JAMA Oncology | Original Investigation

Development and Validation of an 18-Gene Urine Test for High-Grade Prostate Cancer

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IMPORTANCE Benefits of prostate cancer (PCa) screening with prostate-specific antigen (PSA) alone are largely offset by excess negative biopsies and overdetection of indolent cancers resulting from the poor specificity of PSA for high-grade PCa (ie, grade group [GG] 2 or greater).

OBJECTIVE To develop a multiplex urinary panel for high-grade PCa and validate its external performance relative to current guideline-endorsed biomarkers.

DESIGN, SETTING, AND PARTICIPANTS RNA sequencing analysis of 58 724 genes identified 54 markers of PCa, including 17 markers uniquely overexpressed by high-grade cancers. Gene expression and clinical factors were modeled in a new urinary test for high-grade PCa (MyProstateScore 2.0 [MPS2]). Optimal models were developed in parallel without prostate volume (MPS2) and with prostate volume (MPS2+). The locked models underwent blinded external validation in a prospective National Cancer Institute trial cohort. Data were collected from January 2008 to December 2020, and data were analyzed from November 2022 to November 2023.

EXPOSURE Protocolized blood and urine collection and transrectal ultrasound-guided systematic prostate biopsy.

MAIN OUTCOMES AND MEASURES Multiple biomarker tests were assessed in the validation cohort, including serum PSA alone, the Prostate Cancer Prevention Trial risk calculator, and the Prostate Health Index (PHI) as well as derived multiplex 2-gene and 3-gene models, the original 2-gene MPS test, and the 18-gene MPS2 models. Under a testing approach with 95% sensitivity for PCa of GG 2 or greater, measures of diagnostic accuracy and clinical consequences of testing were calculated. Cancers of GG 3 or greater were assessed secondarily.

RESULTS Of 761 men included in the development cohort, the median (IQR) age was 63 (58-68) years, and the median (IQR) PSA level was 5.6 (4.6-7.2) ng/mL; of 743 men included in the validation cohort, the median (IQR) age was 62 (57-68) years, and the median (IQR) PSA level was 5.6 (4.1-8.0) ng/mL. In the validation cohort, 151 (20.3%) had high-grade PCa on biopsy. Area under the receiver operating characteristic curve values were 0.60 using PSA alone, 0.66 using the risk calculator, 0.77 using PHI, 0.76 using the derived multiplex 2-gene model, 0.72 using the derived multiplex 3-gene model, and 0.74 using the original MPS model compared with 0.81 using the MPS2 model and 0.82 using the MPS2+ model. At 95% sensitivity, the MPS2 model would have reduced unnecessary biopsies performed in the initial biopsy population (range for other tests, 15% to 30%; range for MPS2, 35% to 42%) and repeat biopsy population (range for other tests, 9% to 21%; range for MPS2, 46% to 51%). Across pertinent subgroups, the MPS2 models had negative predictive values of 95% to 99% for cancers of GG 2 or greater and of 99% for cancers of GG 3 or greater.

CONCLUSIONS AND RELEVANCE In this study, a new 18-gene PCa test had higher diagnostic accuracy for high-grade PCa relative to existing biomarker tests. Clinically, use of this test would have meaningfully reduced unnecessary biopsies performed while maintaining highly sensitive detection of high-grade cancers. These data support use of this new PCa biomarker test in patients with elevated PSA levels to reduce the potential harms of PCa screening while preserving its long-term benefits.

JAMA Oncol. 2024;10(6):726-736. doi:10.1001/jamaoncol.2024.0455
Published online April 18, 2024.

Supplemental content

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Prostate cancer (PCa) remains the most commonly diagnosed malignancy and a leading cause of cancer death worldwide.[1] The European Randomized Study of Screening for PCa and Göteborg Randomized Prostate Cancer Screening trial showed significant reductions in cancer mortality for men participating in prostate-specific antigen (PSA)–based screening.[2,3] At the same time, these studies confirmed that PSA screening leads to unnecessary invasive biopsies in men without cancer and frequent overdiagnosis of low-grade, indolent cancers (grade group [GG] 1).[4] In response to this, current clinical guidelines offer that men with an elevated PSA level undergo multiparametric magnetic resonance imaging (mpMRI), if available, or biomarker testing for risk stratification prior to biopsy.[5,6]

Indeed, use of prostate mpMRI with targeted biopsy has improved detection of clinically significant, high-grade cancer (ie, cancer of GG 2 or greater) in men with tumors visible on mpMRI.[7] While these data support prebiopsy mpMRI in patients requiring biopsy, the use of negative findings on mpMRI to rule out high-grade cancers in men with elevated PSA levels is not well supported. Population-level data spanning academic and community settings reveal a negative predictive value (NPV) of only 77% for high-grade cancers,[8] and subjective interpretation of mpMRI is highly problematic, with NPVs as low as 63% by site and 40% among radiologists.[9,10] Thus, even following negative findings on mpMRI, its limited sensitivity merits biopsy in a substantial proportion of men. Moreover, there are practical reasons mpMRI may not be feasible for populationwide use after PSA, including its resource burden and limited availability in the community setting.[11,12]

Objective, noninvasive biomarker tests could be a more practical option. Current National Comprehensive Cancer Network (NCCN) guidelines offer 6 blood-based and urine-based biomarker tests, each including 3 or fewer markers of PCa (ie, cancer of any grade).[5] While consistently outperforming PSA alone,[13] these assays have not evolved to reflect current understanding of PCa biology. For one, given the minimal metastatic potential of low-grade cancers, contemporary practice is focused on detecting high-grade cancers, while reducing overdiagnosis of low-grade disease.[5] Thus, assays based solely on markers associated with cancer of any grade have limited biologic specificity for high-grade cancers. Moreover, assays including only 2 to 3 biomarkers simply cannot capture the multitude of diverse molecular pathways driving lethal disease.[14,15]

We hypothesized that augmenting the prior generation of cancer-associated biomarkers with novel molecules selectively expressed by high-grade, aggressive cancers would improve testing accuracy. Leveraging multi-institutional transcriptomic data,[14,16,17] we identified novel genes specifically overexpressed by high-grade cancers. We then adopted multiplex polymerase chain reaction (PCR)–based technology to evaluate 54 candidate markers in a development cohort, deriving an optimal 18-gene assay for standard clinical use. Finally, we performed blinded external validation of the new assay, including direct comparison with currently endorsed biomarker tests.

Key Points

Question Can a new 18-gene urinary test for high-grade prostate cancer (ie, grade group [GG] 2 or greater) improve prostate-specific antigen (PSA) screening outcomes relative to existing biomarker tests?

Findings In this diagnostic study including 761 men in the development cohort and 743 men in the validation cohort, novel cancer-specific and high-grade cancer-specific genes were identified from RNA sequencing data and optimally modeled in a development cohort, yielding an 18-gene test for high-grade prostate cancer. Applying a testing approach with 95% sensitivity for high-grade prostate cancer to an external validation population, use of the 18-gene test would have reduced the number of unnecessary biopsies performed relative to current guideline-endorsed tests.

Meaning The new 18-gene prostate cancer test may reduce more burdensome additional testing (eg, imaging and biopsy) while maintaining highly sensitive detection of high-grade cancer in patients undergoing PSA screening.

Methods

Institutional review board approval was obtained from the University of Michigan Institutional Review Board and at each site, and all participants provided written informed consent. This study followed the Standards for Reporting of Diagnostic Accuracy (STARD) reporting guideline.[18]

Biomarker Discovery

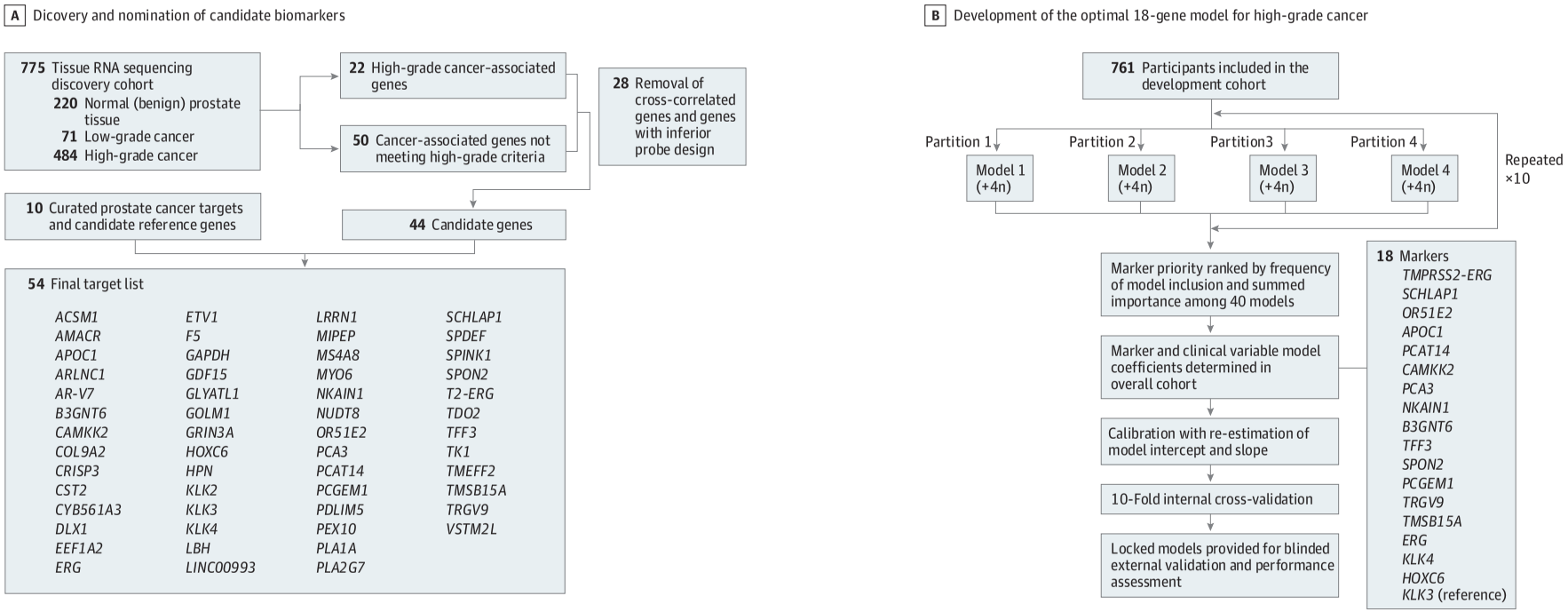
The original MyProstateScore (MPS) test incorporates prostate cancer antigen 3 (*PCA3*) and *TMPRSS2:ERG* gene fusion expression with serum PSA level to estimate risk of high-grade cancers and is endorsed by NCCN guidelines for prebiopsy risk stratification.[5,19] To derive a gene panel for high-grade cancers, we performed differential expression analysis of 58 724 genetic targets in multi-institutional RNA sequencing data (**Figure 1**; eFigures 1 and 2 in Supplement 1 and the eTable in Supplement 2). A total of 72 genes met predefined nomination criteria for cancer (n = 50) or high-grade cancer (n = 22) (eTable 1 in Supplement 1). Removal of collinear genes and those without PCR primers resulted in 44 candidate markers (eFigures 1 to 3 in Supplement 1). These were supplemented with 10 previously described PCa-associated or reference genes, yielding a 54-gene candidate panel.

Model Development

Development Cohort

Prebiopsy urine has been prospectively collected at the University of Michigan Prostate Specialized Program of Research Excellence under a National Cancer Institute (NCI) Early Detection Research Network (EDRN) protocol approved by the University of Michigan Institutional Review Board since 2008. First-catch urine was obtained following digital rectal examination and was mixed with RNA stabilization buffer and frozen at −70 °C.[20] The development cohort included patients

Figure 1. Biomarker Discovery and Development of the MyProstateScore 2.0 Urinary Test for High-Grade Prostate Cancer



A, Discovery and nomination of candidate biomarkers for the multiplex urinary panel. Biomarker discovery was performed using RNA sequencing data from 220 benign prostates, 71 with cancers of grade group 1, and 484 with cancers of grade group 2 or greater available through the Cancer Genome Atlas, the Genotype-Tissue Expression portal, and the University of Michigan. A total of 72 markers met predefined criteria. Of these, quantitative polymerase chain reaction probes could not be successfully designed for 19, and 9 genes were highly cross-correlated, resulting in exclusion from the candidate panel. The remaining 44 transcripts meeting nomination criteria were supplemented with 10 curated genes to yield a 54-gene candidate panel. B, Development of the optimal 18-gene model for high-grade cancer. To avoid multicollinearity in regression models, highly correlated variables were identified and removed with a stepwise procedure. We assessed 3 model-building approaches: (1) logistic regression with stepwise feature selection, (2) logistic regression with recursive feature elimination, and (3) regularized logistic regression with elastic net. Performance of each model-building approach was quantified as the area under the receiver operating characteristic curve on repeated cross-validation (10-fold cross-validation repeated 3 times) with upsampling of the minor class to yield balanced classes. Elastic net modeling yielded the highest median area under the curve and was used for development. Using an ensemble approach, the development set was randomly divided into 4 partitions, and the model yielding the highest area under the curve was identified for each partition. This approach was repeated 10 times with different random seeds, yielding 40 elastic net models in total. For each candidate gene, the frequency of model inclusion and importance to high-grade prostate cancer detection was tabulated across models. Based on analysis of optimal feature size and technical features of the OpenArray platform (Thermo Fisher Scientific), the 17 biomarkers providing optimal discriminative accuracy for prostate cancer of grade group 2 or greater were included with standard clinical variables and the normalization gene *KLK3* in the MyProstateScore 2.0 model (without prostate volume) and MyProstateScore 2.0 Plus model (with prostate volume). Models were calibrated and internally cross-validated prior to external validation.

presenting for 12-core or greater prostate biopsy due to elevated PSA levels (3-10 ng/mL; to convert to micrograms per liter, multiply by 1) from 2008 to 2020. In accordance with guidelines,[5] we excluded patients with PCa. Based on proposed use of this test as a pre-mpMRI, prebiopsy test to rule out the need for mpMRI or biopsy, we excluded men with a history of prostate mpMRI and targeted biopsy.

Multiplex Quantitative PCR OpenArray Profiling

OpenArray technology (Thermo Fisher Scientific) is a high-throughput real-time quantitative PCR (qPCR) method for rapid screening of multiple TaqMan assays. RNA isolation, extraction, and complementary DNA synthesis were performed (eFigure 4 in Supplement 1).

Model Building and Calibration

We assessed the 54-gene candidate panel using multiple model-building strategies (Figure 1). Clinical factors consistently associated with PCa (age, race, digital rectal examination findings, PSA level, family history of prostate cancer, and prior negative biopsy)[21] were locked into models a priori. Because prostate volume improves predictive value[22,23] but is not available for all patients, we developed a second model including volume for use when volume is clinically available (ie, previous biopsy or mpMRI). Test outputs were standardized to represent the percentage likelihood of detecting high-grade cancers (0% to 100%). The optimal 18-gene model without prostate volume (MPS2) and with prostate volume (MPS2+) were calibrated (eFigure 5 in Supplement 1) to account for differences in outcome prevalence between cohorts,[21,24,25] and the calibrated models were locked for external validation. The MPS2 test is owned by LynxDx, which has obtained an exclusive license for commercialization from the University of Michigan.

Model Validation

External Validation Cohort

The validation cohort consisted of patients in the prospective NCI EDRN PCA3 Evaluation Trial. This trial enrolled consecutive patients presenting for biopsy across 11 academic centers, primarily due to elevated PSA levels or abnormal digital rectal examination findings (eTable 2 in Supplement 1).[20] Patient race was self-reported via a questionnaire; selectable options included American Indian or Alaska Native, Asian, Black, Native Hawaiian or Other Pacific Islander, White, other, or unknown race. Black race was considered pertinent to this study based on the known association of Black race with PCa incidence, outcomes, and molecular subtypes.[26] Because such associations are not well established for other racial groups and because racial groups other than Black and White are frequently misclassified,[27] racial groups were categorized as Black or other race.

Specimens and Laboratory Analysis

Deidentified urine specimens were shipped to the University of Michigan for OpenArray profiling. Laboratory procedures were conducted per the identical protocol used in development. We derived a multiplex 2-gene model (*HOXC6* and *DLX1)* and a multiplex 3-gene model (*PCA3*, *ERG*, and *SPDEF)*. These genes are measured in the commercially available SelectMDx and ExoDx Prostate Intelliscore (EPI) tests, respectively. The multiplex models considered herein were independently derived based on gene expression measured using the OpenArray qPCR platform and are not proposed to represent the commercial products. Serum PSA, free PSA, and [−2]proPSA were measured using the Access 2 Immunoassay System (Beckman Coulter) at the Johns Hopkins EDRN Laboratory.

Blinded Validation and Comparative Analysis

Expression data and model coefficients were available to 2 investigators (C.X. and Y. Zheng) at the NCI EDRN for predefined validation. Locked model coefficients from development were used to generate outputs of the derived multiplex 2-gene model, derived multiplex 3-gene model, MPS2, and MPS2+. The original MPS was calculated using qPCR-based *PCA3* and *TMPRSS2:ERG* scores[19]; a subset of data were previously described.[28] Prostate Health Index (PHI) was calculated using the formula ([−2]proPSA/free PSA) × $\sqrt{}$(PSA).

Comparative analysis included PSA, the Prostate Cancer Prevention Trial risk calculator,[21] PHI, the derived multiplex 2-gene and 3-gene models, MPS,[19] MPS2, and MPS2+. The primary outcome was cancer of GG 2 or greater on biopsy; cancer of GG 3 or greater was secondarily assessed. Diagnostic potential was visualized by receiver operating characteristic (ROC) curves and quantified by area under the ROC curve (AUC) using R package pROC.[29] For the development cohort, mean AUC from repeated 10-fold cross-validation was reported. For the validation cohort, 95% CIs of AUCs were computed with 2000 stratified bootstrap.

We sought to illustrate test performance using a straightforward, clinically applicable approach. As described,[30] considering prevalence of high-grade cancers in testing populations (17% to 31%),[31-35] relative harms of false-negative and false-positive testing results,[36] and the proposed role of biomarkers for rule-out testing,[37,38] we assessed thresholds providing 95% sensitivity for high-grade cancer. Performance measures were calculated using the confusionMatrix function of R package caret. Given disparate risk profiles of initial and repeat biopsy populations,[39-41] analyses were carried out in each subpopulation.

Decision curve analysis (DCA) was used to quantify net benefit of each biomarker on the decision to undergo biopsy compared with (1) biopsying all patients and (2) biopsying no patients.[42] Considering a more than 20% risk of high-grade cancer justifies performing biopsy and a less than 5% risk justifies foregoing biopsy in most patients,[43] we assessed threshold probabilities spanning this clinically relevant range. DCA was performed using dca in the R package dcurves.

Statistical Analysis

Statistical analyses were performed using R version 4.1.1 (The R Foundation). Two-tailed tests were used for all comparisons, and *P* values less than .05 were considered statistically significant.

Results

Model Development

Among 815 participants in the development cohort, qPCR yielded valid results in 761 (93.4%) (eFigure 4 in Supple-

ment 1). The median (IQR) age was 63 (58-68) years, and the median (IQR) PSA level was 5.6 (4.6-7.2) ng/mL (**Table 1**). On study biopsy, 293 men (38.5%) had cancer of GG 2 or greater. The contribution of candidate genes to model predictions was quantified across elastic net models (Figure 1; eTable 3 in Supplement 1). The final MPS2 model included clinical variables and the 17 most informative markers, including 13 from the discovery analysis (4 high-grade cancer–specific genes [*APOC1*, *B3GNT6*, *NKAIN1*, and *SCHLAP1*] and 9 cancer-specific genes [*PCGEM1*, *SPON2*, *TRGV9*, *PCA3*, *OR51E2*, *CAMKK2*, *TFF3*, *PCAT14*, and *TMSB15A*]), 4 curated markers (*HOXC6*, *ERG*, *TMPRSS2:ERG*, and *KLK4*), plus the reference gene *KLK3* (eTable in Supplement 3). Model coefficients were determined in the overall cohort (eTable 4 in Supplement 1). Calibration and internal cross-validation were performed (eFigures 5 and 6 in Supplement 1), and the MPS2 models were locked for external validation.

External Validation and Comparative Analysis

Overall Study Population

Of 813 patients in the validation cohort (eFigure 7 in Supplement 1), qPCR was successful in 743 (91.4%). The median (IQR) age was 62 (57-68) years, and the median (IQR) PSA level was 5.6 (4.1-8.0) ng/mL. A total of 95 men (12.8%) were Black and 648 (87.2%) were another race, and 247 men (33.2%) had a previous negative biopsy (Table 1). On study biopsy, 151 men (20.3%) had high-grade PCa. Median (IQR) MPS2 values were significantly higher in men with cancer of GG 2 or greater (0.44 [0.23-0.69]) than in men with negative biopsies (0.08 [0.03-0.19]; $P < .001$) and in men with cancer of GG 1 (0.20 [0.08-0.43]; $P < .001$) (Table 1; eFigure 8 in Supplement 1). Similarly, median (IQR) MPS2+ values were significantly higher in men with PCa of GG 2 or greater (0.54 [0.27-0.79]) relative to those with negative biopsies (0.08 [0.03-0.21]; $P < .001$) or those with cancer of GG 1 (0.25 [0.09-0.48]; $P < .001$). The AUC values for high-grade cancer were 0.60 (95% CI, 54.7-64.6) for PSA alone, 0.66 (95% CI, 61.1-70.7) for the Prostate Cancer Prevention Trial risk calculator, 0.77 (95% CI, 73.0-81.3) for PHI, 0.76 (95% CI, 71.9-80.3) for the derived multiplex 2-gene model, 0.72 (95% CI, 67.0-76.1) for the derived multiplex 3-gene model, and 0.74 (95% CI, 69.4-78.0) for MPS compared with 0.81 (95% CI, 76.9-84.6) for MPS2 and 0.82 (95% CI, 78.1-85.5) for MPS2+ (eFigure 9 in Supplement 1). The observed prevalence of high-grade cancer closely approximated MPS2 and MPS2+ predicted probabilities (**Figure 2**), reflecting good calibration. Critically, the models were particularly well-calibrated for predicted probabilities less than 30%, which are most clinically pertinent.

We assessed clinical consequences of prebiopsy biomarker testing. At a 95% sensitivity testing threshold, the proportions of unnecessary biopsies that would have been avoided using each test were 11% for PSA alone, 20% for the Prostate Cancer Prevention Trial risk calculator, 26% for PHI, 27% for the derived multiplex 2-gene model, 17% for the derived multiplex 3-gene model, and 23% for MPS compared with 37% for MPS2 and 41% for MPS2+. Full performance measures and the estimated numbers of unnecessary biopsies avoided per 1000 patients are listed in **Table 2**. Critically, MPS2 and MPS2+ each provided 99% sensitivity and 99% NPV for cancer of GG 3 or greater.

Initial Biopsy Subpopulation

The initial biopsy population included 496 patients with a median (IQR) PSA level of 5.0 (3.8-6.6) ng/mL (eTable 5 in Supplement 1). On study biopsy, 133 (26.8%) had high-grade cancer. Using a 95% sensitivity threshold, the proportions of unnecessary biopsies avoided were 15% for PSA alone, 27% for the Prostate Cancer Prevention Trial risk calculator, 30% for PHI, 30% for the derived multiplex 2-gene model, 17% for the derived multiplex 3-gene model, and 27% for MPS compared with 35% for MPS2 (Table 2; eTable 6 in Supplement 1). Although patients undergoing initial biopsy often may not have prostate volume available, use of MPS2+ would have avoided 42% of unnecessary biopsies. Performance of MPS2 models with and without clinical factors are provided by subpopulation in eTables 7 and 8 in Supplement 1. An alternative initial biopsy model was developed in the initial biopsy population of the development cohort and similarly validated (eTables 9 and 10 and eFigure 10 in Supplement 1).

Repeat Biopsy Subpopulation

The repeat biopsy population included 247 men with median (IQR) PSA level of 7.2 (5.5-9.8) ng/mL, of which 18 (7.3%) were found to have high-grade cancer (eTable 5 in Supplement 1). At 95% sensitivity, the proportions of unnecessary biopsies that would have been avoided were 15% for PSA alone, 8.7% for PHI, 14% for the derived multiplex 2-gene model, 16% for the derived multiplex 3-gene model, 15% for MPS, 46% for MPS2, and 51% for MPS2+ (Table 2). Accordingly, MPS2 testing would have avoided approximately one-half of unnecessary biopsies while maintaining detection of 94.4% of high-grade cancers.

DCA

DCA was used to evaluate the net benefit of biomarker testing relative to performing biopsy in all patients and performing no biopsies. Across the clinically pertinent threshold probabilities spanning 5% to 20%, use of the MPS2 models would have provided the highest net clinical benefit across all tests (**Figure 3**A). Expressing benefit as net reduction in unnecessary biopsies, use of the MPS2 models would have provided the greatest net reduction in unnecessary biopsies without failing to biopsy a single patient with high-grade cancer (Figure 3B).

Discussion

Acknowledging the indolent nature of low-grade PCa, contemporary guidelines emphasize a narrowed diagnostic focus on high-grade cancers.[5,6,44] Existing biomarkers expressed by all PCa—including low-grade, indolent tumors—therefore offer limited potential to selectively detect high-grade disease. Translating sequencing-based discovery to an expandable qPCR platform, we developed and validated a new urinary test incorporating 17 markers of cancer, and—for the first time, to our knowledge—novel markers uniquely overexpressed by high-grade cancers relative to low-grade cancers. On validation, MPS2 testing with 95% sensitivity for high-grade cancer had 95% to 99% NPV and 35% to 51% specificity

Table 1. Characteristics of the Development and Validation Populations Overall and Stratified by Pathologic Findings on Prostate Biopsy

Characteristic	Median (IQR)									
	Development cohort					External validation cohort				
	Total (n = 761)	Negative (n = 362)	GG 1 (n = 106)	Negative or GG 1 (n = 468)	GG ≥2 (n = 293)	Total (n = 743)	Negative (n = 452)	GG 1 (n = 140)	Negative or GG 1 (n = 592)	GG ≥2 (n = 151)
Age, y	63 (58-68)	62 (57-67)	64 (57-68)	63 (57-68)	64 (58-69)	62 (57-68)	62 (57-67)	63 (57-67)	62 (57-68)	64 (59-70)
Race, No. (%)[a]										
Black	33 (4)	12 (3)	4 (4)	16 (3)	17 (6)	95 (13)	51 (11)	19 (14)	70 (12)	25 (17)
Other race	728 (96)	350 (97)	102 (96)	452 (97)	276 (94)	648 (87)	401 (89)	121 (86)	522 (88)	126 (83)
Positive family history, No. (%)	206 (27)	88 (24)	28 (26)	116 (25)	90 (31)	212 (29)	118 (26)	46 (33)	164 (28)	48 (32)
Previous negative biopsy, No. (%)	163 (21)	105 (29)	22 (21)	127 (27)	36 (12)	247 (33)	196 (43)	33 (24)	229 (39)	18 (12)
Abnormal DRE, No. (%)	104 (14)	34 (9)	4 (4)	38 (8)	66 (23)	139 (19)	72 (16)	16 (11)	88 (15)	51 (34)
Prostate volume, mL[b]	48 (36-66)	56 (42-76)	47 (37-61)	53 (40-72)	41 (30-54)	43 (32-60)	48 (35-69)	40 (29-52)	46 (34-65)	36 (28-47)
PSA, ng/mL	5.6 (4.6-7.2)	5.6 (4.6-6.8)	5.55 (4.6-7.0)	5.6 (4.6-6.9)	5.6 (4.7-7.5)	5.6 (4.1-8.0)	5.5 (4.0-8.0)	5.3 (4.3-7.0)	5.4 (4.0-7.7)	6.2 (4.7-8.9)
PSA density, ng/mL[2c]	0.12 (0.08-0.16)	0.10 (0.07-0.14)	0.12 (0.09-0.16)	0.10 (0.07-0.14)	0.15 (0.10-0.20)	0.12 (0.08-0.19)	0.11 (0.07-0.17)	0.12 (0.09-0.18)	0.11 (0.08-0.17)	0.17 (0.12-0.31)
PHI	NA	NA	NA	NA	NA	40.5 (30.0-55.0)	36.5 (27.7-47.6)	40.8 (32.2-50.7)	37.4 (28.4-49.5)	57.5 (44.9-86.8)
Derived multiplex 2-gene model	NA	NA	NA	NA	NA	0.46 (0.33-0.67)	0.40 (0.28-0.59)	0.45 (0.36-0.64)	0.42 (0.31-0.60)	0.70 (0.49-0.90)
Derived multiplex 3-gene model	NA	NA	NA	NA	NA	0.45 (0.31-0.60)	0.38 (0.28-0.51)	0.53 (0.39-0.66)	0.41 (0.29-0.55)	0.59 (0.46-0.70)
MPS	37 (20-58)	26 (14-42)	42 (24-63)	29 (16-48)	51 (33-72)	35 (17-56)	26 (12-44)	42 (26-65)	30 (15-49)	55 (37-72)
MPS2[d]	0.16 (0.05-0.39)	0.07 (0.03-0.16)	0.15 (0.06-0.30)	0.07 (0.03-0.19)	0.40 (0.20-0.61)	0.13 (0.05-0.37)	0.08 (0.03-0.19)	0.20 (0.08-0.43)	0.10 (0.04-0.24)	0.44 (0.23-0.69)
MPS2+[d]	0.14 (0.05-0.42)	0.06 (0.02-0.14)	0.14 (0.06-0.32)	0.07 (0.03-0.17)	0.44 (0.22-0.68)	0.15 (0.05-0.43)	0.08 (0.03-0.21)	0.25 (0.09-0.48)	0.11 (0.04-0.30)	0.54 (0.27-0.79)

Abbreviations: DRE, digital rectal examination; GG, grade group; MPS, MyProstateScore; MPS2, MyProstateScore 2.0; MPS2+, MyProstateScore 2.0 plus prostate volume; NA, not applicable; PHI, Prostate Health Index; PSA, prostate-specific antigen.

SI conversion factor: To convert PSA to µg/L, multiply by 1.

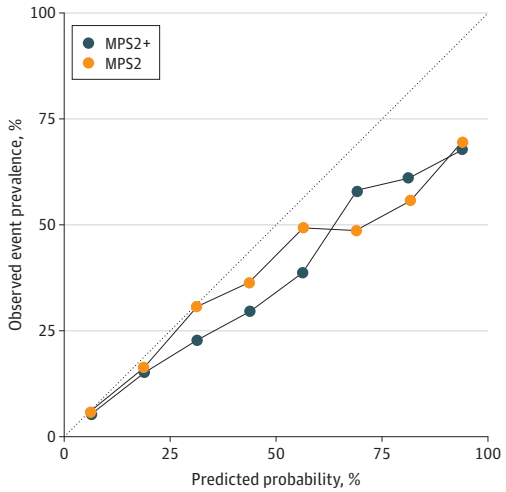
[a] Race was self-reported by participants via a questionnaire. Black race was pertinent to the current study due to the well-established association of race with prostate cancer incidence, outcomes, and tumor molecular subtypes.[26] The other race category includes American Indian or Alaska Native, Asian, Native Hawaiian or Other Pacific Islander, White, other, and unknown race.

[b] Measured by transrectal ultrasound.

[c] PSA density equals serum PSA divided by prostate volume.

[d] MPS2 and MPS2+ values are reported on a continuous scale as the likelihood of cancer of GG 2 or greater detection on biopsy.

Figure 2. Calibration Curves for High-Grade Prostate Cancer for MyProstateScore 2.0 (MPS2) and MPS2 Plus Prostate Volume (MPS2+) in the External Validation Cohort



across subgroups. For individual patients, NPVs approaching 100% provide clear guidance for confident decision-making. For clinicians, uniform use of MPS2 could avoid unnecessary biopsies while preserving immediate detection of 95% of cancers of GG 2 or greater diagnosed using the biopsy all approach. Critically, MPS2 had 99% sensitivity and 99% NPV for cancers of GG 3 or greater, meaning the rare false-negative MPS2 results were almost uniformly more favorable cancers of GG 2 least likely to metastasize.

The 2023 NCCN guidelines for PCa early detection propose consideration of prebiopsy risk stratification with validated biomarker tests, including PHI, SelectMDx, 4Kscore, EPI, MPS, and IsoPSA.[5] These tests have consistently outperformed PSA alone, with aggregate data approximating 90% to 95% sensitivity and 30% to 40% specificity for high-grade cancer.[13,28,32] However, heterogeneity of published data and a lack of head-to-head comparisons have precluded recommendations of any particular testing approach.[45] Using an NCI cohort clinically indicated for biomarker testing, we directly compared the new 18-gene test for high-grade PCa with existing guideline-endorsed tests. Broadly, AUC values for MPS2 models were associated with meaningful improvement compared with currently available options. Using a testing approach with 95% sensitivity for high-grade cancer, MPS2 would have meaningfully reduced unnecessary biopsies performed relative to other tests. These data support use of MPS2 to mitigate the potential harms of screening while preserving its long-term benefits.

Patients with a prior negative biopsy pose a unique challenge.[40] Because most patients undergo initial biopsy due to PSA elevation, the value of repeated PSA testing is particularly limited in this population.[46] In one prior study, among 229 patients undergoing repeat biopsies, the EPI test provided 82% sensitivity and 27% specificity for high-grade cancer.[47] Among 268 patients undergoing repeat biopsies, MPS provided 100% sensitivity and 23% specificity.[48] In the current analysis including 247 patients, at 94.4% sensitivity, MPS2+ provided 51% specificity, compared with 8.7% with PHI, 14% with the derived multiplex 2-gene model, 16% with the derived multiplex 3-gene model, and 15% with MPS. While striking, these findings are plausible, as most current assays include PSA and PSA isoforms, underscoring a continued dependence on PSA. Second, existing assays measure 3 or fewer non-PSA markers. Given the multiple pathways driving lethal PCa,[14,15] it is difficult to conceive that most aggressive cancers would overexpress one of so few markers early in the disease course. By capturing 17 cancer-associated, PSA-independent markers, MPS2 provides roughly 5-fold the breadth of previous tests and offers promise of a new generation of biomarkers not reliant on PSA.

The ideal diagnostic test has been described as safe, accurate, available, and actionable and providing a favorable benefit-to-harm ratio.[49,50] While PSA alone offers favorable practical attributes, its lack of cancer specificity has driven the need for a complementary test to improve screening outcomes.[4] While prebiopsy mpMRI improves detection of high-grade cancer in men with positive findings on mpMRI,[7,51] data describing the use of negative mpMRI findings to rule out significant cancer merit concern. Findings from a statewide collaborative revealed an NPV of only 77% across diverse settings.[8] Even at experienced centers, subjective MRI interpretation yields significant variability, with NPVs as low as 63% at one center and 40% among individual radiologists.[9,10] Moreover, MRI bears an extensive time and resource burden, is not widely available in community settings, and is not an option for some patients, posing critical barriers to widespread use.[11,12] While a valuable component of the diagnostic armamentarium, practical limitations and suboptimal rule-out performance suggest MRI may be best used later in the diagnostic pathway, eg, to improve the yield of biopsy in men most likely to benefit from invasive testing.

The accuracy of MPS2 offers potential for straightforward application at the primary care level (ie, negative test rules out high-grade disease; positive test prompts specialist referral). For specialists, providing patients with early noninvasive molecular tumor data[52-55] could enable more informed, individualized cancer care. For example, in patients indicated for biopsy, the relationship of tumor subtypes with MRI visibility could help identify patients likely to benefit from prebiopsy mpMRI and those better served by immediate biopsy.[56] In men with PCa of GG 1, high-grade markers could signal the presence of occult aggressive tumors, while their absence could obviate the need for scheduled surveillance biopsies.[57] Finally, while biopsy and tissue-based assays rely on the specific tumor foci sampled,[58,59] urine provides a comprehensive assessment of prostatic gene expression—an ideal complement to mitigate the sampling limitations of biopsy.

Limitations

The current study has limitations. For one, there was limited racial diversity in the study population. Thus, it is unclear how our findings could differ in Black men, and we are currently pursuing analyses to ensure optimal testing for all patients. Second, the reference standard was systematic

Table 2. Performance of Prostate-Specific Antigen (PSA) Alone, Prostate Cancer Prevention Trial Risk Calculator, Prostate Health Index (PHI), Derived Multiplex 2-Gene and 3-Gene Models, MyProstateScore (MPS), MPS2, and MPS2 Plus Prostate Volume (MPS2+) in the Validation Cohort

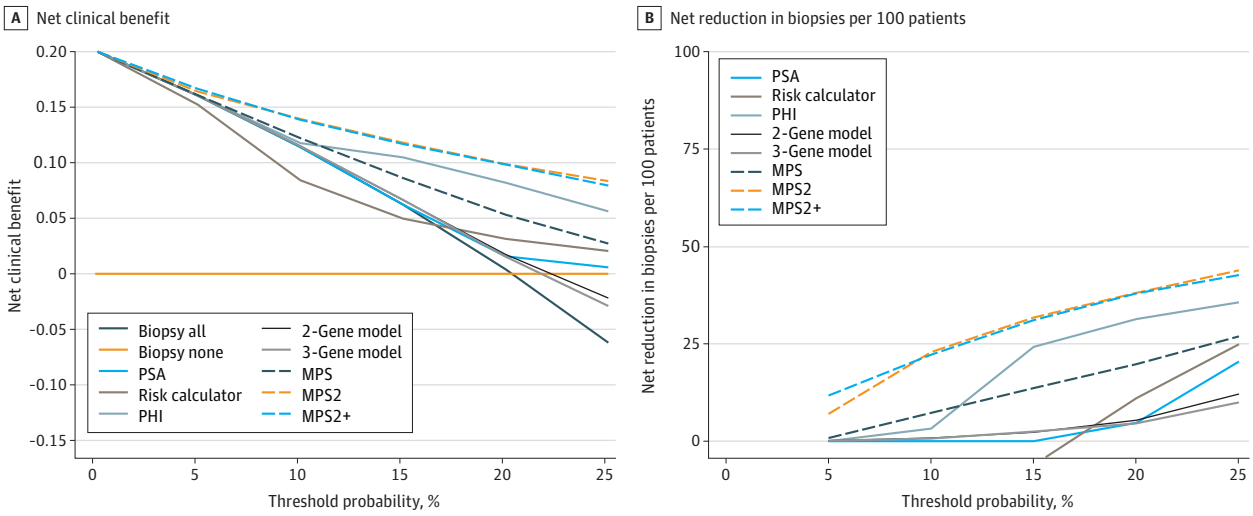
Model	%				Estimated unnecessary biopsies avoided per 1000 patients
	Sensitivity	Specificity	NPV	PPV	
Overall (n = 743)					
PSA	95	11	90	21	108
Prostate Cancer Prevention Trial risk calculator	95	20	94	23	198
PHI	95	26	96	25	258
Derived multiplex 2-gene model	95	27	96	25	270
Derived multiplex 3-gene model	95	17	94	23	171
MPS	95	23	94	24	230
MPS2	95	37	97	28	370
MPS2+	95	41	97	29	405
Initial biopsy (n = 496)					
PSA	95	15	89	29	152
Prostate Cancer Prevention Trial risk calculator	95	27	94	32	267
PHI	95	30	95	33	295
Derived multiplex 2-gene model	95	30	95	33	303
Derived multiplex 3-gene model	95	17	91	30	168
MPS	95	27	93	32	270
MPS2	95	35	95	35	347
MPS2+	95	42	96	37	419
Repeat biopsy (n = 247)					
PSA	94.4	15	97	8.0	148
Prostate Cancer Prevention Trial risk calculator	94.4	21	98	8.6	210
PHI	94.4	8.7	95	7.5	87
Derived multiplex 2-gene model	94.4	14	97	8.0	144
Derived multiplex 3-gene model	94.4	16	97	8.1	162
MPS	94.4	15	97	8.0	148
MPS2	94.4	46	99	12	462
MPS2+	94.4	51	99	13	511

Abbreviations: NPV, negative predictive value; PPV, positive predictive value.

biopsy, which is subject to undersampling that could increase NPV and decrease positive predictive value relative to surgical pathology.[60-62] Nonetheless, sampling misclassification would be expected to impact all tests equally, and we uniquely performed head-to-head comparison of MPS2 with existing biomarker tests. Furthermore, we repeated model development in patients with more definitive pathologic data (eg, radical prostatectomy), and prostatectomy-derived MPS2 models did not differ substantially (eTable 11 in Supplement 1). Notably, the current analysis used the Prostate Cancer Prevention Trial risk calculator due to its extensive validation and recognition by clinicians[63]; other risk calculators could have performed differently.[64]

We acknowledge the limitations of deriving molecular models developed on other platforms. Although the derived multiplex models capture the components of other commercially available tests, these models should not be interpreted as equivalent to the commercial assays, just as no conclusions can be drawn regarding biomarkers not assessed. Still, external comparison of a newly validated test with guideline-endorsed tests has not previously been performed, to our knowledge, and the 18-gene test would have yielded clinically meaningful improvement in accuracy for high-grade PCa relative to current testing options. While encouraging, these findings do not rule out disparate findings in additional cohorts. Moreover, the 95% sensitivity threshold is a single data point that, while illustrative and clinically applicable, may not be ideal for all populations; decision curves presented herein provide a greater breadth of information regarding utility. Finally, this study population was not suitable for comparing biomarkers with mpMRI, which remains a critical knowledge gap. We are currently conducting a prospective multicenter trial for this assessment.[65] Regardless, the externally validated performance of MPS2 supports its effectiveness in accurately ruling out the need for mpMRI and

Figure 3. Decision Curve Analysis for High-Grade Prostate Cancer in the External Validation Cohort



A, Decision curve analysis plots for net clinical benefit of prebiopsy testing with prostate-specific antigen (PSA) alone, the Prostate Cancer Prevention Trial risk calculator, Prostate Health Index (PHI), derived multiplex 2-gene model, derived multiplex 3-gene model, MyProstateScore (MPS), MPS2, and MPS2+ compared with baseline approaches of biopsy all or biopsy none. The threshold probability (x-axis) reflects how the patient and clinician value potential clinical outcomes. For example, a threshold probability of 5% applies to patients that would choose to pursue biopsy if their risk of high-grade cancer is 5% or higher. For high-grade prostate cancer, a 5% threshold probability represents a risk-averse population, such as younger men with a long life expectancy. At a practice level, this implies that the clinician would be willing to perform as many as 20 biopsies to detect an additional high-grade cancer. At the other end of the spectrum, a threshold probability of 20% applies to patients that would choose to pursue biopsy only if their risk of high-grade cancer was 20% or greater. Such a population strongly values avoiding biopsy and is willing to accept a higher risk of delayed detection of high-grade cancer. The unit of net benefit (y-axis) is true positives. A net benefit of 0.15 is equivalent to an approach in which 15 patients per 100 are directed to biopsy based on use of the test, and all 15 patients are found to have high-grade cancer. As illustrated in the figure, the MPS2 and MPS2+ models provided the highest net benefit across the range of clinically pertinent threshold probabilities (5% to 20%). B, Decision curve analysis plots illustrating the net reduction in biopsies performed per 100 patients without missing a single diagnosis of cancer of grade group 2 or greater based on prebiopsy testing with PSA alone, the Prostate Cancer Prevention Trial risk calculator, PHI, the derived multiplex 2-gene model, the derived multiplex 3-gene model, MPS, MPS2, and MPS2+ compared with a baseline approach of biopsying all patients. The MPS2 and MPS2+ models provided the largest net reduction in biopsies performed across clinically pertinent threshold probabilities.

biopsy altogether. Additional studies are needed to corroborate these data and confirm the observed positive impact of MPS2 testing on longer-term outcomes.

Conclusions

In this study, within an external validation population referred for prostate biopsy, an 18-gene urinary test had higher diagnostic accuracy for high-grade PCa beyond currently available testing options. Clinically, use of this test would have safely avoided unnecessary additional testing with imaging or biopsy in 35% to 51% of patients while maintaining high sensitivity for high-grade cancers that stand to benefit from early detection. These findings suggest that use of the test in patients with elevated PSA levels can reduce the potential harms of prostate cancer screening while preserving its long-term benefits.

ARTICLE INFORMATION

Accepted for Publication: December 6, 2023.

Published Online: April 18, 2024. doi:10.1001/jamaoncol.2024.0455

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Obtained funding: Tosoian, Xiao, Niknafs, Tomlins, Chan, Feng, Sanda, Chinnaiyan.
Administrative, technical, or material support: Tosoian, Xiao, Samora, Chopra, Siddiqui, H. Zheng, Herron, Arivoli, Palapattu, Salami, Chan, Srivastava, Feng, Sanda, Wei.
Supervision: Tosoian, Morgan, Palapattu, Srivastava, Feng, Y. Zheng, Chinnaiyan.

Conflict of Interest Disclosures: Dr Tosoian reported personal fees from LynxDx and equity interest from LynxDx outside the submitted work; and has a patent for a novel multiplex urine test for high-grade prostate cancer pending. Dr Zhang reported personal fees from LynxDx outside the submitted work and has a patent for a novel multiplex urine test for high-grade prostate cancer pending. Dr Xiao reported grants from Prostate Cancer Foundation as well as personal fees from LynxDx during the conduct of the study; and has a patent for a novel multiplex urine test for high-grade prostate cancer pending. Dr Niknafs reported personal fees from LynxDx during the conduct of the study; personal fees from LynxDx outside the submitted work; and has a patent for use of some biomarkers as diagnostic tools issued. Dr Trock reported personal fees from Artera during the conduct of the study as well as personal fees from Myriad Genetics outside the submitted work. Dr Salami reported personal fees from Bayer and NRichDx during the conduct of the study. Dr Tomlins reported grants and personal fees from Astellas as well as equity interest from Strata Oncology outside the submitted work; and has a patent for *ETS* gene fusions in prostate cancer issued and licensed to LynxDx. Dr Sokoll reported grants from the National Institutes of Health during the conduct of the study. Dr Feng reported grants from the National Cancer Institute during the conduct of the study. Dr Chinnaiyan reported grants from the National Institutes of Health/National Cancer Institute, Prostate Cancer Foundation, and Howard Hughes Medical Institute; nonfinancial support from the American Cancer Society during the conduct of the study; and equity interest from LynxDx outside the submitted work; and has a patent for a novel multiplex urine test for high-grade prostate cancer pending. No other disclosures were reported.

Funding/Support: This work was funded by the Michigan-Vanderbilt Early Detection Research Network Biomarker Characterization Center (grant U2C CA271854) and the Early Detection Research Network Data Management and Coordinating Center (grant U24 CA086368). The Early Detection Research Network Data Management and Coordinating Center carried out analyses on the blinded validation cohort. Other sources of funding not involved in the design and conduct of the study included the Michigan Prostate Specialized Program of Research Excellence (grant P50 CA186786), National Cancer Institute Outstanding Investigator Award (Dr Chinnaiyan; grant R35 CA231996), Johns Hopkins University Biomarker Reference Laboratory (grant U24 CA115102), National Cancer Institute Early Detection Research Network Clinical Validation Center (grant U01 CA113913), Prostate Cancer Foundation Young Investigator Award (Drs Tosoian and Xiao), Prostate Cancer Foundation, Howard Hughes Medical Institute (Dr Chinnaiyan), and the American Cancer Society (Dr Chinnaiyan).

Role of the Funder/Sponsor: The Early Detection Research Network Data Management and Coordinating Center members had access to the blinded validation cohort analysis; the other funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Group Information: Members of the EDRN-PCA3 Study Group appear in Supplement 4.

Meeting Presentation: These data were presented in abstract form at the American Urological Association 2023 Annual Meeting; April 28, 2023; Chicago, IL.

Previous Posting: This article was posted as a preprint on medRxiv.org.

Data Sharing Statement: See Supplement 5.

Additional Contributions: We thank Stephanie Miner, PhD (Michigan Center for Translational Pathology, University of Michigan, Ann Arbor), for her help in the editing and file preparation of this study. Dr Miner was not compensated for her work.

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