

Assessment of Long-Term Outcomes Associated With Urinary Prostate Cancer Antigen 3 and TMPRSS2:ERG Gene Fusion at Repeat Biopsy

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BACKGROUND: In men with clinically localized prostate cancer who have undergone at least 1 previous negative biopsy and have elevated serum prostate-specific antigen (PSA) levels, long-term health outcomes associated with the assessment of urinary *prostate cancer antigen 3 (PCA3)* and the *transmembrane protease, serine 2 (TMPRSS2):v-ets erythroblastosis virus E26 oncogene homolog (avian) (ERG)* gene fusion (*T2:ERG*) have not been investigated previously in relation to the decision to recommend a repeat biopsy. **METHODS:** The authors performed a decision analysis using a decision tree for men with elevated PSA levels. The probability of cancer was estimated using the Prostate Cancer Prevention Trial Risk Calculator (version 2.0). The use of PSA alone was compared with the use of PCA3 and T2:ERG scores, with each evaluated independently, in combination with PSA to trigger a repeat biopsy. When PCA3 and T2:ERG score evaluations were used, predefined thresholds were established to determine whether the patient should undergo a repeat biopsy. Biopsy outcomes were defined as either positive (with a Gleason score of <7, 7, or >7) or negative. Probabilities and estimates of 10-year overall survival and 15-year cancer-specific survival were derived from previous studies and a literature review. Outcomes were defined as age-dependent and Gleason score-dependent 10-year overall and 15-year cancer-specific survival rates and the percentage of biopsies avoided. **RESULTS:** Incorporating the PCA3 score (biopsy threshold, 25; generated based on the urine *PCA3* level normalized to the amount of *PSA* messenger RNA) or the T2:ERG score (biopsy threshold, 10; based on the urine *T2:ERG* level normalized to the amount of *PSA* messenger RNA) into the decision to recommend repeat biopsy would have avoided 55.4% or 64.7% of repeat biopsies for the base-case patient, respectively, and changes in the 10-year survival rate were only 0.93% or 1.41%, respectively. Multi-way sensitivity analyses suggested that these results were robust with respect to the model parameters. **CONCLUSIONS:** The use of PCA3 or T2:ERG testing for repeat biopsy decisions can substantially reduce the number of biopsies without significantly affecting 10-year survival. *Cancer* 2015;121:4071-9. © 2015 American Cancer Society.

KEYWORDS: prostate cancer antigen 3, prostate cancer, repeat biopsy, survival, TMPRSS2-ERG, transmembrane protease, serine 2-ETS-related gene fusion, urinary biomarkers.

INTRODUCTION

Commonly used diagnostic indicators for the early detection of prostate cancer (PCa) include an abnormal digital rectal examination (DRE) and an elevated prostate-specific antigen (PSA) level. Serum PSA levels from >2.5 to 4 ng/mL and/or suspicious DRE results may indicate the presence of PCa; however, the evaluation PSA alone, with a cutoff of 4 ng/mL, reportedly yielded a positive predictive value of only 24% to 37%,^{1,2} and up to 75% of these men had a negative first biopsy.^{3,4} Furthermore, PCa was detected in 10% to 35% of men who had a negative first biopsy.^{3,4} In clinical practice, it is often uncertain whether or not men who have clinically localized PCa and prior negative biopsy findings should undergo a repeat biopsy. For men who have a negative first biopsy but persistently high PSA levels, the European Association of Urology⁵ guidelines recommend a prostate biopsy; however, among men who have a suspicion of PCa and a prior negative biopsy, approximately 80% of repeat biopsies reportedly are negative. In addition to being costly, biopsies are associated with morbidity, anxiety, discomfort, and complications.³ New biomarkers may increase the diagnostic accuracy of repeat biopsies and reduce the number of unnecessary biopsies, but their impact on long-term health outcomes remains unclear.

Results from recent studies have demonstrated the potential clinical utility of the urine-based PROGENSA prostate cancer antigen 3 (PCA3) assay (Gen-Probe Inc, San Diego, Calif) to predict repeat biopsy outcomes in men with elevated

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serum PSA levels and previous negative biopsy findings.⁶⁻¹⁵ Those results indicate that an increasing PCA3 score corresponds to an increasing probability of a positive repeat biopsy. Some studies have demonstrated that the PCA3 test is superior to serum PSA measurement in predicting biopsy outcome,^{6,16,17} and the test has been included in recently developed nomograms.¹⁸⁻²⁰ A recent literature review reported current evidence suggesting that the PCA3 test is clinically useful for selecting which patients should undergo repeat biopsy.²¹ Several studies have determined that urine assessment of the *transmembrane protease, serine 2 (TMPRSS2):v-ets erythroblastosis virus E26 oncogene homolog (avian) (ERG) gene fusion (T2:ERG)* is also associated with biopsy outcome²²⁻²⁸ and may be better at discriminating between low-grade and high-grade cancers.²² Although there are studies supporting increased diagnostic accuracy for both biomarkers, the ideal thresholds to trigger a repeat biopsy and the resulting increase in survival and decrease in unnecessary biopsies remain unknown.

We used decision analysis to evaluate the clinical value of PCA3 and T2:ERG scores in men with clinically localized PCa who had at least 1 prior negative biopsy. We performed head-to-head comparisons of protocols that used either PCA3 or T2:ERG in combination with PSA in terms of the incremental change in 10-year overall survival and the rate of negative biopsies. Furthermore, we considered 15-year cancer-specific survival as an endpoint in our analyses. Here, we present results for both expected 10-year survival and 15-year cancer specific survival and the repeat biopsy rate for each biomarker. We also present results from our sensitivity analysis of clinical variables, such as PSA level, the biopsy detection rate, and patient age, to provide evidence regarding which patients benefit most from the use of an additional biomarker.

MATERIALS AND METHODS

Study Population

The decision-analysis model for this study was based on results from a prospectively collected cohort design. For the study cohort, post-DRE urine was prospectively collected from 1977 men who presented for diagnostic prostate biopsies at 3 US academic institutions ($n = 733$) and 7 community clinics ($n = 1244$). The vast majority of men had elevated levels of serum PSA. Because this cohort reflected actual clinical practice, no specific indication for repeat biopsy was required; however, for the vast majority of the cohort, the repeat biopsy was triggered by persistently ele-

vated serum PSA levels. Exclusion criteria included the following: previously attempted curative therapy (radical prostatectomy, radiation therapy, androgen-deprivation therapy, or brachytherapy), surgical treatment of the prostate within 6 months of urine collection (or previous biopsy within 6 weeks), receipt of 5 α -reductase inhibitors or testosterone within 3 months of urine collection, or prostatitis at the time of urine collection. All urine specimens were obtained with institutional review board approval.

Specimen Collection and Processing: Urine T2:ERG and PCA3 Assay Procedure

Urine processing for the determination of PCA3 and T2:ERG scores was performed as described in previous studies.^{22,23,29} Urine specimens were obtained immediately after attentive DRE, refrigerated, processed within 4 hours by mixing with an equal volume of urine transport medium, and stored below -70°C until they were analyzed. Amounts of urine *PCA3*, *T2:ERG*, and *PSA* messenger RNA (mRNA) were determined using transcription-mediated amplification assays. To generate a T2:ERG score, the amount of *T2:ERG* mRNA was normalized to the amount of *PSA* mRNA, which was calculated using the following formula: $(100,000 \times \text{average urine } TMPRSS2:ERG \text{ copies/mL}) / (\text{average urine PSA copies/mL})$. Samples with an average of $>10,000$ urine *PSA* copies/mL were considered informative for urine T2:ERG scores. Urine T2:ERG scores were assessed as described previously using the final T2:ERG transcription-mediated amplification assay^{23,27,29} or an earlier generation assay²² that yielded equivalent T2:ERG scores.

The PROGENSA PCA3 assay similarly quantitates *PCA3* and *PSA* mRNA in post-DRE urine. The PCA3 score was calculated with the following formula: $1000 \times (\text{average urine } PCA3 \text{ copies/mL}) / (\text{average urine } PSA \text{ copies/mL})$. Samples with average urine *PSA* copies/mL $>10,000$ copies/mL were considered informative. Identical primers for quantifying urine *PSA* are used in the PROGENSA PCA3 assay and the T2:ERG assay.

All urine PCA3 and T2:ERG analyses were performed at the University of Michigan or at Gen-Probe, Inc, with a subset of samples assessed at both locations to ensure concordance. In total, 1936 urine samples had sufficient urine *PSA* mRNA ($>10,000$ copies/mL) to provide informative PCA3 and T2:ERG scores, and these samples were considered for analysis. The final study population consisted of 140 men who had informative urine PCA3 and T2:ERG scores and a history of at least 1 previous negative biopsy and who were diagnosed with PCa in their study biopsy.

Decision Tree

We constructed a decision tree for patients with elevated PSA levels to compare the expected 10-year survival and 15-year cancer-specific survival for protocols that use 1 of the urinary biomarkers versus those that do not. The complete decision-tree schema is illustrated in Supporting Figure 1 (see online supporting information). The initial decision is whether to use an additional biomarker (yes or no). If no additional biomarker is used, then we consider 2 cases: repeat biopsy or no repeat biopsy. Therefore, the decision tree has 3 separate decision branches. *Branch 1* represents the protocols that incorporate a urinary biomarker into repeat biopsy decisions; *branch 2* represents the protocol that does not involve any additional indication for repeat biopsy (thus, it is assumed that every patient undergoes a biopsy regardless of his clinical parameters [age, serum PSA level, etc]); and *branch 3* represents the protocol in which no patient undergoes a repeat biopsy.

In the decision tree, men who have detected and undetected, clinically localized PCa are assumed to have a 10-year survival rate consistent with that of men who undergo radical prostatectomy at diagnosis and men who receive conservative treatment (who are managed without surgery or radiation), respectively. Although the decision tree focuses on a 1-time repeat biopsy decision, occurrences of delayed biopsy, histologic reclassification, and future treatment are reflected in the survival estimates. The risk of PCa was derived from the Prostate Cancer Prevention Trial Risk Calculator, version 2.0 (PCPTRC 2.0), which incorporates age, race, PSA level, family history of PCa, DRE, and history of a negative biopsy.³⁰ The decision tree accounts for the different tumor grades based on each patient's Gleason score (GS) (GS <7, 7, and >7). The probability for each grade was estimated based on the proportion of each outcome in the study population.

The biopsy decision in branch 1 of the decision tree is determined by a prespecified threshold for the urinary biomarker. The probability that the biomarker score exceeds this threshold is grade-dependent and is estimated from the study population (Supporting Table 1; see online supporting information). In our analyses, the probability of a positive repeat biopsy is estimated according to Haas et al.³¹ The primary endpoint of each branch is the 10-year overall survival rate estimated according to Tewari et al.³² and depends on tumor grade, patient age, serum PSA level, race, and Charlson comorbidity index (CCI). We did not have CCI data for the patients in our study cohort; thus, we assumed that they were healthy men with a CCI that ranged from 0 to 1. Outcomes for patients

without PCa also were estimated according to Tewari et al.³² The look-up tables for 10-year overall survival were constructed separately for black and white men; however, Tewari et al.³² did not identify race as an independent predictor of survival, and most of the patients in our study population were white. Thus, we considered the 10-year overall survival estimates in white men with clinically localized PCa.

We conducted similar analyses using 15-year PCa-specific survival as the primary endpoint in the decision tree. We used cancer-specific survival because, to our knowledge, there is no study in the literature that estimates 15-year overall survival. We obtained the 15-year cancer-specific survival estimates for men with untreated, clinically localized PCa from Johansson et al.³³ and the 15-year cancer-specific survival estimates after radical prostatectomy from Stephenson et al.³⁴

There is no consensus about the most appropriate thresholds for the PCA3 and T2:ERG tests. The US Food and Drug Administration recommends a PCA3 threshold of 25, but a threshold of 35 is commonly used.^{6-8,15,33-36} Although some studies have indicated that a cutoff score of 25 provides a good balance between sensitivity and specificity,³⁷⁻⁴⁰ others have supported the use of different thresholds, eg 17,^{18,19} 43,⁴¹ and 51.²⁰ In our current study, we considered thresholds of 25, 35, and 100 for PCA3. For the T2:ERG threshold, Tomlins et al.²² considered specimens with T2:ERG scores >50 as positive, and Leyten et al.²⁸ used a threshold of 10 in their multivariate regression analysis although these assays were not the same as those used herein. In the current study, to provide a diverse set of thresholds, we considered T2:ERG thresholds of 7, 10, 30, 50, and 100.

Survival Estimates

We conducted a literature review to obtain estimates of overall survival in men with clinically localized PCa. Relevant studies were based on retrospective cohorts of men with clinically localized PCa who were not screened for PSA and who had survival outcomes reported. Several use nomograms (for example, see Cowen et al.⁴²) that could not be adapted to our study because complete information for all clinical variables was not available for the study cohort. Some reports, such as that by Walz et al.,⁴³ lacked the clinicopathologic information that was used in our analysis. Albertsen et al.⁴⁴ reported survival outcomes, but their study considered men aged 66 years and older, and 38% of our study population was younger than age 66 years. Therefore, for our analysis, we used the overall 10-year survival estimates reported by Tewari et al.,³² which

quantify the impact of treatment modality on the overall survival of men with clinically localized PCa.

The 15-year survival estimates reported by Johansson et al³³ were based on a cohort of patients who had early, untreated PCa before the PSA screening era and were given for grade 1, 2, and 3 tumors according to the *World Health Organization Classification of Malignant Diseases*. We interpreted grade 1 PCa tumors as GS 2 through 4 PCa, grade 2 tumors as GS 5 through 7 PCa, and grade 3 tumors as GS 8 through 10 PCa, as noted by Johansson et al.³³ The 15-year cancer-specific, postprostatectomy survival estimates published by Stephenson et al³⁴ were derived from a study cohort of patients who underwent radical prostatectomy for localized PCa during the era of PSA screening and were given according to GS as <7, 7, and >7. We assumed that the 15-year survival rate without PCa was same as the 15-year cancer-specific survival rate for GS 2 through 4 PCa, as estimated from Johansson et al.³³ Additional details about the analysis are provided in the online supporting information.

Probabilistic Sensitivity Analyses

We conducted multi-way, probabilistic sensitivity analyses around model parameters (biopsy sensitivity, sensitivity of biomarkers at different thresholds for different tumor grades, and 10-year survival under different treatments) and clinical parameters (serum PSA and age). We did not conduct multi-way sensitivity analyses representing the uncertainty around clinical parameters in the analysis of 15-year cancer-specific survival, because 15-year survival estimates are not available by PSA and age. Additional details about the ranges and assumed distributions are provided in the online supporting information. We sampled the parameters 1000 times drawn from independent distributions and computed the additional 10-year survival and percentage of men biopsied for each resulting decision tree. We chose 4 and 30 ng/mL as the lower and upper bounds for serum PSA, respectively, and 50 and 75 years as the lower and upper bounds for age, respectively.

RESULTS

Study Population

Table 1 provides the characteristics of the 420 men who had previous negative biopsies. The men with positive biopsies were older (statistically significant), had lower prostate volumes, and had higher mean PCA3 and T2:ERG scores than the men with negative repeat biopsies. Mean serum PSA levels did not change significantly between men with negative versus positive biopsies.

Among the men who had positive biopsies, 78.6% and 20% had clinical stage T1 and T2 tumors, respectively; 88.6% had a biopsy GS of 6 or 7; and 75% had $\leq 33\%$ positive biopsy cores.

Additional details of the study population are provided in the online supporting information. Supporting Table 1 presents our estimates of the probability that a man's biomarker scores will exceed different thresholds based on his grade of PCa (see online supporting information). Among 420 men with stage T1 or T2 PCa, 140 (33.3%) had cancer on repeat biopsy. Of the 140 men who had a positive repeat biopsy, 82 (58.6%) had GS <7 PCa, 42 (30%) had GS 7 PCa, and 16 (11.4%) had GS >7 PCa. On the basis of univariate analysis, all prebiopsy clinical variables were associated with a positive repeat biopsy ($P < .04$; data not shown). PCA3 demonstrated the highest accuracy in predicting a positive repeat biopsy (area under the concentration-time curve, 0.652) compared with PSA (area under the concentration-time curve, 0.54), as detailed in Supporting Table 2 (see online supporting information).

Supporting Table 3 lists PCa detection rates for various PSA, PCA3, and T2:ERG thresholds; the number of prostate biopsies that would be avoided; and the number of GS ≥ 7 cancers that would be missed if a urinary biomarker (PCA3 or T2:ERG) were used to select men for repeat biopsies. PCA3 thresholds of ≥ 25 and ≥ 35 would detect 95 (67.9%) and 69 (49.3%) cancers, respectively; and T2:ERG thresholds of ≥ 7 and ≥ 10 were similar, detecting 78 (55.7%) and 71 (50.7%) cancers, respectively. A PCA3 threshold of ≥ 25 would identify 42 of 58 (72.4%) GS ≥ 7 cancers and would avoid 52.4% of repeat biopsies; and a PCA3 threshold of ≥ 35 would identify 32 (55.2%) GS ≥ 7 cancers, but 66.4% of all biopsies could have been avoided. Similarly, a T2:ERG threshold of ≥ 7 would identify 35 of 58 (60.3%) GS ≥ 7 cancers and would avoid 56.2% of repeat biopsies; and a T2:ERG threshold of ≥ 10 would identify 33 (56.9%) GS ≥ 7 cancers, but 62.1% of all biopsies could have been avoided.

Base-Case Analysis

We considered a base-case patient with the following characteristics: white, age 65 years, the most recent serum PSA was 6.3 ng/mL (based on the mean PSA of patients in the study cohort), a CCI of 0, no family history of PCa, normal DRE, and a previous negative biopsy. Table 2 presents 10-year survival and biopsy rates for the protocols with various biopsy thresholds. Table 2 indicates that branch 2 (repeat biopsy) would yield better 10-year survival than branch 1 (biomarker at repeat biopsy) under

TABLE 1. Baseline Characteristics of the Study Population

Characteristic	Mean \pm SD or No. (%)		P	Mean \pm SD or No. (%) All Men, n = 420
	Men With Negative Biopsies, n = 280	Men With Positive Biopsies, n = 140		
Age, y	65.4 \pm 8.1	68.2 \pm 8.9	.002 ^a	66.3 \pm 8.5
Serum PSA, ng/mL	7.2 \pm 5.4	8.4 \pm 6.5	.0766 ^b	7.5 \pm 5.8
<4	55 (19.6)	24 (17.1)		79 (18.8)
4-10	177 (63.2)	83 (59.3)		260 (61.9)
>10	48 (17.1)	33 (23.6)		81 (19.3)
Ethnicity			.0113 ^c	
African American	12 (4.3)	15 (10.7)		27 (6.4)
Other	268 (95.7)	125 (89.3)		393 (93.6)
DRE result			.0783 ^c	
Normal	242 (86.4)	110 (78.6)		352 (83.8)
Abnormal	33 (11.8)	28 (20)		61 (14.5)
Not available	5 (1.8)	2 (1.4)		7 (1.7)
Prostate volume ^d	69.3 \pm 39.8	58.2 \pm 33.1	.0016 ^b	65.6 \pm 38.0
PCA3 ^e	32 \pm 36.4	61 \pm 78.4	< .0001 ^b	41.7 \pm 55.8
T2:ERG ^f	32.8 \pm 110.4	127.5 \pm 678.3	.0006 ^b	64.4 \pm 403.4

Abbreviations: DRE, digital rectal examination; PCA3, prostate cancer antigen 3; PSA, prostate-specific antigen; SD, standard deviation; T2:ERG, the transmembrane protease, serine 2 (TMPRSS2): v-ets erythroblastosis virus E26 oncogene homolog (avian) (ERG) fusion.

^aThis P value was determined using the t test.

^bThis P value was determined using the Wilcoxon rank-sum test.

^cThis P value was determined using the chi-square test.

^dInformation on prostate volume was available for 273 men with negative biopsy, for 136 men with positive biopsy, and for 409 men overall.

^eThe PCA3 score was generated based on the urine PCA3 level normalized to the amount of PSA messenger RNA.

^fThe T2:ERG score was generated based on the urine T2:ERG level normalized to the amount of PSA messenger RNA.

TABLE 2. Ten-Year Survival and the Percentage of Men Biopsied for the Base-Case Patient at Various Biopsy Thresholds for Prostate Cancer Antigen 3 and the TMPRSS2:ERG Gene Fusion

Biomarkers at Different Thresholds ^a	Percentage of Men Biopsied at This Threshold	10-Year Survival, %	Percentage Change in Survival (95% CI)	
			Branch 1 vs Branch 2 ^b	Branch 1 vs Branch 3 ^c
Branch 1 ^d				
PCA3				
≥25	44.63	83.98	0.93 (0.66–1.14)	2.00 (1.42–2.45)
≥35	31.25	83.44	1.47 (1.04–1.78)	1.46 (1.04–1.79)
T2:ERG				
≥7	42.05	83.63	1.27 (0.91–1.56)	1.65 (1.17–2.03)
≥10	35.95	83.50	1.41 (1.00–1.73)	1.51 (1.07–1.86)
≥30	23.70	83.03	1.88 (1.33–2.30)	1.05 (0.75–1.29)
≥50	19.28	83.84	2.07 (1.47–2.53)	0.86 (0.61–1.06)
≥100	10.11	82.54	2.36 (1.68–2.90)	0.57 (0.40–0.70)
Branch 2 ^e	100	84.91	—	—
Branch 3 ^f	0	81.98	—	—

Abbreviations: CI, confidence interval; PCA3, prostate cancer antigen 3; T2:ERG, transmembrane protease, serine 2-ETS-related fusion gene.

^aPCA3 and T2:ERG scores were generated based on urine PCA3 or T2:ERG levels normalized to the amount of PSA messenger RNA.

^bThis value is the absolute difference between branches 1 and 2. Numbers in parentheses were calculated using 95% CIs estimated according to Tewari 2004³² for 10-year survival after radical prostatectomy and conservative management.

^cThis value is the absolute difference between branches 1 and 3. Numbers in parentheses were calculated using 95% CIs estimated according to Tewari 2004³² for 10-year survival after radical prostatectomy and conservative management.

^dBranch 1 represents protocols that incorporate a urinary biomarker into repeat biopsy decisions.

^eBranch 2 represents protocols that do not involve any additional indication for repeat biopsy.

^fBranch 3 represents protocols in which no patients undergo repeat biopsy.

every protocol with various PCA3 and T2:ERG thresholds. Similar results were obtained in the analysis of 15-year cancer-specific survival (Supporting Table 4; see online supporting information).

Probabilistic Sensitivity Analyses

Our multi-way, probabilistic sensitivity analyses consisted of 2 steps. The first step involved varying the model parameters. The results summarized in Table 3 indicate that

TABLE 3. Multi-way, Probabilistic Sensitivity Analysis Representing the Uncertainty Around Model Parameters

Biomarkers at Different Thresholds ^a	Percentage of Men Biopsied at This Threshold (95% CI)	10-Year Survival (95% CI), %	Percentage Change in Survival (95% CI)	
			Branch 1 vs Branch 2 ^b	Branch 1 vs Branch 3 ^c
Branch 1 ^d				
PCA3				
≥ 25	45.46 (44.91-46.01)	83.91 (83.83-84)	0.92 (0.91-0.94)	1.99 (1.96-2.01)
≥ 35	32.36 (31.92-32.79)	83.38 (83.30-83.47)	1.45 (1.43-1.48)	1.45 (1.43-1.48)
T2:ERG				
≥ 7	42.82 (42.29-43.35)	83.57 (83.48-83.65)	1.27 (1.25-1.29)	1.66 (1.63-1.68)
≥ 10	36.79 (36.35-37.30)	83.43 (83.35-83.52)	1.40 (1.38-1.42)	1.52 (1.50-1.54)
≥ 30	24.59 (24.23-24.95)	82.97 (82.89-83.05)	1.87 (1.84-1.89)	1.05 (1.04-1.07)
≥ 50	21.93 (21.60-22.27)	82.79 (82.71-82.87)	2.05 (2.02-2.07)	0.86 (0.85-0.88)
≥ 100	8.54 (8.74-8.35)	82.50 (82.41-82.58)	2.34 (2.31-2.37)	0.58 (0.57-0.59)
Branch 2 ^e	100	84.84 (84.75-84.93)	—	—
Branch 3 ^f	0	81.93 (81.84-82.01)	—	—

Abbreviations: CI, confidence interval; PCA3, prostate cancer antigen 3; T2:ERG, transmembrane protease, serine 2-ETS-related fusion gene.

^aPCA3 and T2:ERG scores were generated based on urine PCA3 or T2:ERG levels normalized to the amount of PSA messenger RNA.

^bThe difference is calculated as the absolute difference between branches 1 and 2 in the decision tree.

^cThe difference is calculated as the absolute difference between branches 1 and 3 in the decision tree.

^dBranch 1 represents protocols that incorporate a urinary biomarker into repeat biopsy decisions.

^eBranch 2 represents protocols that do not involve any additional indication for repeat biopsy.

^fBranch 3 represents protocols in which no patients undergo repeat biopsy.

the confidence interval for each protocol was relatively narrow, and the magnitude of effect difference for each protocol was not changed when uncertainty was incorporated for the base-case patient. In the second step, we performed a sensitivity analysis in which we included the uncertainty around serum PSA level and age of the base-case patient and varied the model parameters (Table 4). Multi-way sensitivity analyses demonstrated that branch 2 (repeat biopsy) yielded better 10-year survival than branch 1 (biomarker at repeat biopsy) under every protocol with various PCA3 and T2:ERG thresholds. Similar results were obtained in the analysis of 15-year cancer-specific survival (Supporting Table 5; see online supporting information).

DISCUSSION

There is no definitive criterion for deciding whether to perform a repeat prostate biopsy. Typically, the decision for a repeat biopsy is based on serum PSA measurement and DRE findings. The use of diagnostic biomarkers like urine PCA3 and T2:ERG may help clinicians make better decisions about repeat biopsies. In this respect, PCA3 and T2:ERG have demonstrated promising results, and the studies available in the literature support use of the PCA3 test for patients with persistent suspicions of PCa who have had previous negative biopsy results. However, those studies were focused on diagnostic performance and not

on health outcomes. In the current study, we investigated the value of PCA3 and T2:ERG for improving overall 10-year survival and reducing unnecessary repeat biopsies in the challenging subgroup of patients with previous negative biopsies and persistently elevated PSA levels.

On the basis of multi-way sensitivity analyses for the base-case patient, protocols that used a PCA3 threshold of ≥ 25 and a T2:ERG threshold of ≥ 10 to decide in favor of a repeat biopsy resulted in 54.4% and 63.2% reductions, respectively, in the total number of biopsies performed compared with protocols that indicated a biopsy for every man with a suspicion of PCa; whereas the losses in 10-year survival were 0.9% and 1.4%, respectively. Multi-way sensitivity analyses in which we varied the base-case patient's age and serum PSA level in addition to the model parameters demonstrated that incorporating PCA3 or T2:ERG into repeat biopsy decisions produced large reductions in the total number of biopsies (53.2% and 62% reductions with a PCA3 threshold of ≥ 25 and a T2:ERG threshold of ≥ 10 , respectively) and resulted in a small change ($< 2.1\%$) in 10-year overall survival compared with the case in which every man underwent a biopsy. Reductions in the number of biopsies increased as the threshold for biomarkers increased, whereas the loss in 10-year survival also increased slightly. In the analysis of 15-year cancer-specific survival, multi-way sensitivity analyses for the base-case patient revealed that the

TABLE 4. Multi-way Probabilistic Sensitivity Analyses Representing the Uncertainty Around Model and Clinical Parameters (Serum Prostate-Specific Antigen and Age)

Biomarkers at Different Thresholds ^a	Percentage of Men Biopsied at the Threshold (95% CI)	10-Year Survival (95% CI), %	Percentage Change in Survival (95% CI)	
			Branch 1 vs Branch 2 ^b	Branches 1 vs Branch 3 ^c
Branch 1 ^d				
PCA3				
≥25	46.80 (46.27-47.32)	82.16 (81.72-82.61)	1.36 (1.31-1.42)	2.92 (2.82-3.03)
≥35	33.53 (33.10-33.96)	81.38 (80.92-81.85)	2.14 (2.22-2.06)	2.14 (2.06-2.22)
T2:ERG				
≥7	43.55 (43.04-44.07)	81.65 (81.19-82.11)	1.88 (1.81-1.95)	2.41 (2.32-2.50)
≥10	37.99 (37.51-38.47)	81.44 (80.98-81.91)	2.09 (2.01-2.17)	2.20 (2.12-2.28)
≥30	25.47 (25.12-25.83)	80.77 (80.29-81.26)	2.75 (2.65-2.86)	1.53 (1.48-1.59)
≥50	22.66 (22.34-22.98)	80.49 (80-80.98)	3.04 (2.92-3.15)	1.25 (1.20-1.30)
≥100	9.34 (9.13-9.54)	80.07 (79.57-80.57)	3.46 (3.59-3.33)	0.83 (0.80-0.86)
Branch 2 ^e	100	83.53 (83.12-83.94)	—	—
Branch 3 ^f	0	79.24 (78.71-79.77)	—	—

Abbreviations: CI, confidence interval; PCA3, prostate cancer antigen 3; T2:ERG, the transmembrane protease, serine 2 (TMPRSS2); v-ets erythroblastosis virus E26 oncogene homolog (avian) (ERG) fusion.

^a PCA3 and T2:ERG scores were generated based on urine PCA3 or T2:ERG levels normalized to the amount of PSA messenger RNA.

^b The difference is calculated as the absolute difference between branches 1 and 2 in the decision tree.

^c The difference is calculated as the absolute difference between branches 1 and 3 in the decision tree.

^d Branch 1 represents protocols that incorporate a urinary biomarker into repeat biopsy decisions.

^e Branch 2 represents protocols that do not involve any additional indication for repeat biopsy.

^f Branch 3 represents protocols in which no patients undergo repeat biopsy.

protocols using a PCA3 threshold of ≥ 25 and a T2:ERG threshold of ≥ 10 to decide in favor of a repeat biopsy resulted in losses in 15-year cancer-specific survival of 1.5% and 2.5%, respectively. Similar to the 10-year overall survival analysis, reductions in the number of biopsies increased as the threshold for biomarkers increased, and the loss in 15-year cancer-specific survival also increased slightly.

In our study, we did not address the cost implications of protocols that incorporated biomarkers into repeat biopsy decisions. Some insight can be gained by considering the cost of biomarkers and biopsy in branch 1 of the decision tree. The cost of a biopsy and a PSA test would be approximately \$904⁴⁵ and \$31,⁴⁶ respectively. There is no established, independent cost for a T2:ERG test; therefore, we assumed that the institutional costs of testing for PCA3 and T2:ERG markers would be the same, and we used the bundled cost of \$749 for the Mi-Prostate Score (or MiPS; developed at the University of Michigan Comprehensive Cancer Center; commercially available from the University of Michigan MLabs, Ann Arbor, Mich), which is an early detection test for PCa that combines PSA, PCA3, and T2:ERG.⁴⁷ On the basis of these cost estimates, the expected cost of using PCA3 with a threshold of 25 and T2:ERG with threshold of 10 would be \$782 and \$753, respectively, compared with \$904 for branch 2 in the decision tree.

This study has some limitations. We examined a relatively small proportion of patients with clinically insignificant PCa, which raises the question of whether our study cohort is representative. However, we need to emphasize that the data were prospectively collected from multiple centers; thus, selection bias is minimal. Additional limitations of this study are related to model inputs, such as the use of 10-year survival and 15-year cancer-specific survival estimates and biopsy sensitivity. Limitations of the studies that provided estimates of overall survival include nonrandomized treatment assignment and retrospective design. Also, the studies evaluated overall survival within 10 years of treatment, and a cohort of patients with longer follow-up (>10 years) would provide more accurate estimates of long-term outcomes. We assumed that overall survival was independent of biomarker test scores, because there were no studies providing survival estimates that considered PCA3 and T2:ERG test results. An alternative would be to base the model on expected lifespan or quality-adjusted lifespan rather than survival; however, such considerations would require a Markov model, which would require many assumptions about follow-up to the repeat biopsy decision.

These limitations notwithstanding, our study has several strengths as well as important clinical and policy implications regarding application of the PCA3 assay and T2:ERG in repeat biopsy decisions. We performed a

head-to-head comparison of these biomarkers in providing supporting information to guide repeat biopsy decisions, and the PCA3 assay and T2:ERG appeared to provide an incremental improvement in the ability to increase the specificity while resulting in a slight decrease in overall 10-year survival relative to the case in which every man undergoes a biopsy regardless of his clinical parameters. In addition to the effect on health care use, avoiding unnecessary repeat biopsies will reduce the discomfort, pain, and other complications associated with repeat biopsies.

Conclusions

In this study, for the first time, the value of using PCA3 and T2:ERG in the diagnosis of PCa at repeat biopsy was investigated by comparing losses in overall survival with gains in the repeat biopsy rate. The results suggest that PSA alone is ineffective for recommending patients undergo repeat biopsy after previous negative biopsy results. The addition of PCA3 or T2:ERG tests to the decision-making process for recommending a repeat biopsy can reduce the number of biopsies substantially; however, this is associated with some reduction in 10-year overall survival and 15-year cancer-specific survival. Decisions about whether to use PCA3 or T2:ERG at repeat biopsy should weigh these competing considerations.

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CONFLICT OF INTEREST DISCLOSURES

Dr. Tomlins reports personal fees from Roche/Ventana Medical Systems outside the submitted work; also, he is a coinventor on a patent issued to the University of Michigan on ETS gene fusions in prostate cancer, with royalties paid to Hologic/Gen-Probe and Roche/Ventana Medical Systems.

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