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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 2–10 ng/ml at Initial Biopsy

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Abstract

Background: Discriminating indolent from clinically significant prostate cancer (PCa) in the initial biopsy setting remains an important issue. Prospectively evaluated diagnostic assays are necessary to ensure efficacy and clinical adoption.
Objective: Performance and utility assessment of ExoDx Prostate (IntelliScore) (EPI) urine exosome gene expression assay versus standard clinical parameters for discriminating Grade Group (GG) ≥2 PCa from GG1 PCa and benign disease on initial biopsy.
Design, setting, and participants: A two-phase adaptive clinical utility study (NCT03031418) comparing EPI results with biopsy outcomes in men, with age ≥50 yr and prostate-specific antigen (PSA) 2–10 ng/ml, scheduled for initial prostate biopsy. After EPI performance assessment during phase I, a clinical implementation document (ie, CarePath) was developed for utilizing the EPI test in phase II, where the biopsy decision is uncertain.
Outcome measurements and statistical analysis: Performance evaluation of the EPI test in patients enrolled in phase I and publication of a consensus CarePath for phase II.
Results and limitations: In a total of 503 patients, with median age of 64 yr, median PSA 5.4 ng/ml, 14% African American, 70% Caucasian, 53% positive biopsy rate (22% GG1, 17% GG2, and 15% ≥ GG3), EPI was superior to an optimized model of standard clinical parameters with an area under the curve (AUC) 0.70 versus 0.62, respectively, comparable with previously published results (n = 519 patients, EPI AUC 0.71). Validated cut-point 15.6 would avoid 26% of unnecessary prostate biopsies and 20% of total biopsies, with negative predictive value (NPV) 89% and missing 7% of ≥GG2 PCa. Alternative cut-point 20 would avoid 40% of unnecessary biopsies and 31% of total biopsies, with NPV 89% and missing 11% of ≥GG2 PCa. The clinical investigators reached consensus recommending use of the 15.6 cut-point for phase II. Outcome of the decision impact cohort in phase II will be reported separately.
Conclusions: EPI is a noninvasive, easy-to-use, gene expression urine assay, which has now been successfully validated in over 1000 patients across two prospective validation trials to stratify risk of ≥GG2 from GG1 cancer and benign disease. The test improves identification of patients with higher grade disease and would reduce the total number of unnecessary biopsies.
Patient summary: It is challenging to predict which men are likely to have high-grade prostate cancer (PCa) at initial biopsy with prostate-specific antigen 2–10 ng/ml. This study further demonstrates that the ExoDx Prostate (IntelliScore) test can predict ≥GG2 PCa at initial biopsy and defer unnecessary biopsies better than existing risk calculator's and standard clinical data.

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1. Introduction

There are over a million prostate needle biopsies performed in the USA including repeat biopsies every year, mostly as a direct result of fluctuating or increasing levels of prostate-specific antigen (PSA) [1]. Unfortunately, the low specificity of PSA for prostate cancer (PCa) has led to an increase in either benign unnecessary biopsies or the diagnosis of clinically indolent tumors, which subject approximately 50% of men to some form of (over) treatment including surgery, radiation, or additional biopsies as part of an active surveillance program [1–5]. Furthermore, the biopsy procedure is not completely benign, with an increasing incidence of infections (3–5%) and the potential for serious complications requiring hospitalization [6].

In 2012, the United States Preventive Services Task Force (USPSTF) recommended against PSA screening [7]; however, to avoid missing clinically significant PCa, they produced an updated draft recommendation in 2017 to promote age-specific shared-decision PSA testing for men aged 55–69 yr (https://screeningforprostatecancer.org). The emphasis on "smart screening" has produced a variety of PSA-derived blood tests (eg, 4Kscore, phi) and post-digital rectal exam (DRE) urine-based gene assays (eg, MiProstate [MiPS], SelectMDx). However, these tests have been developed in cohorts largely outside the PSA 2–10 ng/ml grey zone population of biopsy-naïve patients, and for some requiring inclusion of PSA levels and other clinical standard of care variables in their test algorithm (eg, 4Kscore, MiPS, SelectMDx) to generate respective risk scores [8–13].

We have previously published on the development of a urine exosome gene expression test, ExoDx Prostate (IntelliScore) (EPI), which utilizes exosomal RNA expression levels of three genes to predict the likelihood of having high-grade PCa (HGPCa) of Grade Group (GG) 2 or greater [14]. To our knowledge, EPI is the only test for this indication, with the exception of multiparametric magnetic resonance imaging (mpMRI) that does not incorporate PSA or a PSA derivative in the test algorithm. As such, EPI is a "standalone" test that does not rely on any other parameters to calculate the score compared with the exosomal RNA markers measured in the urine sample. This allows clinicians to use the test result in conjunction with other clinical variables, including clinical nomograms, mpMRI, or standard of care risk calculators (RCs). The EPI test was previously validated in 519 men from 22 community practice and academic urology clinic sites in the USA, all in the intended use population of 50 yr or older with PSA values 2–10 ng/ml presenting for their initial biopsy [14,15]. The validation study demonstrated good assay performance with a pre-determined cut-point (15.6) yielding a negative predictive value (NPV) >90%, and a sensitivity of 92%, with 27% of patients having an EPI score below the cut-point [14]. Using an alternative cut-point of 20, the assay missed 6% of ≥GG3 PCas and would have potentially avoided 37% of biopsies.

To further assess the performance of the EPI test in the intended use population, we initiated a two-phase, prospective adaptive decision impact trial (NCT03031418), with phase I constituting a second validation cohort of 500 patients leading to a cut-point recommendation and agreement among the principal investigators on a clinical implementation plan (ie, a "CarePath"). The CarePath will be used in a subsequent phase II 500-patient cohort consisting of men aged ≥50 yr with a PSA 2–10 ng/ml for which the decision to perform an initial biopsy is uncertain. We now report on the performance of EPI in cohort 1 and the outcome of the clinical investigator CarePath consensus. Outcome of the second utility phase of the study will be reported separately.

2. Patients and methods

2.1. Study setting and population

We report on the second validation of a prospective, two-cohort, adaptive clinical implementation and utility study of the EPI urine exosome gene expression assay comparing EPI results with biopsy outcomes. Eligible participants had not been diagnosed with PCa, were aged ≥50 yr with a PSA 2–10 ng/ml, and scheduled for their initial prostate needle biopsy. After completion of cohort 1, a clinical implementation document (ie, CarePath) was developed for utilizing the EPI score in a second phase patient cohort, where the biopsy decision is uncertain.

2.2. Study design

This report constitutes the planned prospective phase I analysis correlating the EPI test result with biopsy outcomes on 500 eligible patients, cohort 1, (ie, age of ≥50 yr, PSA 2–10 ng/ml, and scheduled for initial biopsy) in the multisite, expanded validation adaptive utility trial (NCT03031418). At the time of this phase I analysis, 14 clinical sites in the USA participated from May 2016 to August 2017 (Supplementary Table 1). All cohort 1 patients underwent an EPI test independent to the prostate needle biopsy outcome. All personnel involved in operational activities of the validation studies including execution of the EPI test were blinded to the outcome. We have adopted the more patient-centric prognostic GGs developed at Johns Hopkins Hospital and endorsed by the International Society of Urological Pathology: GS2-6 = GG1, GS 3 + 4 = GG2, GS4 + 3 = GG3, GS8 = GG4 and GS9-10 = GG5 [15–17]. Clinical features (ie, PSA, age, race, DRE, and family history) available at the time of enrollment were obtained. Men with a history of a prior biopsy, invasive treatment for benign prostatic disease, or taking medications that influence serum PSA levels within 6 mo were excluded. Men with active prostatitis on antibiotics were also excluded. Pathologic examination of biopsies, blinded to EPI test result, was performed by urologic pathologists at each study site.

The study protocol was approved by local institutional review boards (IRBs); all participants provided written informed consent and were not compensated for participating. The protocol and statistical analysis plan were agreed upon by all investigators prior to patient enrollment and study data collection. Analyses of test results with biopsy outcomes including performance of the previously validated and alternative cut-points were evaluated by the principal investigators and pre-identified external content experts. A consensus opinion, CarePath, was developed for using the EPI test in the phase II population, where the biopsy decision is uncertain.

2.3. Assay methods

2.3.1. EPI test

EPI is a non-DRE urine-based liquid biopsy test indicated for men aged ≥50 yr with a PSA 2–10 ng/ml being considered for an initial prostate

needle biopsy. All sites received a urine collection vessel and shipping kits. First-catch 15–20 ml urine samples were collected from all enrolled patients and stored at 4 °C for up to 5 d prior to shipping to a central laboratory (Exosome Diagnostics, Inc, Waltham, MA) for EPI test analysis. Description of methods used in exosome isolation, RNA extraction, and reverse transcriptase polymerase chain reaction have been analytically validated and previously published [13,14]. Briefly, the test result is calculated based on the relative gene expression of three genes, without inclusion of other clinical parameters, and provides a risk score (scale 0–100) that predicts the presence of HGPCa (≥GG2). Men with a score ≥15.6 (or 20) are at increased risk for having HGPCa on a subsequent biopsy.

2.4. Statistical methods

One of the primary objectives of this scheduled phase I analysis was to perform a second validation of the EPI test for predicting HGPCa on initial biopsy for men with a PSA 2–10 ng/ml in a prospective, multisite trial. Besides EPI alone, logistic regression models from McKiernan et al. [14] with a standard of care model (SOCm, including PSA level, age, race, and family history), with (SOCm + EPI) and without (SOCm) the EPI score, were used to predict the biopsy result for HGPCa (ie, biopsy negative and GG1 vs ≥GG2). We also employed both the Prostate Cancer Prevention Trial (PCPT 2.0) and European Randomized Study of Screening for Prostate Cancer (ERSPC) RCs to further characterize the investigated cohort for risk of PCa using existing standard of care clinical parameters [18,19]. As prostate volume is not routinely provided in the enrolled patient populations, we used the ERSPC RC which incorporates PSA and DRE for men without previous biopsy [20]. Receiver operating characteristics (ROCs) for all models assessed clinical performance. Missing DRE results were imputed as nonsuspicious.

Cohort 1 second validation results were compared with performance of the previously published validation cohort utilizing area under the curve (AUC) of the ROC, assessment of the cut-point sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV). Analyses were performed as outlined above. Where applicable, confidence intervals were calculated using Clopper-Pearson method. DeLong's test was applied to assess the significance of AUC differences between analyses. The clinical value of the EPI test was assessed with a decision curve analysis to evaluate the net health benefit of the urine exosome gene expression assay for predicting HGPCa.

2.5. Consensus conference

After completion of phase I of the trial, a panel of clinicians and risk modeling experts (J.M., M.J.D., E.M., A.P., B.C., G.B., N.S., G.A., R.E., I.T. and P. C.) convened at a Consensus Conference to review the performance of the EPI test in cohort 1 and to compare the results with previous validation study results using both the validated and alternative cut-points of 15.6 and 20, respectively. A consensus recommendation was reached and documented in an EPI clinical CarePath, which was submitted for IRB approval for use in the decision impact phase II of the trial.

3. Results

3.1. Study population

Of the 532 patients enrolled in cohort 1, 503 were evaluable; two patients were removed for PSA >10 ng/ml, one patient was aged <50 yr, two had urine volumes <15 ml, and 26 patients (5%) had gene expression and/or internal quality control levels outside assay acceptance limits. Four sites enrolled >70% of the patients representing three community practice centers (Urology Center of Englewood New Jersey, USA; Delaware Valley Urology, New Jersey, USA; and Associated Urologists of North Carolina, USA) and one large academic medical center (Johns Hopkins University, Baltimore, MD, USA; see Supplementary materials for complete trial site list and patient enrollment). The participating clinical sites between the original and current validation studies were comparable with respect to geography and general patient characteristics. Although 65.5% of patients in the original validation study and 57.3% of patients in the present cohort 1 were enrolled at the same urology centers, this trial represents a second, independent prospective study. The present study was performed 2 yr after the original study, following separate collection protocols and was not an extension of the first. In the original validation study, 6% of patients were enrolled at academic sites in contrast to 22% in the present study. In both studies, the remaining patients were enrolled at local community practices, reflecting real-world experience in the USA. The median age was 64 yr, with a median PSA of 5.4 ng/ml; 14% of patients self-reported a positive family history of PCa and 70% identified as Caucasian with 14% African American, 4% Asian, 5% Hispanic, and 7% not available or other. DRE was nonsuspicious in 75%, suspicious in 12.5%, and not available in 12.5%. MRI-guided biopsies were performed in a minority (9.3%) of patients in the current study. Complete demographic and clinical characteristics of final evaluable cohort are summarized in Table 1.

Table 1 – Demographic and clinical characteristics of cohort 1

	Registration cohort
Total	503
Age median, IQR	64 (59–69)
PSA median, IQR	5.4 (4.4–6.7)
Family history, *n* (%)	
Yes	72 (14.3)
No	262 (52.1)
NA	169 (33.6)
Ethnicity, *n* (%)	
African American	71 (14.1)
Asian/Pacific Islander	18 (3.6)
Caucasian	350 (69.6)
Hispanic	25 (5)
Native American	2 (0.4)
Other	25 (5)
NA	12 (2.4)
DRE, *n* (%)	
Nonsuspicious	379 (75.3)
Suspicious	63 (12.5)
NA	61 (12.1)
Grade Group, *n* (%)	
Benign	234 (46.5)
GG 1 (ISUP1, GS3 + 3)	111 (22.1)
GG 2 (ISUP2, GS3 + 4)	86 (17.1)
GG 3 (ISUP3, GS4 + 3)	26 (5.2)
GG 4 (ISUP4, all GS8)	26 (5.2)
GG 5 (ISUP5, >GS8)	20 (4)
≥GG 3 (ISUP3, GS4 + 3)	72 (14.3)

DRE = digital rectal exam; GG = Grade Group; GS = Gleason score; IQR = interquartile range; ISUP = International Society of Urological Pathology; PSA = prostate-specific antigen.

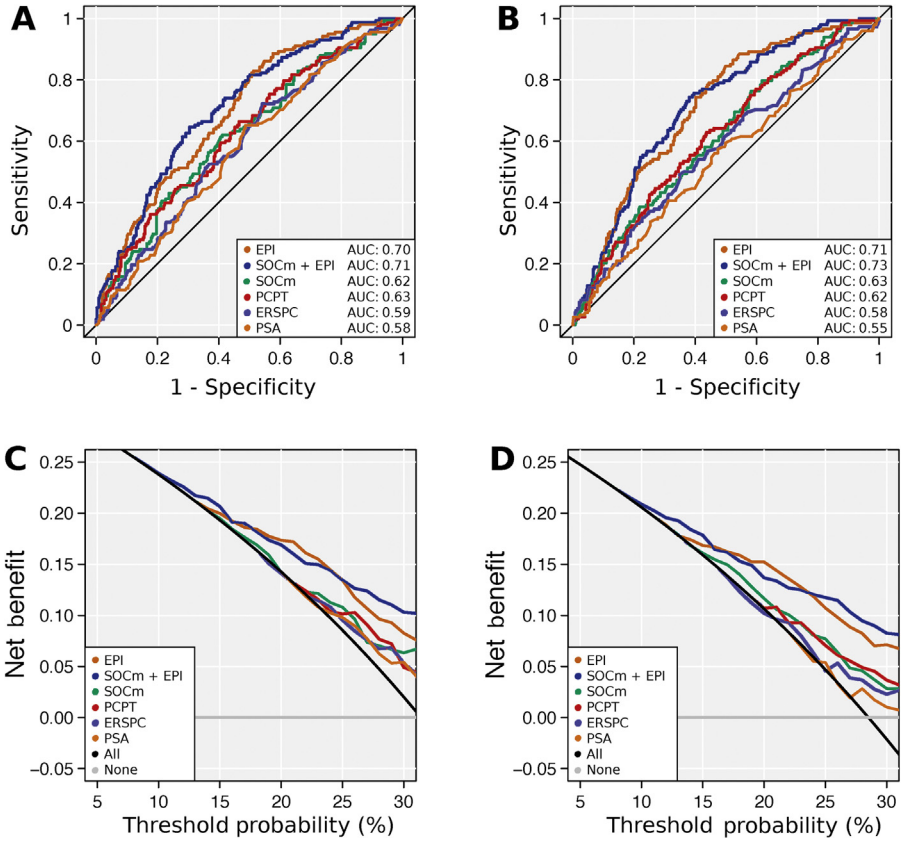

Fig. 1 – Area under receiver operating characteristic (AUC) curves are shown to compare performances of EPI in (A) the current cohort (n = 503) and (B) the previous validation cohort (n = 519) with and without the SOCm, PCPT-RC, ERSPC-RC, and PSA alone. The corresponding net benefit analysis for the two cohorts is shown (C) for current validation cohort and (D) for previous validation cohort.
EPI = ExoDx Prostate (IntelliScore); ERSPC-RC = European Randomized Study of Screening for Prostate Cancer risk calculator; PCPT-RC = Prostate Cancer Prevention Trial risk calculator; PSA = prostate-specific antigen; SOCm = standard of care model.

3.2. *Biopsy outcome (GG classification)*

Of the 503 patients, 88% underwent a 12-core, transrectal ultrasound-guided prostate needle biopsy with diagnosis performed at individual site-designated pathology practices. There was no central pathology diagnosis rendered during the trial. The total positive biopsy rate was 53%: 22% GG1 and 31% ≥GG2 (Table 1). Although PSA and age were quite comparable between the previous validation study and the current cohort (year 2014 vs 2016), there was a 5% increase in the positive biopsy rate (48% to 53%) and a 3% increase in the diagnosis of ≥GG2 PCa (28% to 31%). Of possible significance, the ≥GG3 was slightly increased (12% vs 14%).

3.3. *EPI as a predictor of ≥GG2 PCa*

On comparing the performance of the EPI test with alternative models, the EPI test was superior to SOCm, ERSPC-RC, PCPT-RC, and PSA alone for predicting ≥GG2 PCa in both the second and first validation cohort (Fig. 1A and 1B; Table 2). The DeLong test comparing differences between AUC curves further demonstrated good independent performance of the EPI test.

Table 2 – Performance comparison (AUC, 95% CI) of the EPI test to alternative models and DeLong test for significance

Test/model	Current cohort (n = 503)		Original validation cohort (n = 519)	
	AUC (95% CI)	p value	AUC (95% CI)	p value
EPI	0.70 (0.65–0.75)	–	0.71 (0.66–0.76)	
EPI + SOCm	0.71 (0.66–0.76)	0.1457	0.73 (0.68–0.77)	0.3629
SOCm	0.62 (0.57–0.67)	0.0140	0.63 (0.58–0.68)	0.0027
PCPT-RC	0.63 (0.58–0.68)	0.0209	0.62 (0.57–0.67)	0.0032
ERSPC-RC	0.59 (0.54–0.64)	0.0014	0.58 (0.52–0.63)	<0.0001
PSA	0.58 (0.53–0.63)	0.0005	0.55 (0.49–0.60)	<0.0001

AUC = area under curve; CI = confidence interval; EPI = ExoDx Prostate (IntelliScore); ERSPC-RC = European Randomized Study of Screening for Prostate Cancer risk calculator; PCPT-RC = Prostate Cancer Prevention Trial risk calculator; PSA = prostate-specific antigen; SOCm = standard of care model.
EPI has a significantly higher AUC than all alternative models, except the combined model of EPI + SOCm. AUCs from the cohort of n = 503 patients are nearly identical to the original validation cohort of n = 519.

By applying an EPI cut-point of 15.6 for predicting ≥GG2 PCa, the EPI test demonstrated an NPV of 89% with a sensitivity of 93% and would have avoided 20% of all

Table 3 – Performance of the EPI test with a cut-point of 15.6 in the second validation cohort 1

	EPI ≥ cut-point	EPI < cut-point	Total	Performance, %	(95% CI)
Biopsy Positive/ ≥ GG2	147	11	158	Sensitivity, 93	(87.9–96.5)
Biopsy Negative/GG1	255	90	345	Specificity, 26.1	(21.5–31.1)
Total	402	101	503	PPV, 36.6	(31.8–41.5)
				NPV, 89.1	(81.3–94.4)
Prevalence	31.4%	Predicted negative	20.1%		

CI = confidence interval; EPI = ExoDx Prostate (IntelliScore); GG = Grade Group; NPV = negative predictive value; PPV = positive predictive value.

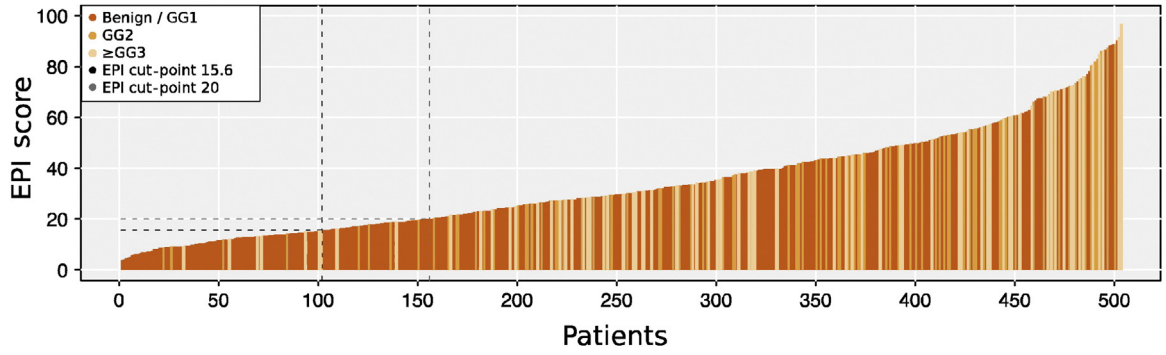

Fig. 2 – Waterfall plot of the EPI scores in relation to prostate biopsy outcomes across cohort 1 (*n* = 503). EPI scores are shown on Y-axis and the Grade Group biopsy results illustrated in blue, yellow, or red.
EPI = ExoDx Prostate (IntelliScore); GG = Grade Group.

biopsies (*n* = 503) or 26% of unnecessary, negative/GG1 biopsies (*n* = 345; Table 3). The assay missed 11 of 158 (7%) ≥GG2 cancers of which seven were ≥GG3. Of note, the 15.6 cut point in the original validation cohort produced a similar NPV of 91% and sensitivity of 92%, while avoiding 27% of biopsies, and 12 of 148 (8%) ≥GG2 cancers were missed of which three were ≥GG3.

We also investigated the clinical value of the EPI test relative to alternative models using a decision curve analysis (Fig. 1C and 1D). EPI's performance was superior (highest net benefit) to all models tested, including SOCm, PCPT-RC, ERSPC-RC, and PSA. The distribution of biopsy results from patients with different EPI scores is also shown in a waterfall plot from the current validation cohort, illustrating the distribution of HGPCa above and below the cut-point (Fig. 2).

Further, we previously reported that the EPI test performed equally well with an alternative cut-point of 20. In this second validation cohort, an EPI score of 20 produced an NPV of 89% with a sensitivity of 89% and would avoid 31% of all biopsies and 40% of negative/GG1 biopsies (Table 4). The assay missed 17 of 158 (11%) ≥GG2 cancers of which nine were ≥GG3. For completeness, we have also included a table of results across a range of different cut-points (Supplementary Table 2).

A direct comparison of cut-point performances relative to available alternatives was performed at a 90% fixed sensitivity analysis of EPI against PSA and standard of care RCs (Table 5).

3.4. *Consensus CarePath*

As outlined in the study protocol, a panel of principal investigators and pre-identified external content experts convened to reach consensus on CarePath for EPI test implementation in phase II of the current adaptive utility trial. The panel first confirmed that the demographics and phenotypic characteristics of the patients enrolled in the previous and current validation studies were similar. The panel took note of the observed 5% increase in the positive biopsy rate and discussed that this could be a reflection of the 2012 USPSTF reduced PSA screening recommendations but that this difference did not appear to impact performance of the EPI test. The panel agreed that the primary

Table 4 – Performance of the EPI test with a cut-point of 20 in the second validation cohort 1

	EPI ≥ cut-point	EPI < cut-point	Total	Performance, %	(95% CI)
Biopsy Positive/ ≥ GG2	141	17	158	Sensitivity, 89.2	(83.3–93.6)
Biopsy Negative/GG1	207	138	345	Specificity, 40	(34.8–45.4)
Total	348	155	503	PPV, 40.5	(35.3–45.9)
				NPV, 89	(83.0–93.5)
Prevalence	31.4%	Predicted negative	30.8%		

CI = confidence interval; EPI = ExoDx Prostate (IntelliScore); GG = Grade Group; NPV = negative predictive value; PPV = positive predictive value.

Table 5 – Performance comparison of EPI to alternative models at 90% fixed sensitivity in the current cohort of *n* = 503 patients

Method	Sensitivity	Specificity	NPV	PPV	% classified negative
EPI	90 (84.1–94.1)	38.6 (33.4–43.9)	89.3 (83.1–93.7)	40.1 (35.0–45.4)	29.6 (25.7–35.7)
SOCm + EPI	90 (84.1–94.1)	33.6 (28.7–38.9)	87.9 (81.1–92.9)	38.8 (33.3–43.4)	26.2 (22.4–30.3)
SOCm	90 (84.1–94.1)	19.1 (15.1–23.7)	80.5 (70.3–88.4)	33.7 (29.2–38.5)	16.3 (13.2–19.8)
PCPT	90 (84.1–94.1)	21.4 (17.2–26.2)	82.2 (72.7–89.5)	34.4 (29.8–39.2)	17.9 (14.6–21.5)
ERSPC	90 (84.1–94.1)	18.8 (14.9–23.4)	80.2 (69.9–88.3)	33.6 (29.2–38.4)	16.1 (13.0–19.6)
PSA	90 (84.1–94.1)	19.7 (15.6–24.3)	81.0 (70.9–88.7)	33.9 (29.4–38.6)	16.7 (13.5–20.3)

EPI = ExoDx Prostate (IntelliScore); ERSPC = European Randomized Study of Screening for Prostate Cancer; NPV = negative predictive value; PPV = positive predictive value; PCPT = Prostate Cancer Prevention Trial; PSA = prostate-specific antigen; SOCm = standard of care model.
Confidence intervals as determined by the Clopper-Pearson method are given in parentheses for each performance measure.

endpoint of validating EPI performance was met and that the EPI test was superior to the standard of care for predicting HGPCa in the intended use population.

After a comprehensive review of the phase I outcome and the previously published validation study, the panel unanimously recommended to use the 15.6 cut point for men aged ≥50 yr, with a PSA of 2–10 ng/ml presenting for their initial biopsy in the second phase of the study. The panel determined that while the alternative cut-point of 20 avoided additional biopsies, it also produced more false-negatives, and to reduce risk, there was uniform agreement to proceed with the more conservative cut-point in phase II of the study, where biopsy decision is uncertain. There was uniform agreement that the EPI test would not be used in a "clinical vacuum" and since the EPI score does not incorporate standard clinical parameters, the real-world experience would likely yield fewer missed GG2 biopsies. The EPI score is not a dichotomous variable that leads to an absolute biopsy decision but rather a risk stratification tool (high vs low risk of HGPCa) that can be added to the shared decision-making process between a patient and his urologist regarding appropriate next steps. A CarePath document was subsequently authored by the panel and submitted for approval by the respective IRBs (Supplementary materials). Upon approval, the CarePath was provided to the clinical investigators enrolling patients in the decision impact phase II of the trial.

4. Discussion

PCa remains the most common nondermatologic malignancy among men in the USA and Europe. It is a major health concern, especially in developed countries with a greater proportion of elderly men in the general population [21,22]. The use of PSA for opportunistic and general screening has produced an overall reduction in PCa mortality but resulted in a substantial increase in unnecessary biopsies along with the detection of clinically low-risk asymptomatic cancers [23]. Furthermore, more than a million biopsies performed every year in the USA alone has produced an increase in infectious complications; according to some studies, the 30-d hospitalization rate rose from 1.0% in 1996 to 4.1% in 2005 and 75% were for sepsis [24]. Although the published frequency of these complications is quite variable (0–6.5%) [6], the number of men requiring medical intervention continues to rise, especially for those with fluoroquinolone-resistant intestinal flora [25].

The decreased PSA screening recommendation by the 2012 USPSTF has reduced the overall number of biopsies performed and has also reportedly increased the positive biopsy rate; however, nationally representative studies are lacking [26–29]. Furthermore, there has been an observed increase in the diagnosis of more clinically significant disease, suggesting that potentially important cancers are not being diagnosed early enough. From the original (2014) to current trial (2016), we observed a 5% increase in the positive biopsy rate (48% to 53%) with an increase in GG3 by 2%, suggesting that such trends may have impacted the cohort composition. The challenges associated with the use of PSA have emphasized the need to develop more objective measures for identifying clinically significant PCa while continuing to reduce the sequence of over-diagnosis and over-treatment. Such efforts have given rise to several blood- and urine-based commercial assays, notably Prostate Health Index (phi, Beckman Coulter, Brea, California, USA), 4Kscore (Opko, Miami, Florida, USA), PCA3 (Gen-Probe, San Diego, CA, USA), and SelectDx (MDx Health, Herstal, Belgium).

We have previously validated a noninvasive, urinary three-gene expression assay, EPI, to discriminate ≥GG2 cancer from GG1 and benign disease for men aged ≥50 yr, undergoing initial biopsy with PSA levels 2–10 ng/ml [14]. In the current trial, the EPI test underwent an additional independent prospective validation on 503 men, and the results replicated the original study (AUC 0.70 vs AUC 0.71, respectively) including superiority to well-established RCs, such as PCPT-RC (AUC 0.63), ERSPC-RC (AUC 0.58), and PSA alone (AUC0.58). The present EPI validation study targeted the intended use population of men with a PSA 2–10 ng/ml at initial biopsy, where standard of care parameters are less informative. Furthermore, EPI alone had an AUC of 0.70, while EPI combined with the SOCm was 0.71 (95% confidence interval: 0.66–0.76), supporting independent performance of EPI from clinical variables. Of note, to effectively evaluate the performance of EPI with other available commercial assays would, however, require an assessment within this intended use population.

In the second validation cohort in this study (*n* = 503), an EPI score <15.6 would have avoided biopsies in 101 men (20%), or 26% unnecessary biopsies while missing 11 men (7%) with ≥GG2 PCa of which seven patients had ≥GG3. These results are comparable with the original validation study of 8% ≥GG2 false-negatives. Applying a cut point of 20 in this second validation cohort would potentially avoid

31% or 40% unnecessary biopsies while missing 17 (11%) men with ≥GG2 of which nine (12%) had ≥GG3.

Importantly, in the decision curve analyses, EPI when compared with PSA and clinical-only models (ie, SOCm, PCPT-RC, and ERSPC) demonstrated a higher net benefit beginning at a biopsy threshold probability of 10%, which was maintained up to a maximum of 30%. It is understood that the determination of acceptable risk and biopsy threshold for missing high-grade disease is based on a shared decision-making process between the patient and urologist. This is further complicated in the initial biopsy setting with PSA in the 2–10 ng/ml gray zone where patient anxiety is most likely increased. As EPI performance is independent of clinical variables, it may be combined with other parameters such as clinical preference and comorbidities for developing a more personalized risk assignment.

A major strength of the present study is that it represents an additional prospective validation of the EPI test on a clinically similar cohort of patients as the original validation study (ie, men aged ≥50 yr, undergoing initial biopsy with a PSA 2–10 ng/ml). There are, however, a few limitations including absence of a mpMRI feature for the majority (>90%) of enrolled patients. To address this point, additional clinical trials are currently in progress to include mpMRI outcomes correlated with the EPI score. Other limitations include the absence of a free PSA and prostate volume, neither of which are routinely performed for the majority of contemporary patients in the USA. Finally, we did not have a central pathology review on all diagnostic biopsies; however, our main goal was to evaluate EPI performance based on routine laboratory practices.

We convened a multidisciplinary panel of experts including principal investigators, external non-trial urologists, and computational biologists with experience in clinical risk assessment models to review results of the independent second and previous validation studies. Utilizing a prepared dossier of EPI performance characteristics, the consensus group confirmed the accuracy of the EPI test for discriminating HGPCa of ≥GG2 from benign and GG1 biopsies in men aged ≥50 yr, with a PSA 2–10 ng/ml presenting for their initial biopsy. After a comprehensive assessment of cut-point performance, specifically the false-negative rate, the consensus group recommended that a cut-point of 15.6 be utilized for the intended use population in cohort 2 utility trial participants. A document was provided to the respective IRBs for implementation and guidance.

5. Conclusions

In summary, the EPI test performed equally well in a second prospective independent second validation study for predicting HGPCa of ≥GG2 in men presenting for their initial biopsy with a PSA 2–10 ng/ml. In an analysis of over 1000 men from two cohorts, the EPI test was superior to both the PCPT and ERSPC RCs for predicting clinically significant PCa on initial biopsy. An expert consensus panel determined that an EPI score of >15.6 be used to discriminate patients as high-risk for GG2 PCa on their initial biopsy. This assessment was incorporated into a guidance document for phase II patients in the decision impact trial.

Author contributions: Michael Joseph Donovan had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Obtaining funding: Donovan, Skog, Noerholm.
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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.eururo.2018.08.019.

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