Clinical utility of multiple secondary combined tests in prostate cancer screening

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Introduction: *The clinical utility of concurrent Prostate Health Index (PHI) and ExosomeDx Prostate Intelliscore (EPI) testing is unclear. We sought to examine the performance of combined PHI and EPI testing on men undergoing elevated PSA work up.*

Materials and methods: *Patients who received both EPI and PHI testing were identified from an institutional database of men referred to urology for an elevated total PSA. Cut points of EPI > 15.6 and PHI ≥ 36 were used to denote a positive test. Patients were placed into one of four groups determined by combination of EPI and PHI results. Demographic variables and biopsy recommendations were compared between groups. The concordance of test positivity between EPI and PHI was compared by Cohen's kappa. Demographic variables and secondary testing results were compared between patients' compliant and non-compliant with prostate biopsy recommendation. Biopsy pathology was compared between groups.*

Results: *A total of 162 patients had both EPI and PHI testing. Median age was 65 years, with a median PSA of 6.64 ng/mL. Age (p = 0.001), PSA (< 0.001) and biopsy recommendation (< 0.001) differed between combined secondary screening test result groups. Seventy-five percent of patients with both a positive EPI and PHI were found to have prostate cancer, with 54.2% being ≥ Gleason 7. Cohen's kappa was 0.19, indicating poor concordance. The AUC of EPI and PHI for clinically significant cancer was 0.563 (95% CI: 0.4331-0.6923) and 0.685 (95% CI: 0.569-0.8) (p = 0.147).*

Conclusions: *Concurrently positive EPI and PHI indicate increased prostate cancer risk, with combined usage potentially influencing biopsy recommendation and compliance.*

Key Words: prostate cancer, Prostate Health Index, ExosomeDx, screening, biomarkers

Introduction

Prostate cancer remains the second leading cause of cancer-related mortality in men.[1] Prostate cancer survival is improved with early diagnosis leading to potentially curative treatment of lethal disease; however, screening is controversial due to the risk of over-diagnosing indolent tumors.[2] Current AUA recommendation is that men aged 55-69 should be offered prostate cancer screening with prostate-specific antigen (PSA) testing with or without digital rectal exam (DRE) based on shared decision-making.[3] Though PSA is the focal point of screening, it is an imperfect biomarker with a high false positive rate.[4] There have been a number of secondary screening tests developed to improve the accuracy of prostate cancer screening.[5,6] In 2012, the Food and Drug Administration approved

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the Prostate Health Index (PHI) test (Beckman Coulter, INC. Chaska, MN, USA) to aid in prostate cancer diagnosis for patients with PSA values from 4-10 ng/mL. PHI combines total PSA, free PSA and [-2]proPSA to give risk percentage/estimate of harboring prostate cancer.[7] PHI has been associated with nearly a 3-fold improvement in prostate cancer detection compared to total PSA testing alone[8]; yet it may still be susceptible to confounders elevating the PSA level such as BPH and inflammatory conditions.

The ExoDx Prostate Intelliscore test (EPI) (Exosome Diagnostics, Waltham, MA, USA) is a noninvasive gene expression assay developed to isolate RNA from exosomes found in urine to predict the probability of clinically significant prostate cancer (Gleason ≥ 7).[9,10] Previously, EPI combined with standard of care (SOC) factors was demonstrated to be more predictive than SOC alone for differentiating clinically significant prostate cancer (Gleason ≥ 7) from Gleason 6 or benign disease when utilizing a validated cutoff score of > 15.6.[11]

While secondary tests such as PHI and EPI have individually demonstrated utility to enhance selection for prostate biopsy recommendation, it is currently unknown if utilizing multiple secondary screening assays provides additional clinical value. As PHI and EPI assess different biomarkers, it is possible that the assays may provide synergistic results with regards to cancer risk in an individual patient when used concurrently.

We sought to retrospectively review our institutional experience among patients receiving both EPI and PHI testing to assess the performance of these tests and clinical utility of concurrent screening biomarkers.

Materials and methods

Study cohort

This retrospective cross-sectional study was approved by our institutional review board. We queried the electronic health record of all patients at our institution undergoing secondary prostate cancer screening who had both PHI and EPI testing between February 2018 and January 2022. We identified and included all 162 patients who had numeric results for both biomarkers. These tests were ordered by urology providers at our institution for further evaluation of elevated total PSA. The decision to obtain secondary testing and undergo prostate biopsy was based on shared decision-making. For patients who underwent prostate biopsy, histopathologic results were included for analysis.

EPI and PHI testing

First catch urine samples were collected and submitted in compliance with established protocol to Exosome Diagnostic Laboratory (Waltham, MA, USA) to obtain the EPI score. Urine was either processed in clinic or via home testing. For EPI, the established cut point of 15.6 was used to denote a positive test; for PHI testing, a score of ≥ 36 was used to denote a positive test. Patients were then stratified into four groups for analysis by results of combined EPI and PHI testing: negative EPI and PHI, positive EPI and PHI, positive EPI with negative PHI, and positive PHI with negative EPI.

Statistical analysis

Clinical and demographic variables were compared between secondary testing result groups, categorical variables were compared with Chi-square and Fisher's exact tests and continuous variables were compared with the Kruskal-Wallis test. Post-hoc comparisons to evaluate for differences between individual testing result groups were made with the pairwise Fisher's test and Dunn's test with Holm adjustment. Variables of interest included age and PSA as continuous variables; race, prior biopsy, and biopsy recommendation were analyzed as categorical variables. Concordance of test positivity between EPI and PHI results was measured with Cohen's kappa. Sensitivity and specificity of PHI and EPI were calculated. Receiver operator characteristic (ROC) curves, area under the curve (AUC), sensitivity and specificity were evaluated for both biomarkers' performance for detecting all prostate cancers and clinically significant (> GG1) prostate cancer. The ROC curves of PHI and EPI were compared using Delong's test. Pathologic results were presented as cancer positive, Gleason Grade 6 and ≥ Gleason Grade 7; stratified by both EPI and PHI positivity, and by test score. Biopsy compliant patients were compared to non-compliant patients by Chi-square and Fisher's exact testing, with variables of interest including the previously described demographic variables and secondary test result groups as a categorical variable.

Statistical tests were two tailed, and statistical significance was defined as a p value of < 0.05. Analyses were performed using R version 4.1.1 with R studio 2021.09.0 with the following packages installed: FSA, lubridate, rstatix, psych, tidyverse, tableone, pROC.

Results

There were 162 patients with both EPI and PHI tests available for analysis. Demographic and clinical information for the overall cohort and after stratification by EPI and PHI results are available in Table 1. Median age was 65 years (IQR 59-69), with

TABLE 1. **Cohort demographics**

	Overall (n = 162)	**EPI ≤ 15.6 PHI < 36 (n = 27)**	**EPI > 15.6 PHI ≥ 36 (n = 68)**	**EPI > 15.6 PHI < 36 (n = 58)**	**EPI ≤ 15.6 PHI ≥ 36 (n = 9)**	**p value**
Age (median [IQR])	65.00 [59.00, 69.00]	62.00 [51.50, 65.50]	67.00 [63.00, 72.00]	66.50 [59.25, 69.00]	66.00 [63.00, 69.00]	0.001
PSA (median [IQR])	6.64 [4.49, 9.64]	4.42 [3.27, 5.36]	8.27 [5.88, 10.54]	5.80 [3.92, 8.18]	9.29 [6.03, 12.07]	< 0.001
Race (%)						0.154
White	118 (72.8)	22 (81.5)	49 (72.1)	41 (70.7)	6 (66.7)	
Black	38 (23.5)	2 (7.4)	18 (26.5)	15 (25.9)	3 (33.3)	
Other	6 (3.7)	3 (11.1)	1 (1.5)	2 (3.4)	0 (0.0)	
Biopsy recommended (%)	106 (65.4)	8 (29.6)	56 (82.4)	36 (62.1)	6 (66.7)	< 0.001
Prior biopsy (%)	38 (23.5)	4 (14.8)	19 (27.9)	12 (20.7)	3 (33.3)	0.454

a median PSA of 6.64 ng/mL (IQR 4.49-9.64). Of the 162 patients analyzed, 118 (72.8%) were white and 38 (23.5%) were black. Thirty-eight patients (23.5%) had a previous negative prostate biopsy.

Of the total cohort, 27/162 (16.7%) had both a negative EPI and PHI, 68/162 (41.9%) had both a positive EPI and PHI, 58/162 (35.8%) had a positive EPI and negative PHI and 9/162 (5.6%) had a positive PHI and negative EPI. Age ($p = 0.001$), PSA ($p < 0.001$) and biopsy recommendation ($p < 0.001$) were found to differ between groups. On post-hoc testing, median age was found to significantly differ between the EPI + PHI negative group (62.00, IQR: 51.50-65.50) and the EPI + PHI positive group (67.00, IQR: 64.00-72.00), and between the PHI + EPI negative group (62.00, IQR: 51.50-65.50) and positive EPI + negative PHI group (66.50, IQR:59.25-69.00). Post-hoc comparisons for median PSA found all group comparisons to be significantly different except between the PHI + EPI positive (8.27, IQR:5.88-10.54) and PHI positive + EPI negative (9.29, 6.03-12.07) groups. For biopsy recommendation, the only between group comparison found to be significantly different on post-hoc testing was between the EPI + PHI negative group (29.6%) and the EPI + PHI positive group (82.4%).

Among patients recommended for biopsy, 90/106 (84.9%) underwent biopsy. The histopathologic results stratified by secondary screening test results are available in Table 2.

Figure 1 visualizes the concordance between EPI with a cut off of > 15.6 and PHI with a cut off of ≥ 36. Cohen's kappa between EPI and PHI was estimated to be 0.19, indicating poor concordance. Overall, EPI and PHI were discordant in 67/162 (41.4%) of patients.

ROC curves for detection of clinically significant cancer and all cancer are presented in Figure 2. The AUC of EPI and PHI was 0.563 (95% CI: 0.4331-0.6923) and 0.685 (95% CI: 0.569-0.8), respectively ($p = 0.147$). For detection of all cancer the AUC of PHI and EPI was 0.668

TABLE 2. **Pathologic results of prostate biopsy stratified by secondary testing results**

	EPI ≤ 15.6 PHI < 36	**EPI > 15.6 PHI ≥ 36**	**EPI > 15.6 PHI < 36**	**EPI ≤ 15.6 PHI ≥ 36**
No. biopsies	4	48	33	5
Ca. positive (%)	1 (25.0)	36 (75.0)	16 (48.5)	3 (60.0)
Gleason 6 (%)	0 (0.0)	10 (20.8)	8 (24.2)	1 (20.0)
Gleason 7 or greater (%)	1 (25.0)	26 (54.2)	8 (24.2)	2 (40.0)

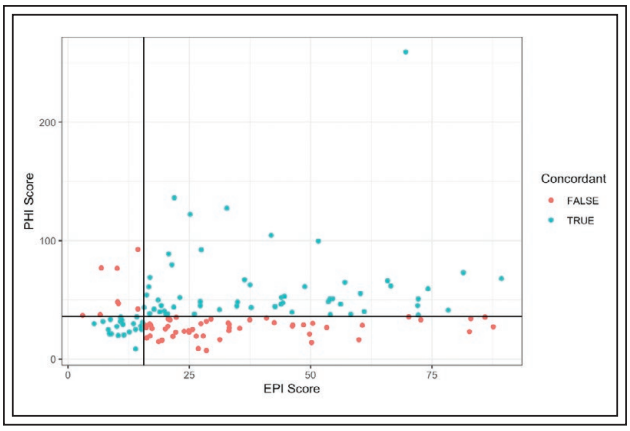

Figure 1. Scatterplot demonstrating concordance between EPI score and PHI score, at thresholds of positive test of > 15.6 for EPI (vertical line) and ≥ 36 for PHI (horizontal line) respectively. Blue dots represent concordant rest results, red dots represent non-concordant results.

(95% CI: 0.555-0.782) and 0.563 (95% CI: 0.553-0.777) (p = 0.971). The sensitivity for clinically significant disease for PHI and EPI was 0.75 and 0.91, with specificity of 0.53 and 0.11. For any cancer the sensitivity of PHI and EPI was 0.70 and 0.93, with a specificity of 0.58 and 0.14.

Only combined PHI and EPI result groups differed between biopsy-compliant and biopsy non-compliant patients (p = 0.039), Table 3. No between group comparison of screening result groups reached statistical significance on post-hoc testing for biopsy compliance.

Discussion

In the modern era of prostate cancer screening, clinicians have multiple secondary screening assays to help guide decision making. However, more testing can potentially lead to greater uncertainty for both the physician and patient, and the value of utilizing multiple screening biomarkers is unknown. To our knowledge, this study is the first to examine the performance and clinical utility of concurrent PHI and EPI as secondary prostate cancer screening tests and is the first to report cancer detection rates when utilizing these tests in elevated PSA work up. We report 75% of patients with both a positive EPI and PHI test to have prostate cancer with 54.2% of these cancers being high grade. These findings provide insight to clinicians counseling patients about cancer risk and indicate that co-biomarker usage may have a role in future prostate cancer screening paradigms. However, we have found a lack of concordance between the assays which is an

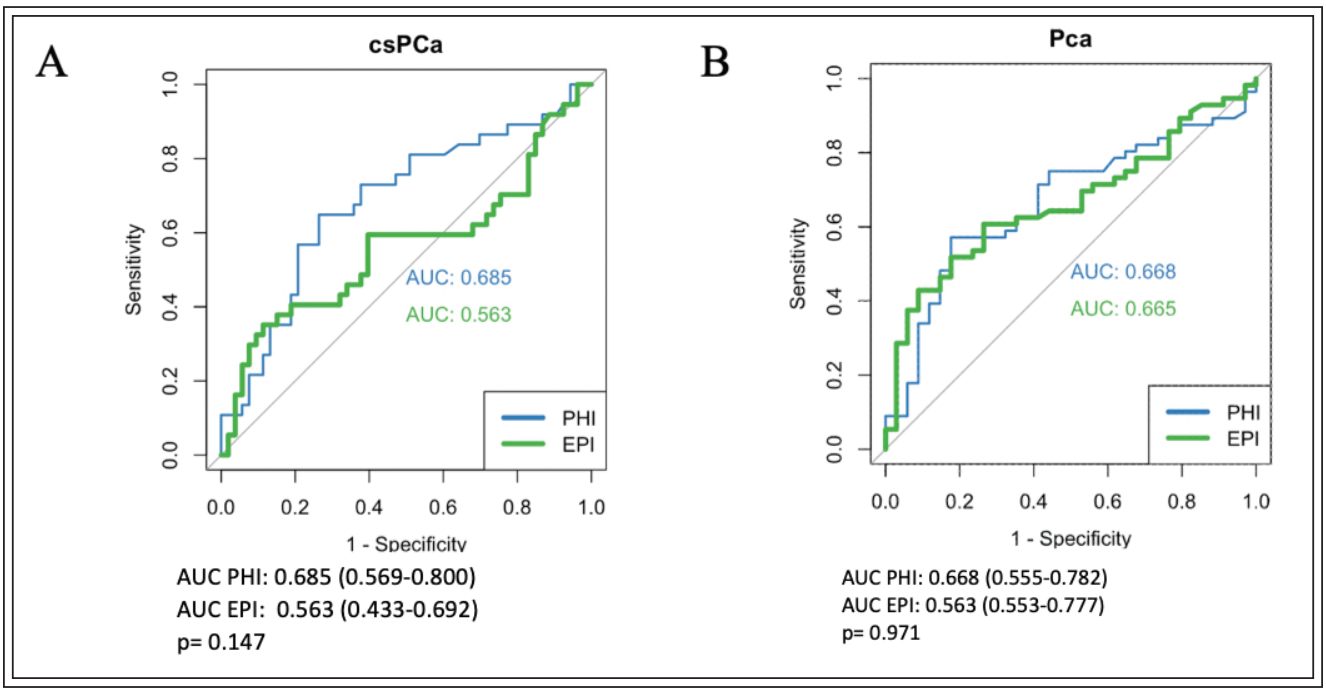

Figure 2. ROC curves for PHI and EPI for detection of **(A)** clinically significant prostate cancer and **(B)** any prostate cancer.

TABLE 3. **Comparison of biopsy compliance**

	Biopsy non-compliant (n = 16)	**Biopsy compliant (n = 90)**	**p value**
Age (median [IQR])	63.50 [58.75, 68.00]	65.50 [59.25, 70.00]	0.344
PSA (median [IQR])	8.34 [4.66, 10.07]	7.19 [5.42, 10.42]	0.958
Prior biopsy (%)	4 (25.0)	18 (20.0)	0.739
Race (%)			0.43
White	12 (75.0)	61 (67.8)	
Black	3 (18.8)	26 (28.9)	
Other	1 (6.2)	3 (3.3)	
EPI > 15.6 (%)	11 (68.8)	81 (90.0)	0.056
PHI ≥ 36 (%)	9 (56.2)	53 (58.9)	1
Secondary testing results			0.039
EPI ≤ 15.6, PHI < 36	4 (25.0)	4 (4.4)	
PI > 15.6, PHI ≥ 36	8 (50.0)	48 (53.3)	
EPI > 15.6, PHI < 36	3 (18.8)	33 (36.7)	
EPI ≤ 15.6, PHI ≥ 36,	1 (6.2)	5 (5.6)	

important practical consideration when evaluating the results of these tests.

PHI has been demonstrated to aid in prostate cancer detection,[12,13] and be a cost-effective screening tool.[14] Notably, PHI does not stratify results by risk of clinically significant disease and has been recommended for use in conjunction with other clinical factors to aid in decision making.[15] Nonetheless, prior studies have demonstrated that PHI can have utlity in detection of clinically signifant cancer. Loeb et al found the AUC for Phi to detect Gleason 7 or greater cancer to be 0.707 (0.655- 0.729), significantly greater than any of the component biomarkers of PHI.[12] Tosoian et al found PHI to have an AUC of 0.767 (0.681-0.852) for detection of ≥ Grade Group 2 cancer.[16] We report an AUC of 0.685 for clinically significant disease, in line with prior studies. Our study shows a trend of both increased overall cancer positivity and clinically significant cancer positivity with increased PHI, among both EPI positive and negative patients.

EPI has shown promise in providing a noninvasive option for high-grade prostate cancer detection and is unlike other screening assays as it does not rely on clinical features.[10] Margolis et al found that EPI could have avoided 23% of biopsies and found a negative predictive value of 90% and an AUC of 0.70 (0.67- 0.73) for ≥ GG2 cancer.[17] A potential limitation of EPI is its PPV which was found to be 36.4% at the cut-point of 15.6 in a pooled meta-analysis of multiple validation studies.[17] When faced with a negative EPI result, the high NPV of the test can reassure patients to avoid biopsy, however with a positive result there is room for improved risk-stratification of whom ultimately needs biopsied. Our trend towards increasing clinically significant prostate cancer rates with increasing PHI score among EPI positive patients indicates that PHI could potentially fill this role, although larger prospective trials are needed. Novel biomarkers, including EPI, have been previously evaluated for use in screening algorithms to stratify patients who should undergo mpMRI,[18] but further evaluation of the optimal timing of when and which screening tests should be used in decisions for imaging and biopsy is necessary. Furthermore, EPI testing may improve patient compliance with recommended biopsy. Tutrone et al found a biopsy compliance rate of 72% among patients with an EPI score > 15.6 and 56% among patients with an EPI ≤ 15.6 when EPI results were made available for decision making.[19] Our findings show that combined results of PHI and EPI may influence patients' compliance with recommended biopsy, but larger randomized trials are necessary to account for the many aspects of patient compliance that were unable to be captured in this retrospective study.

While we have found poor concordance between EPI and PHI, this result may not be surprising as the assays utilize different biomarkers and were designed with different functional endpoints. PHI utilizes PSA and PSA derivatives, providing a risk of finding any prostate cancer on biopsy.[7] EPI utilizes exosome RNA expression of PCA3, ERG and SPDEF,

non-PSA biomarkers, and is designed to provide risk of Gleason 7 or greater cancer.[19] With these differences taken into consideration, the concordance of these tests was felt to be important to report as it highlights the potential clinical scenarios faced when ordering these tests concurrently. For a test to provide actionable information in practice, the outcome of the test should affect clinical decision making. In the case of concurrent testing of EPI and PHI, the result of each test must be considered both independently and combined. As we have shown, these tests will often produce discordant results and the ordering clinician must take this into account when considering the necessity of ordering both tests. These results expand on prior work comparing the concordance of urine and blood biomarkers; de la Calle et al found 4Kscore and EPI to be concordant in 37/62 (59.7%) of patients.[18]Additionally, 4kScore and SelectMDx were shown to be discordant in 46.5% of patients at the 7.5% clinically significant prostate cancer predicted detection rate.[20] These discordance rates may reflect differing tumor biology, and further study into which test is right for an individual patient is warranted.

While our study represents a novel experience of concurrent EPI and EPI testing for prostate cancer screening, we do recognize several limitations. This was a real-world evaluation of EPI and PHI usage in our practice but lacked a true control arm. Similarly, there may be selection bias related to which men were offered secondary screening tests and who ultimately underwent biopsy. Our cohort was limited by its sample size, and any findings require validation in larger, ideally prospective studies. While PHI is reported as a 4-tier outcome based on score, reflecting probability of prostate cancer present: 0-26.9, 27-35.9, 36-54.9, and 55.0+, representing a prostate cancer risk of 9.8%, 16.8%, 33.3%, and 50.1%, respectively; we felt using a PHI cut-point of ≥ 36 to denote a positive test to be a clinically relevant grouping as these patients are more likely to harbor cancer and require biopsy. Interestingly, we found cancer positivity rates were higher than expected for PHI risk category among patients with a positive EPI score. This study did not factor other patient characteristics, such as family history, socioeconomic status or other comorbidities that may influence compliance with recommenced biopsy. We included both biopsy-naïve patients and those with prior negative biopsy, as this represented a real-world utilization of these tests. Patients were seen at a tertiary care center, which may affect generalizability of our findings. Nonetheless, we do report a novel experience with combined PHI and EPI testing and provide groundwork for further investigation of optimal prostate cancer screening.

Conclusion

Patients with concurrently positive PHI and EPI had increased clinically significant cancer detection rates, however there was poor concordance of test positivity between the two biomarkers.

Disclosures

JM has received compensation for speaking/consulting from: Exosome Diagnostics, Dendreon, Janssen, Sanofi, Myovant, Astellas, Bayer, Lantheus, Theralogix and Accord Biosciences. TP has received compensation for speaking/consulting from AngioDynamics and Progenics Pharmaceuticals. All other authors report no disclosures. □

References

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *CA: Cancer J Clin* 2022;72 (1):7-33.
2. Hugosson J, Roobol MJ, Månsson M et al. A 16-yr follow-up of the European randomized study of screening for prostate cancer. *Eur Urol* 2019;76(1):43-51.
3. Carter HB, Albertsen PC, Barry MJ et al. Early detection of prostate cancer: AUA guideline. *J Urol* 2013;190(2):419-426.
4. Kilpeläinen TP, Tammela TL, Roobol M et al. False-positive screening results in the European randomized study of screening for prostate cancer. *Eur J Cancer* 2011;47(18):2698-2705.
5. Moul JW. Secondary screening tests for prostate cancer: is more information better? Which test is best? *Can J Urol* 2020; 27(1):10086.
6. Haese A, Trooskens G, Steyaert S et al. Multicenter optimization and validation of a 2-gene mRNA urine test for detection of clinically significant prostate cancer before initial prostate biopsy. *J Urol* 2019;202(2):256-263.
7. Loeb S, Catalona WJ. The Prostate Health Index: a new test for the detection of prostate cancer. *Ther Adv Urol* 2014;6(2):74-77.
8. White J, Shenoy BV, Tutrone RF et al. Clinical utility of the Prostate Health Index (phi) for biopsy decision management in a large group urology practice setting. *Prostate Cancer Prostatic Dis* 2018;21(1):78-84.
9. Nilsson J, Skog J, Nordstrand A et al. Prostate cancer-derived urine exosomes: a novel approach to biomarkers for prostate cancer. *Br J Cancer* 2009;100(10):1603-1607.
10. McKiernan J, Noerholm M, Tadigotla V et al. A urine-based Exosomal gene expression test stratifies risk of high-grade prostate cancer in men with prior negative prostate biopsy undergoing repeat biopsy. *BMC Urol* 2020;20(1):138.
11. McKiernan J, Donovan MJ, Margolis E et al. A prospective adaptive utility trial to validate performance of a novel urine Exosome gene expression assay to predict high-grade prostate cancer in patients with prostate-specific antigen 2–10ng/ml at initial biopsy. *Eur Urol* 2018;74(6):731-738.

12. Loeb S, Sanda MG, Broyles DL et al. The prostate health index selectively identifies clinically significant prostate cancer. *J Urol* 2015;193(4):1163-1169.
13. Zhang G, Li Y, Li C et al. Assessment on clinical value of prostate health index in the diagnosis of prostate cancer. *Cancer Med* 2019;8(11):5089-5096.
14. Huang D, Yang X, Wu Y et al. Cost-effectiveness analysis of Prostate Health Index in decision making for initial prostate biopsy. *Front Oncol* 2020;10:565382
15. Press B, Schulster M, Bjurlin MA. Differentiating molecular risk assessments for prostate cancer. *Rev Urol* 2018;20(1):12-18.
16. Tosoian JJ, Druskin SC, Andreas D et al. Use of the Prostate Health Index for detection of prostate cancer: results from a large academic practice. *Prostate Cancer Prostatic Dis* 2017;20(2): 228-233.
17. Margolis E, Brown G, Partin A et al. Predicting high-grade prostate cancer at initial biopsy: clinical performance of the ExoDx (EPI) Prostate Intelliscore test in three independent prospective studies. *Prostate Cancer Prostatic Dis* 2022;25(2): 296-301.
18. de la Calle CM, Fasulo V, Cowan JE et al. Clinical utility of 4Kscore(®), ExosomeDx™ and magnetic resonance imaging for the early detection of high grade prostate cancer. *J Urol* 2021; 205(2):452-460.
19. Tutrone R, Donovan MJ, Torkler P et al. Clinical utility of the exosome based ExoDx Prostate(IntelliScore) EPI test in men presenting for initial Biopsy with a PSA 2-10 ng/mL. *Prostate Cancer Prostatic Dis* 2020;23(4):607-614.
20. Wysock JS, Becher E, Persily J, Loeb S, Lepor H. Concordance and performance of 4Kscore and SelectMDx for informing decision to perform prostate biopsy and detection of prostate cancer. *Urology* 2020;141:119-124.