining high sensitivity and specificity meanwhile avoiding unnecessary biopsy. Present indicators for prostate biopsy are mainly based on serum tPSA levels and DRE. In view of the unsatisfactory accuracy of these two diagnostic exams, researches have been focused on finding novel markers to improve pre-biopsy PCa detection while using biopsy as a golden standard.

Lazzeri M et al. conducted a multicentric prospective study recruited 646 patient with tPSA levels of 2.0-10.0 ng/ml and scheduled for initial biopsy, at a PHI cut-off of 27.6 and a p2PSA cut-off of 1.22, a total of 100 (15.5%) and 111 (17.0%) biopsies could have been avoided respectively, and 90% of cancers could have been detected. The specificity of PHI and %p2PSA is 19.4% and 22.8% respectively, better than that of tPSA (15.7%) [26]. In the same multicentric study involved 222 patients with 1-2 repeat biopsies, the authors reported that at a sensitivity of 90%, cutoff for %p2PSA is 1.23 and for PHI is 28.8, both of them significantly increased the specificity of tPSA and %fPSA by 31.8, 22.5 and 16.6%, 7.3% respectively. 153 (68.9%) and 116 (52.25%) biopsies could have been avoided [24]. Filella X et al. led a study with a total of 354 patients received prostate biopsy. Results showed the highest specificity at sensitivity around 90% was obtained for PHI (27.4%). Using a cut-off of 31.94 for PHI and a cut-off of 1.21 for %p2PSA, a reduction of 19% and 12.7% biopsies could be obtained respectively [8]. The results are in agreement with two other retrospective studies [27, 28].

In summary, compared with tPSA and %fPSA, use PHI and %p2PSA could get better diagnostic specificity and reduce unnecessary biopsies in PCa detection, meanwhile maintain high sensitivity. The specificity and cut-off point of each predictor at 90% sensitivity for PCa detection was summarized in **Table 2**.

Clinical use in predicting aggressiveness of prostate cancer

Currently, most commonly used assessments for PCa aggressiveness are Gleason Score and clinical tumor stage, which are subject to intra and inter-observer biases [15]. Therefore, detecting of aggressive tumors became one of the main concerns in context of PCa biomarkers.

Sokoll et al. analyzed the serum samples of 566 men (43% cancer, 57% without cancer on biopsy) who were prospectively enrolled in a multicenter study to investigate the potential correlation between p2PSA and PCa aggressiveness, they found increased p2PSA and %p2PSA were associated with increased Gleason score and worsening disease characteristics [29]. In another large multicenter study which involved 892 men who underwent biopsy, the risk of having PCa with a Gleason score ≥ 7 was 1.6-fold higher in patients PHI value above 55 compare with those with PHI less than 25 [21]. This result was subsequently confirmed by another multicenter trial with more patients, showing that PHI had the best discriminative power to detect aggressive PCa compared with tPSA or %fPSA [20].

The ability of %p2PSA and PHI in predicting PCa characteristics in final pathology reports after radical prostatectomy was also examined. Guazzoni G et al. conducted a prospective study which recruited 350 consecutive men diagnosed with localized PCa who underwent radical prostatectomy [30]. By performing tPSA, fPSA, %fPSA, p2PSA, %p2PSA and PHI measurements on preoperative serum samples of these patients, they showed that %p2PSA and PHI were significantly higher in patients with pT3 disease, pathological Gleason ≥ 7 score or those that had Gleason sum upgrading (all $P < 0.001$), suggesting that preoperative serum measurement of %p2PSA and PHI appear to be able to predict patients bearing more aggressive diseases and be useful in the preoperative counseling with patients [30]. For the similar goal, Heidegger et al. led a study with 381 patients who undergoing radical prostatectomy. They found %p2PSA levels were significantly higher in the cancer group than in the benign group 4 years before PCa was diagnosed. Patients with high p2PSA levels are at a higher risk of having aggressive PCa [31]. More recently, a study found that the cutoff level of 22.5

Clinical significance of %p2PSA and PHI in PCa

pg/ml of p2PSA can accurately distinguish organ-confined from advanced PCa [32].

The above data suggested that %p2PSA and PHI play critical roles not only in improving detection but also in predicting aggressiveness of PCa. With the further study of these aggressiveness-related biomarkers, we may be able to distinguish the clinically significant and indolent PCa preoperatively and subsequently help to decide the best treatment option for individual patient.

Performance in following up of prostate cancer undergo active surveillance

The tPSA-based PCa screening has led to the excess detection of non-aggressive PCa and subsequent overtreatment due to its low specificity. Active surveillance has been set up as a strategy to reduce the risk of overtreatment in clinically confined, low-risk PCa patients. However, patients enrolled in active surveillance program still have to receive invasive repeated prostate biopsy every 1-2 years and suffer from biopsy-related complications including pain, bleeding, rarely acute infection and even death [33]. Additionally, due to the multifocal-genesis nature of PCa and intra-tumoral heterogeneity, sometimes a conventional biopsy are not able to provide complete characteristics of the tumor [34]. In approximately 25-40% of patients who are eligible for active surveillance, the biopsy pathology underestimated tumor grade or extent resulting in improper treatment for aggressive disease [35, 36]. Thus, it is necessary to discover more reliable biomarkers to help predicting unfavorable tumor pathology in the setting of active surveillance. This may reduce the frequency of surveillance biopsies and enhance acceptance of active surveillance.

Preliminary studies showed encouraging potential clinical applications of serum p2PSA and its derivatives in active surveillance of PCa. Makarov et al. evaluated the association between serum proPSA levels and unfavorable biopsy conversion on annual surveillance examination at Johns Hopkins Hospital by using serum and prostatic biopsy samples from 71 men who under active surveillance program. They found that %p2PSA was significantly higher in men who developed unfavorable biopsies [19]. Further investigation in the same patient

cohort suggested that, when combined with biopsy tissue DNA content, the accuracy of PHI and %p2PSA in predicting unfavorable biopsy conversion were improved to 70% at annual surveillance biopsy examination [37]. Later, these findings were validated in another study including 167 men and with long-term follow up. Compared with tPSA and %fPSA, %p2PSA and PHI measurements provided the best predictive value for high-grade cancer [38]. Recently, a similar multicenter cohort study was performed in Japan, and the results were consistent with the previously findings [39].

To date, studies indicated that p2PSA and PHI have a potential role in identifying disease progress in men with PCa and enrolled in active surveillance program that might be at an increased risk of disease progression. However, the sample sizes of all these studies are relative small. More studies with larger sample size are required to conclude how PHI or %p2PSA would benefit PCa patients who under active surveillance programs, and how clinically meaningful thresholds could be defined and incorporated into the follow-up schedule appropriately.

Conclusion and prospective

Over the past decades, the use of serum PSA has significantly improved the clinical management of PCa and decreased PCa-specific mortality despite its unsatisfactory specificity and sensitivity. As the novel markers, PHI and %p2PSA have been suggested the most cancer-specific serum biomarkers in men with PCa in comparison with other currently available test (tPSA, fPSA and %fPSA), especially in patients with PSA < 10 ng/ml. Incorporating PHI or %p2PSA into fPSA and tPSA yielded more promising results in predicting PCa. More importantly, increased %p2PSA and PHI levels were strongly associated with patients harboring more aggressive diseases. These roles were validated by both prostatic biopsy and final histology reports after radical prostatectomy. Furthermore, %p2PSA and PHI demonstrated potential ability to identify the progress of low-risk localized cancer under active surveillance. PHI and p2PSA also showed potential association with probability of metastatic progression and biochemical recurrence after radical prostatectomy [40]. Studies showed that if PHI and %p2PSA were added to the current PCa

Clinical significance of %p2PSA and PHI in PCa

screening strategies, the overall reductions in cost can be achieved due to the index reduced the total number of office visits, laboratory tests and unnecessary biopsy [41].

Growing body of evidence suggested that %p2PSA and PHI are more accurate in distinguishing indolent PCa from more aggressive diseases. However, the limitations of those studies must be noticed. First, the recommended cut-off point of these indexes among those studies varied widely (**Table 2**). So far, the standard thresholds of PHI and %p2PSA to identify PCa and aggressive disease have not been set up. Secondly, for men with low-risk biopsy pathology under active surveillance, using PHI and %p2PSA as markers to monitor the progress is still at an early stage. Lastly, most studies were performed with a tPSA cut-off < 10 ng/ml, this may exaggerate the predictive accuracy of these novel markers in PCa diagnosis. Therefore, prior to use these novel markers to aid the diagnosis of PCa in routine clinical practice, more large multicentre, randomized prospective clinical trials are required.

In summary, studies suggested that both PHI and %p2PSA enhanced the accuracy of detection, reduced the number of unnecessary biopsies and helped predicting the aggressiveness of PCa when compared with tPSA and fPSA. The use of these markers might help physicians to counsel with patient and decide the optimal management.

Disclosure of conflict of interest

The authors declare that they have no competing interests.

Address correspondence to: Chin-Lee Wu, Department of Urology, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114, USA. Tel: 617-726-8454; Fax: 617-724-6564; E-mail: CWU2@PARTNERS.ORG; Wei-De Zhong, Department of Urology, Guangzhou First People's Hospital, Guangzhou, Guangdong 510180, China. E-mail: zhongwd2009@live.cn (Wei-De Zhong); hyq128@126.com (Ya-Qiang Huang)

References

- [1] Siegel R, Naishadham D, Jemal A. Cancer statistics 2013. *CA Cancer J Clin* 2013; 63: 11-30.
- [2] Smith RA, Cokkinides V, Eyre HJ. Cancer screening in the United States. 2007: a review

- of current guidelines, practices, and prospects. *CA Cancer J Clin* 2007; 57: 90-104.
- [3] Hugosson J, Carlsson S, Aus G, Bergdahl S, Khatami A, Lodding P, Pihl CG, Stranne J, Holmberg E, Lilja H. Mortality results from the Göteborg randomised population-based prostate-cancer screening trial. *Lancet Oncol* 2010; 11: 725-32.
- [4] Schröder FH, Hugosson J, Roobol MJ. Prostate-cancer mortality at 11 years of follow-up. *N Engl J Med* 2012; 366: 981-90.
- [5] Le BV, Griffin CR, Loeb S, Carvalhal GF, Kan D, Baumann NA, Catalona WJ. [-2]Proenzyme prostate specific antigen is more accurate than total and free prostate specific antigen in differentiating prostate cancer from benign disease in a prospective prostate cancer screening study. *J Urol* 2010; 183: 1355-59
- [6] Thompson IM, Pauler DK, Goodman PJ, Tangen CM, Lucia MS, Parnes HL, Minasian LM, Ford LG, Lippman SM, Crawford ED, Crowley JJ, Coltman CA Jr. Prevalence of prostate cancer among men with a prostate-specific antigen level < or = 4.0 ng per milliliter. *N Engl J Med* 2004; 350: 2239-46.
- [7] Hori S, Blanchet JS, McLoughlin J. From prostate-specific antigen (PSA) to precursor PSA (proPSA) isoforms: a review of the emerging role of proPSAs in the detection and management of early prostate cancer. *BJU Int* 2013; 112: 717-28.
- [8] Filella X, Foj L, Augé JM, Molina R, Alcover J. Clinical utility of %p2PSA and prostate health index in the detection of prostate cancer. *Clin Chem Lab Med* 2014; 52: 1347-55.
- [9] Postma R, Schröder FH. Screening for prostate cancer. *Eur J Cancer* 2005; 41: 825-33.
- [10] Draisma G, Etzioni R, Tsodikov A, Mariotto A, Wever E, Gulati R, Feuer E, de Koning H. Lead time and overdiagnosis in prostate-specific antigen screening: importance of methods and context. *J Natl Cancer Inst* 2009; 101: 374-83.
- [11] Jansen FH, van Schaik RH, Kurstjens J. Prostate-specific antigen (PSA) isoform p2PSA in combination with total PSA and free PSA improves diagnostic accuracy in prostate cancer detection. *Eur Urol* 2010; 57: 921-7.
- [12] Guazzoni G, Nava L, Lazzeri M, Scattoni V, Lughezzani G, Maccagnano C, Dorigatti F, Ceriotti F, Pontillo M, Bini V, Freschi M, Montorsi F, Rigatti P. Prostate-specific antigen (PSA) isoform p2PSA significantly improves the prediction of prostate cancer at initial extended prostate biopsies in patients with total PSA between 2.0 and 10 ng/ml: results of a prospective study in a clinical setting. *Eur Urol* 2011; 60: 214-22.
- [13] Mikolajczyk SD, Millar LS, Wang TJ, Rittenhouse HG, Marks LS, Song W, Wheeler TM, Slawin KM. A precursor form of prostate-specific

Clinical significance of %p2PSA and PHI in PCa

- ic antigen is more highly elevated in prostate cancer compared with benign transition zone prostate tissue. *Cancer Res* 2000; 60: 756-9.
- [14] Catalona WJ, Bartsch G, Rittenhouse HG, Evans CL, Linton HJ, Horninger W, Klocker H, Mikolajczyk SD. Serum pro-prostate specific antigen preferentially detects aggressive prostate cancers in men with 2 to 4 ng/ml prostate specific antigen. *J Urol* 2004; 171: 2239-44.
- [15] Jansen FH, Roobol M, Jenster G, Schröder FH, Bangma CH. Screening for prostate cancer in 2008 II: the importance of molecular subforms of prostate-specific antigen and tissue kallikreins. *Eur Urol* 2009; 55: 563-74.
- [16] Mikolajczyk SD, Rittenhouse HG. Pro PSA: a more cancer specific form of prostate specific antigen for the early detection of prostate cancer. *Keio J Med* 2003; 52: 86-91.
- [17] Mikolajczyk SD, Marks LS, Partin AW, Rittenhouse HG. Free prostate-specific antigen in serum is becoming more complex. *Urology* 2002; 59: 797-802.
- [18] Mikolajczyk SD, Marker KM, Millar LS, Kumar A, Saedi MS, Payne JK, Evans CL, Gasior CL, Linton HJ, Carpenter P, Rittenhouse HG. A truncated precursor form of prostate-specific antigen is a more specific serum marker of prostate cancer. *Cancer Res* 2001; 61: 6958-63.
- [19] Makarov DV, Isharwal S, Sokoll LJ, Landis P, Marlow C, Epstein J, Partin AW, Carter HB, Veltri RW. Pro-prostate-specific antigen measurements in serum and tissue are associated with treatment necessity among men enrolled in expectant management for prostate cancer. *Clin Cancer Res* 2009; 15: 7316-21.
- [20] Stephan C, Vincendeau S, Houlgatte A, Cammann H, Jung K, Semjonow A. Multicenter evaluation of [-2]proprostate-specific antigen and the prostate health index for detecting prostate cancer. *Clin Chem* 2013; 59: 306-14.
- [21] Catalona WJ, Partin AW, Sanda MG, Wei JT, Klee GG, Bangma CH, Slawin KM, Marks LS, Loeb S, Broyles DL, Shin SS, Cruz AB, Chan DW, Sokoll LJ, Roberts WL, van Schaik RH, Mizrahi IA. 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* 2011; 185: 1650-5.
- [22] Na R1, Ye D, Liu F, Chen H, Qi J, Wu Y, Zhang G, Wang M, Wang W, Sun J, Yu G, Zhu Y, Ren S, Zheng SL, Jiang H, Sun Y, Ding Q, Xu J. Performance of serum prostate-specific antigen isoform [-2]proPSA (p2PSA) and the prostate health index (PHI) in a Chinese hospital-based biopsy population. *Prostate* 2014; 74: 1569-75
- [23] Perdonà S, Bruzzese D, Ferro M, Autorino R, Marino A, Mazzarella C, Perruolo G, Longo M, Spinelli R, Di Lorenzo G, Oliva A, De Sio M, Damiano R, Altieri V, Terracciano D. Prostate health index (phi) and prostate cancer antigen 3 (PCA3) significantly improve diagnostic accuracy in patients undergoing prostate biopsy. *Prostate* 2013; 73: 227-35.
- [24] Lazzeri M, Briganti A, Scattoni V, Lughezzani G, Larcher A, Gadda GM, Lista G, Cestari A, Buffi N, Bini V, Freschi M, Rigatti P, Montorsi F, Guazzoni G. Serum index test %[-2]proPSA and Prostate Health Index are more accurate than prostate specific antigen and %fPSA in predicting a positive repeat prostate biopsy. *J Urol* 2012; 188: 1137-43
- [25] Scattoni V, Lazzeri M, Lughezzani G, De Luca S, Passera R, Bollito E, Randone D, Abdollah F, Capitanio U, Larcher A, Lista G, Gadda GM, Bini V, Montorsi F, Guazzoni G. Head-to-head comparison of prostate health index and urinary PCA3 for predicting cancer at initial or repeat biopsy. *J Urol* 2013; 190: 496-501
- [26] Lazzeri M, Haese A, de la Taille A, Palou Redorta J, McNicholas T, Lughezzani G, Scattoni V, Bini V, Freschi M, Sussman A, Ghaleh B, Le Corvoisier P, Alberola Bou J, Esquena Fernández S, Graefen M, Guazzoni G. 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* 2013; 63: 986-94.
- [27] Ito K, Miyakubo M, Sekine Y, Koike H, Matsui H, Shibata Y, Suzuki K. Diagnostic significance of [-2]pro-PSA and prostate dimension-adjusted PSA-related indices in men with total PSA in the 2.0-10.0 ng/mL range. *World J Urol* 2013; 31: 305-11.
- [28] Ng CF, Chiu PK, Lam NY, Lam HC, Lee KW, Hou SS. 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* 2014; 46: 711-17
- [29] Sokoll LJ, Sanda MG, Feng Z, Kagan J, Mizrahi IA, Broyles DL, Partin AW, Srivastava S, Thompson IM, Wei JT, Zhang Z, Chan DW. A prospective, multicenter, National Cancer Institute Early Detection Research Network study of [-2] proPSA: improving prostate cancer detection and correlating with cancer aggressiveness. *Cancer Epidemiol Biomarkers Prev* 2010; 19: 1193-200.
- [30] Guazzoni G, Lazzeri M, Nava L, Lughezzani G, Larcher A, Scattoni V, Gadda GM, Bini V, Cestari A, Buffi NM, Freschi M, Rigatti P, Montorsi F. 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. *Eur Urol* 2012; 61: 455-66.

Clinical significance of %p2PSA and PHI in PCa

- [31] Heidegger I, Klocker H, Steiner E, Skradski V, Ladurner M, Pichler R, Schäfer G, Horninger W, Bektic J. [-2]proPSA is an early marker for prostate cancer aggressiveness. *Prostate Cancer Prostatic Dis* 2014; 17: 70-4.
- [32] Eminaga O, Bögemann M, Breil B, Titze U, Wötzel F, Eltze E, Bettendorf O, Semjonow A. Pre-operative prostate-specific antigen isoform p2PSA \leq 22.5pg/ml predicts advanced prostate cancer in patients undergoing radical prostatectomy. *Urol Oncol* 2014; [Epub ahead of print]. doi: 10.1016/j.urolonc.2014.04.018.
- [33] Zaytoun OM, Anil T, Moussa AS, Jianbo L, Fareed K, Jones JS. Morbidity of prostate biopsy after simplified versus complex preparation protocols: assessment of risk factors. *Urology* 2011; 77: 910-4.
- [34] Cai Y, Wang J, Ren C, Ittmann M. Frequent heterogeneous missense mutations of GGAP2 in prostate cancer: implications for tumor biology, clonality and mutation analysis. *PLoS One* 2012; 7: e32708.
- [35] Tosoian JJ, JohnBull E, Trock BJ, Landis P, Epstein JI, Partin AW, Walsh PC, Carter HB. Pathological outcomes in men with low risk and very low risk prostate cancer: implications on the practice of active surveillance. *J Urol* 2013; 190: 1218-22.
- [36] Porten SP, Whitson JM, Cowan JE, Cooperberg MR, Shinohara K, Perez N, Greene KL, Meng MV, Carroll PR. Changes in prostate cancer grade on serial biopsy in men undergoing active surveillance. *J Clin Oncol* 2011; 29: 2795-800.
- [37] Isharwal S, Makarov DV, Sokoll LJ, Landis P, Marlow C, Epstein JI, Partin AW, Carter HB, Veltri RW. ProPSA and diagnostic biopsy tissue DNA content combination improves accuracy to predict need for prostate cancer treatment among men enrolled in an active surveillance program. *Urology* 2011; 77: 763-6.
- [38] Tosoian JJ, Loeb S, Feng Z, Isharwal S, Landis P, Elliot DJ, Veltri R, Epstein JI, Pa AW, Carter HB, Trock B, Sokoll LJ. Association of [-2]proPSA with biopsy reclassification during active surveillance for prostate cancer. *J Urol* 2012; 188: 1131-6.
- [39] Hirama H, Sugimoto M, Ito K, Shiraishi T, Kakehi Y. 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* 2014; 140: 257-63.
- [40] Sottile A, Ortega C, Berruti A, Mangioni M, Saponaro S, Polo A, Prati V, Muto G, Aglietta M, Montemurro F. A pilot study evaluating serum pro-prostate-specific antigen in patients with rising PSA following radical prostatectomy. *Oncol Lett* 2012; 3: 819-824.
- [41] Nichol MB, Wu J, An JJ, Huang J, Denham D, Frencher S, Jacobsen SJ. Budget impact analysis of a new prostate cancer risk index for prostate cancer detection. *Prostate Cancer Prostatic Dis* 2011; 14: 253-61.