Clinical Performance of the ExoDx (EPI) Prostate Intelliscore Test to Predict High-grade Prostate Cancer at Initial Biopsy: A Pooled Analysis of Three Independent Prospective Studies.

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Research article

Keywords: Prostate Cancer, Epi Test, Exosomes, pooled analysis

DOI: https://doi.org/10.21203/rs.3.rs-131551/v1
Abstract

BACKGROUND The ability to discriminate indolent from clinically significant prostate cancer (PC) in the initial biopsy setting remains an important health issue. ExoDx Prostate(IntelliScore) (EPI), is a non-invasive exosome based liquid biopsy test that quantifies three RNA targets in exosomes from urine. The EPI test is used to help stratify patients for risk of high grade prostate cancer, HGPC (≥ GG2 PC) in men 50 years or older with PSA in the gray zone (2–10 ng/mL). The EPI test has been extensively validated and is included in the NCCN guidelines for prostate cancer early detection. Here we present a pooled meta-analysis from three independent prospective validation studies in men presenting for initial biopsy decision. Age range and PSA level subgroups were also analyzed for EPI performance.

METHODS Pooled data from two prospective multi-site validation studies and the control arm of a clinical utility study were analyzed. The data from these three independent trials is presented for men 50 years or older with PSA 2–10 ng/ml presenting for their initial prostate biopsy as well as a subgroup of patients between 55–69 years as recommended by the USPSTF (United States Preventative Services Task Force) and PSA greater than 3 ng/mL as per NCCN 2020 guidelines. Diagnostic needle biopsy outcomes were compared with the EPI score, PSA and both the Prostate Cancer Prevention Trial (PCPT 2.0) and the European Randomized Study of Screening for Prostate Cancer (ERSPC) risk calculators. Performance was evaluated using the area under the receiver operating characteristic curve (AUC), negative predictive value (NPV), positive predictive value (PPV), sensitivity and specificity for discriminating ≥ GG2 from GG1 and benign pathology.

RESULTS The combined cohort (n = 1212) of initial biopsy subjects had a median age of 63 years, median PSA 5.2 ng/mL and 17% African ancestry. The positive biopsy rate was 52% for ≥ GG1, 30% ≥GG2 and 14% ≥GG3. The EPI AUC of 0.70 was superior to PSA (AUC:0.56), PCPTRC (AUC: 0.62), and ERSPC (AUC: 0.59), (all p-values < 0.001) for discriminating GG2 from GG1 and benign histology. The previously validated cut-point of 15.6 (or alternative 20) would avoid 23% (or 34%) of all prostate biopsies and 30% (or 43%) of “unnecessary” (benign or Gleason 6/GG1) biopsies, with an NPV of 90% (89%). Across the total cohort (n = 1212), only 2.3% (28/1212) or 3.8% (46/1212) of patients would experience delayed detection of ≥ GG2 at the < 15.6 or < 20 threshold, respectively and for GG3, 1% (12/1212) and 1.5% (19/1212) at either cut-point would be delayed. Comparable results were identified when either the USPSTF 55–69 year age limit or NCCN PSA greater than 3 ng/mL were applied.

CONCLUSIONS EPI is a non-invasive, easy to use, urine exosome-RNA assay that has been validated across 3 independent prospective multi-center clinical trials with 1212 patients. The test can discriminate high-grade (≥GG2) from low-grade (GG1) cancer and benign disease and performs equally well in the larger cohort as well as across the USPSTF and NCCN restricted subgroups. EPI effectively guides the biopsy decision process and improves identification of HGPC independent of PSA and other standard of care factors.

Background
Prostate cancer (PC) is the most common cancer in men and the second most common cause of cancer related death in the United States. In 2020, it is estimated that approximately 192,000 men will be diagnosed with prostate cancer and 33,000 will die of their disease. (1) As mentioned in the NCCN, 2020 Prostate Cancer Early Detection guidelines, during this same time period nearly 20 million men in the U.S. will be involved in PC early detection discussions based on a variety of factors including anxiety associated with fluctuating PSA levels, a positive family history, and race.

Importantly, since PSA is not a reliable biomarker for the identification of clinically significant GG2 and higher disease there will continue to be an overall increase in either non-diagnostic unnecessary biopsies or of clinically indolent (GG1) tumors. This cycle will result in a fairly high percentage of men experiencing procedures such as surgery, radiation or additional biopsies as part of an active surveillance program (1–6). Furthermore, although there have been improvements in the prostate biopsy process including the use of prophylactic antibiotics, the procedure is not entirely benign and rare complications may result including a relatively high infection rate of 3–5% with the potential of emergency room visits and hospitalizations (7).

The EPI test (Exosome Diagnostics, Waltham, MA) is a first-catch urine exosome-based liquid biopsy assay which has been independently validated in two prospective multi-site studies and the standard of care control arm of a utility study which evaluated EPI in the biopsy decision process (8–10). Utilizing the expression levels from three genes (i.e. PCA3, ERG and SPDEF) EPI provides a risk score predicting the probability of whether a patient presenting for their first biopsy with an equivocal PSA from 2–10 ng/mL is likely to have Grade group 2 or greater (high-grade) prostate cancer. The EPI risk score is independent of all clinical variables and is performed without the need for a digital rectal exam or prostate massage. (11) The absence of clinical variables in the EPI risk algorithm represents an important differentiator from other assays predicting high-grade prostate cancer, including 4K (OPKO Diagnostics, Miami, FL) and SelectMDX (MDx Health, Irvine, CA). The performance of EPI is based only on the exosome RNA signature instead of relying on clinical factors already included in the biopsy consideration process (such as a prior negative biopsy) and known to have a positive impact on algorithm performance. (12–14)

The 2020 National Comprehensive Cancer Network (NCCN) Prostate Cancer Early Detection V2.2020 guidelines and the updated United States Preventive Services Task Force, USPSTF (15) both acknowledge the benefits of informed and patient-specific (i.e. age, life expectancy, patient history) PSA screening as a mechanism to reduce over-diagnosis and over-treatment of indolent prostate cancer. This has resulted in the need for additional non-invasive tests that not only reduce the number of unnecessary biopsies but also maintain a sufficient sensitivity to identify clinically significant disease. Pooled results from three independent multi-site, prospective validation studies in the intended use population confirmed performance of the EPI test in identifying clinically significant prostate cancer on initial biopsy.

Methods

Study design and population
This paper reports on the pooled analysis of two prospective validation studies and the control arm of a clinical utility study comparing EPI results with biopsy outcomes. All appropriate individuals had never been diagnosed with PC before, were 50-years or older with PSA 2–10 ng/mL and scheduled for their first prostate needle biopsy. The three study protocols were each previously approved by the respective local institutional review boards and all subjects had provided a written informed consent. At each of the sites the developed protocol and associated statistical analysis plan(s) were agreed upon by individual principal investigators prior to patient consent and trial data collection. Correlation of test results with biopsy pathology report diagnoses including an assessment of the 15.6 and 20 EPI test result cut-points were evaluated by the principal investigators and appropriate site-based individuals.

### Assay methods

First-catch urine samples (15–20 ml) were collected and stored at 4 °C for up to 14 days (majority were received within 5 days) prior to shipping to a central laboratory (Exosome Diagnostics, Inc., Waltham, MA). All sites received a urine collection vessel and shipping kits; men from the first validation study received a pediatric urine cup with instructions to only collect urine within the requested volume range, while a standardized 20 ml volume-restricted vessel was used in the second validation cohort and the utility study. Previous methods used in exosome isolation, extraction of RNA, and reverse transcriptase polymerase chain reaction (RT-PCR) were previously published (8–11). The test result provides a binary low or high risk score based on a validated cut-point of 15.6 (scale 0-100) that predicts the presence of Grade group (GG) 2 or higher prostate cancer. The 15.6 cut-point was also externally confirmed by five urologists and a machine learning biostatistician using training and pre-validation clinical cohorts with an emphasis on percentage of missed GG2 and GG3. Since the level of acceptable risk for missing HGPC vs. benefits of reducing biopsies is different among urologists, an alternative cut-point of 20 was also assessed. We had previously demonstrated that men with a score \( \leq 15.6 \) (or \( \leq 20 \)) are less likely to have GG2 or higher prostate cancer on a subsequent biopsy.

### Statistical methods

The primary objective of this pooled analysis was to evaluate combined performance of the EPI test for predicting GG2 or higher PC on a first biopsy for men with a PSA 2–10 ng/mL in a merged cohort that consists of three prospective, multi-site trials. We also employed the PSA measurements alone as well as the Prostate Cancer Prevention Trial risk calculator 2.0 (PCPTRC 2.0) and the European Randomized Study of Screening for Prostate Cancer (ERSPC) RC to further establish and compare the diagnostic risk of prostate cancer (16). Receiver operating characteristics for all models assessed clinical performance. Subgroup analyses including restricted age of 55–69 years as per United States Preventative Services Task Force (15) and the 3 ng/mL PSA cut-off as per NCCN, 2.2020 guidelines were also evaluated for EPI performance.

A pooled ‘meta-analysis’ was conducted on the merged cohort. Area under the curve (AUC) of the ROC was assessed for EPI, PCPT-RC, ERSPC, and PSA with TRUS biopsy outcome being used for subject
labeling. Sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV) are reported for the EPI cut-points of 15.6 and 20, respectively. Where applicable, confidence intervals were calculated using Clopper-Pearson method. DeLong's test was applied to assess the significance of AUC differences between analyses. We also evaluated the net health benefit of the EPI test for predicting GG2 PC across a range of clinical preferences which represents how doctors value different outcomes for their patients. The datasets analyzed during the current study may be available from the corresponding author on reasonable request.

Results

Study Population

There were 1212 eligible study subjects enrolled from three prospective trials: Validation 1, n = 519, Validation 2, n = 503, and Validation 3, n = 190 enrolled across 23 community practice sites and 4 academic medical centers (i.e. Columbia University Medical Center, NYC NY, Johns Hopkins University, Baltimore, MD, University of Michigan, Detroit, MI, and NYU Langone, NYC, NY, see Supplemental Table S1 for a complete listing of sites). The median age was 63 years with a median PSA of 5.2 ng/mL, 18% of patients self-reported a positive family history of PCa and 70% identified as Caucasian and 17% African American. The digital rectal exam (DRE) was non-suspicious in 87%, and 13% suspicious. See Table 1 for complete demographic and clinical characteristics of individual and pooled validation subjects.
Table 1  
Pooled cohort demographic and clinical characteristics.

|                  | Pooled Cohort |
|------------------|---------------|
| Total patient N  | 1212          |
| Age median, IQR  | 63 (58, 69)   |
| PSA median, IQR  | 5.2 (4.3, 6.6)|
| Family History   |               |
| Yes              | 223 (18.4%)   |
| No               | 987 (81.4%)   |
| NA               | 2 (0.2%)      |
| Ethnicity        |               |
| African American | 204 (16.8%)   |
| Asian/Pacific Islander | 37 (3.1%) |
| Caucasian        | 854 (70.5%)   |
| Other            | 96 (7.9%)     |
| NA               | 21 1.7(%)     |
| DRE              |               |
| Non-Suspicious   | 888 (73%)     |
| Suspicious       | 155 (13%)     |
| NA               | 169 (14%)     |
| Grade Group      |               |
| Benign           | 585 (48.3%)   |
| GG 1 (GS3 + 3)   | 261 (21.5%)   |
| GG 2 (GS3 + 4)   | 198 (16.3%)   |
| GG 3 (GS4 + 3)   | 84 (6.9%)     |
| GG 4 (GS8)       | 39 (3.2%)     |
| GG 5 (> GS8)     | 45 (3.7%)     |

Biopsy Gleason grading and Grade Group, GG Classification
The median number of TRUS-guided biopsy cores was 12 for all 1212 patients with diagnosis performed at individual site-designated pathology practices without a central pathology review. The total positive biopsy rate was 52%: 21.5% GG 1 and 30% ≥GG2, 13.8% ≥GG3 (See Table 1). Although PSA and age were quite comparable between all studies there was a 5% increase in the positive biopsy rate (48–56%) and a 3% increase in the diagnosis of ≥ GG2 PCa (28–31%). Of note, the GG3 or greater was also increased (12% vs. 17%) from the initial to the third validation. These mild increases may reflect the 2012 USPSTF recommendation against PSA screening (17).

**EPI as a predictor of ≥ GG2 Prostate Cancer in the merged cohort**

The EPI test in the merged 1212 study group exhibited comparable performance as in the previous individual validation studies. Pooled cohort EPI AUC 0.70 (95%CI 0.67–0.73) was superior to the PCPT-RC 2.0 AUC 0.62 (95%CI 0.59–0.66), ERSPC-RC AUC 0.59 (95%CI 0.56–0.63) and PSA AUC 0.56 (95%CI 0.53–0.60) (Fig. 1). The DeLong test comparing differences between AUC curves further demonstrated good independent performance of the EPI test. (Table 2).

| Test/Model   | N = 1022 | AUC (95%CI) | p-value |
|--------------|----------|-------------|---------|
| EPI          |          | 0.70 (0.67–0.73) | -       |
| PCPT2.0-RC   |          | 0.62 (0.59–0.66) | < 0.001 |
| ERSPC        |          | 0.59 (0.56–0.63) | < 0.001 |
| PSA          |          | 0.56 (0.53–0.60) | < 0.001 |

The pooled EPI validated cut-point of 15.6 cut point would avoid 23% of all biopsies (i.e. <15.6 cut-point) and 30% (i.e. specificity) of the true negative biopsies with an NPV of 90%, PPV 36.4% and a sensitivity of 92%. (Table 3) The alternate cut point of 20 increased the avoided biopsies to 34% with an NPV of 89%, PPV of 40% and a sensitivity of 87%. (Table 4) Across the total cohort (n = 1212), only 2.3% (28/1212) or 3.8% (46/1212) of patients would experience delayed detection (≥ GG2) at the < 15.6 or < 20 threshold, respectively.
### Table 3
Performance of the EPI test with a cut point of 15.6 in the pooled cohort.

|                      | EPI ≥ cut point | EPI < cut point | Total | Performance | (95%CI) |
|----------------------|----------------|----------------|-------|-------------|---------|
| Biopsy Positive/ ≥ GG2 | 338            | 28             | 366   | Sensitivity, 92.3% | (89.1–94.9) |
| Biopsy Negative/GG1  | 591            | 255            | 846   | Specificity, 30.1% | (27.1–33.4) |
| Total                | 929            | 283            | 1212  | PPV, 36.4%   | (33.3–39.6) |
| Prevalence           | 30.2%          | Predicted negative | 23.3% | NPV, 90.1%   | (86.0–93.3) |

### Table 4
Performance of the EPI test with a cut point of 20 in the pooled cohort.

|                      | EPI ≥ cut point | EPI < cut point | Total | Performance, % | (95%CI) |
|----------------------|----------------|----------------|-------|----------------|---------|
| Biopsy Positive/ ≥ GG2 | 320            | 46             | 366   | Sensitivity, 87.4 | (83.6–90.6) |
| Biopsy Negative/GG1  | 486            | 360            | 846   | Specificity, 42.6 | (39.2–46.0) |
| Total                | 806            | 406            | 1212  | PPV, 39.7      | (36.3–43.2) |
| Prevalence           | 30.2%          | Predicted negative | 33.5% | NPV, 88.7      | (85.2–91.6) |

Performance of the 15.6 or 20 cut-point with respect to GG3 or higher disease was also evaluated. An EPI score of ≥ 15.6 identified 93% (156/168) of GG3 while 12 patients or 7% were false negatives. If the EPI score was <15.6 the chance of avoiding detection of a ≥ GG3 biopsy was 4% (12/283). Alternatively, a cut-point of ≥ 20 identified 88% (149/168) of GG3 with 19 patients or 11% false negatives. If the EPI score was <20 the chance of avoiding detection of a ≥ 3 biopsy was 5% (19/406). The 12 or 19 patients represent 3% and 5% of the overall ≥ GG2 population (12/366; 19/366, respectively) and 1% and 1.5% of the entire 1212 cohort.

As previously established, the percentage of delayed GG3 or higher cancers in the pooled study further supports the use of a 15.6 cut-point in the recommended EPI CarePath for the majority of patients.
Similar results were observed for either the $\geq$ GG2 or $\geq$ GG3 with either the USPSTF age restrictions of 55–69 years: $n = 833$ study patients, AUC 0.69 (0.66–0.73), PSA AUC 0.57 (0.53–0.62), PCPT-RC 2.0 AUC 0.61 (0.56–0.65), ERSPC-RC AUC 0.60 (0.56–0.64); and focusing on the 15.6 cut-point: NPV 91.5% (86.5–95.1), sensitivity 93.3% (89.4–96.1) and specificity 29% (25.3–32.8) or when applying the NCCN PSA of 3 ng/mL and age 45–75 guidelines: $n = 1097$, AUC 0.70 (0.67–0.73), PSA AUC 0.56 (0.52–0.60), PCPT-RC 2.0 AUC 0.61 (0.58–0.65), ERSPC-RC AUC 0.59 (0.55–0.63); NPV 89.1% (84.6–92.6), PPV 35.5% (32.2–38.8), sensitivity 91.4% (87.8–94.2) and specificity 29.7% (26.5–33.1). The % false negative rate for $\geq$GG3 with the 15.6 cut-point was <5% in both sub-groups. (See supplemental Figure S1, S2 and Tables S2, S3).

The relative frequency (probability) of finding $\geq$GG2 cancer based on the EPI score was determined in the pooled cohort. As illustrated in Fig. 2, EPI score and risk or probability of finding GG2 or higher PCA has a linear relationship through an EPI score of 100 with a prominent slope at an EPI score 15.6–20 representing a 12–35% probability. It is important to note that the likelihood of finding GG2 or higher PCA is limited by the 12-core TRUS biopsy which has a sensitivity of approximately 48–50% for finding HGPC cancer. (18) In contrast, PSA groups 2–4/mL or 4–10 ng/mL, did not provide the necessary patient specific risk discrimination to be clinically useful (Fig. 3).

We also investigated the net clinical value of the EPI test using a decision curve analysis by comparing EPI scores with PCPT-RC V2.0, ERSPC-RC and PSA variables over a range of probabilities where a patient would likely decide to have a biopsy. (Fig. 4) The net benefit is the sum of true positives minus false negatives in reference to the biopsy decision threshold. In this analysis, the EPI test had the highest net benefit across the 10%-40% biopsy decision threshold, demonstrating a significant clinical utility when compared to more traditional methods.

In summary, the EPI test maintained performance for predicting GG2 or higher prostate cancer on initial biopsy for over 1000 men across three independent prospective studies. Higher EPI scores were associated with a greater probability of GG2 on a subsequent biopsy with an overall clinical net benefit when compared with a standard risk calculator and PSA. The risk of missing GG3 was stable at 3% in the pooled analysis with the 15.6 cut-point. The EPI score gives a personalized risk assessment, stratifying patients into low or higher risk for HGPC in a population where PSA and other clinical factors are non-discriminatory.

**Discussion**

The prostate biopsy decision process to rule out cancer is multifactorial and encompasses an integration of clinical variables including a PSA history, DRE exam, family history, race and patient / urologist anxiety. PSA has merits as a screening biomarker with a positive impact on PC mortality; however, the negative side has been an increase in the overall number of prostate biopsies performed in combination with the overdiagnosis of low grade (presumably) non-clinically significant disease. Noteworthy is that the use of PSA for general screening has produced an overall reduction in PC mortality but resulted in a
A substantial increase in unnecessary biopsies along with the detection of clinically low-risk asymptomatic cancers. With approximately 2 million transrectal ultrasonography-guided prostate biopsies (TRUS-Bx) performed each year in the United States and Europe (19) there is increasing concern over the risk of multi-drug resistant infections which was recently reported to be approximately 10% (20). Collectively, there is much to be gained by introducing more objective biomarker-driven assays into the biopsy decision paradigm and while clinical assessment tools, such as the PCPTRC, have value in assessing risk, these clinical calculators are not designed to be patient specific.

A screening strategy that preferentially targets GG2 PC and higher, with an additional emphasis on ≥ GG3 disease while avoiding detection of GG1 and benign pathology has the potential to maintain the mortality reduction while reducing harm from over-detection of indolent PC. We have previously confirmed clinical performance of a noninvasive, urine-based gene expression assay, EPI, to discriminate ≥ GG2 cancer from GG1 and benign disease for men aged ≥ 50 yrs., undergoing initial biopsy with PSA levels 2–10 ng/ml (8–10). In the current analysis we confirm and extend the previous studies with pooled data from three independent validation studies representing 1212 subjects and found that the EPI test, a gene signature within exosomes analyzed from voided urine, was consistently predictive of GG2 PC with AUC’s greater than PSA and the PCPT-RC V2.0 (AUC 0.70 > 0.56 and 0.62, respectively) and then with the 15.6 validated cut-point, achieved an NPV of 90%; PPV, 36%; sensitivity, 92%; and specificity, 30%. The NPV and sensitivity performance of the pooled analysis was comparable to the previous independent analyses (cut-point 15.6; V1, NPV 91%, sensitivity 92%; V2 NPV 89%, sensitivity 93% and V3 NPV 87%, sensitivity 92%). (8–10)

The urine exosome signature is derived from genes known to play a role in prostate cancer initiation and progression including total ERG, PCA3, and SPDEF. (11) To address some of the recent developments in PSA screening we further evaluated the 59–65 year age restriction as proposed by the USPSTF and the PSA cut-off of 3 ng/mL by NCCN and also found comparable performance. The 2018 USPSTF adjusted recommendation was designed to foster a more personalized, patient specific approach to PSA screening (15). A test that is able to reduce the “diagnosis” of low-grade and/or low-risk disease on a patient-specific basis should have a positive effect on individual urologist practice pattern variability.

Commercially available assays in the NCCN 2020 guidelines, specifically in the initial biopsy setting, including Prostate Health Index (PHI) (21) (Beckman Coulter), SelectMDx (MDxHealth) (14) and the 4K Score (OPKO Inc) (13) have varying accuracy to predict HGPC. Collectively the assays are also limited by composition of their respective validation cohorts, specificity issues of the kallikrein family, especially in the PSA 2–10 ng/mL range (eg, PHI, 4K), relative importance of clinical features in test (algorithm) performance, requirement of a DRE prior to collection and additional specimen processing (e.g. Progensa, SelectMDx). Furthermore, there is limited data to support reliable discrimination of GG2 vs GG3 on initial biopsy. Additional challenges include the integration into busy clinical practices, and whether the respective assays have been recommended for both the initial and repeat biopsy setting. Of note, neither of the well-established on-line clinical risk calculators (i.e. PCPT-RC, v2.0 and ERSPC-RC) were able to effectively discriminate HG in the current meta-analysis.
In the pooled cohort, by applying the 15.6 cut point would avoid 23% of all biopsies (i.e. <15.6 cut-point) and 30% (i.e. specificity) of the true negative biopsies with an NPV of 90, PPV 36.4 and a sensitivity of 92. The alternate cut point of 20 increased the avoided biopsies to 34% with an NPV 89, PPV 40 and a sensitivity of 87. What is clinically important is that across the total cohort (n = 1212), only 2.3% or 3.8% of patients would experience delayed detection of ≥ GG2 at the < 15.6 or < 20 threshold, respectively and for GG3, only 1-1.5% would potentially have a delayed detection at either cut-point. The results are comparable to prior validation studies for missing both ≥ GG2 and ≥ GG3 disease. The ability to subclassify GS7 into both GG2 and GG3 categories supports the important clinical distinction of a dominant pattern 4 which impacts directly on patient outcome and management plan. (22). It is widely accepted that not all GG2 cancers behave similarly and the volume of the dominant pattern 4 dictates disease course. The implication is that patients with a low volume < 10% pattern 4 cancer are most likely to clinically behave as a dominant pattern 3 and are appropriate for active surveillance protocols. (23)

Additional studies are underway to further refine this exosome-genetic signature including the introduction of additional clinical variables such as race, and tumor genetics.

EPI was able to provide an overall net clinical benefit (i.e. predicting ≥ GG2) when compared with standard clinical tools. This was further elucidated in the decision curve analysis, where EPI when compared with important clinical variables such as the level of PSA and the clinical risk calculators, PCPT-RC and ERSPC, demonstrated a higher net benefit beginning at a biopsy decision threshold probability of 10%, which was maintained up to a maximum of 30%. As EPI performance is based on gene expression only there is always the option for the urologist to introduce other parameters such as obesity, underlying genetics and race for developing a more personalized risk assignment.

The pooled data also illustrates that as the EPI score increases above 20, the risk or probability of GG2 prostate cancer increases to 30% and remains at this level and higher through EPI score of 60 where the probability of finding HGPC is just over 50%. It becomes challenging to have a linear improvement of a biomarker beyond 50% since TRUS biopsy is known to miss about 50% of HGPC.

Limitations of the validation studies included the absence of a central pathology review; however, the intent was to assess real-world experience of the assay with site-directed pathologists. Another missing component was the lack of a multi-parametric MRI (mp-MRI) as part of the overall assessment process. The majority of sites across the three studies did not routinely use MRI in the initial biopsy setting during this period. Future studies will incorporate the role of EPI in determining both the use of MRI and possibly incorporation of the EPI risk score into an algorithm that includes the PI-RADS designation and other clinical variables.

**Conclusion**

In summary, the EPI test performed equally well across three independent prospective studies involving some 23 community practices and 4 academic medical centers geographically distributed across the U.S. The EPI test was superior to PSA, PCPT RC and ERSPC for predicting clinically significant GG2 and
GG3 PC on initial biopsy. The results further support that increasing levels of the EPI score above 20 linearly increase the probability for finding HGPC upon an initial TRUS biopsy, up to a 50% risk at EPI scores >50.

**Abbreviations**

PCA3, prostate cancer antigen 3;

ERG, ETS (erythroblast transformation-specific) – related gene;

SPDEF, SAM pointed domain-containing ETS transcription factor.

**Declarations**

**Ethics approval and consent to participate:**

All study protocols were previously approved by the Western Institutional Review Board, Olympia, WA and individual academic institutional review boards (Johns Hopkins Hospital, Columbia University, University of Michigan and New York University, Langone); all study participants provided written informed consent and were not compensated for participating in the study.

**Consent for publication:**

Not Applicable

**Availability of data and materials:**

The datasets during and / or analyzed during all reported studies may be available from the corresponding author on request.

**Acknowledgements**

Acquisition, analysis, or interpretation of data: EM,GB,AP,BC,JM,MD,PT,JS,VT

Administrative, technical, or material support: JM,AP,RT,JS,PT,MD

All authors have read and approved the manuscript.

**Author Contribution’s:**
MD, PT, MK, VT, JS had full access to all data in the study and take responsibility for integrity of the data and the accuracy of the data analysis. Drs EM, GB, AP, BC, RT and JM contributed equally to this research and article.

**Competing interests / Conflict of Interest**

Critical revision of the manuscript for important intellectual content: MD, JS, PT, AP, JM, RT

Drafting of the manuscript: MD, PT, JS, VT.

**Funding / Support**

Exosome Diagnostics, the developer and owner of the ExoDx Prostate IntelliScore (EPI) exosome gene expression assay used in this study, provided all financial and material support for the work reported in this article.

P Torkler, M Noerholm, V Tadigotla, J Skog are employees of Exosome Diagnostics, a Bio-Techne Brand; M Donovan is a consultant to Exosome Diagnostics.

Statistical analysis: PT, VT, JS, MD

Study concept and design: MD, PT, JS, MN

Study supervision: EM, AP, JM, MD, JS

We would like to thank the various urology practices, investigators and laboratory support personnel for assisting in patient accrual, data collection and EPI report generation.

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