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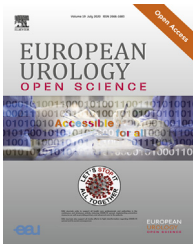

European Association of Urology

Prostate Cancer

Association of Urinary MyProstateScore, Age, and Prostate Volume in a Longitudinal Cohort of Healthy Men: Long-term Findings from the Olmsted County Study

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Abstract

Background: Serum prostate-specific antigen (PSA), used in prostate cancer screening, is nonspecific for cancer and is affected by age and prostate volume. More specific biomarkers could be more accurate for early detection of prostate cancer and reduce unnecessary prostate biopsies.
Objective: To evaluate the association of age and prostate volume with urinary MyProstateScore (MPS) in a screened, longitudinal cohort without evidence of prostate cancer.
Design, setting, and participants: The Olmsted County Study included men aged 40–79 yr who underwent biennial prostate cancer screening. PSA $\geq$4.0 ng/ml or abnormal rectal examination triggered prostate biopsy, and patients with cancer were excluded. The remaining men submitted urinary specimens for PCA3 and TMPRSS2:ERG testing.
Outcome measurements and statistical analysis: MPS was calculated using the validated, locked model for grade group $\geq$2 cancer that includes serum PSA, urinary PCA3, and urinary TMPRSS2:ERG. The associations of age and volume with biomarkers were assessed in multivariable regression models. The *t* statistic was used to quantify the strength of associations independent of the unit of measurement, and R^2 values were used to estimate the proportion of biomarker variance explained by each factor.

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Results and limitations: The study included 314 screened men without evidence of cancer. In multivariable models including age and volume, PCA3 score was significantly associated with age ($t = 7.51$; $p < 0.001$), while T2:ERG score was not associated with age or volume. MPS was significantly associated with both age ($t = 7.45$; $p < 0.001$) and volume ($t = 3.56$; $p < 0.001$), but accounting for age alone explained the variability observed ($R^2 = 0.29$) in a similar way to the model including age and volume ($R^2 = 0.31$). The variability of PCA3, T2:ERG, and MPS was less dependent on age and volume than the variability for PSA ($R^2 = 0.45$).

Conclusions: In a cohort of longitudinally screened men without evidence of cancer, we found that MPS demonstrated less variability with noncancer factors (age, prostate volume) than PSA did. These findings support the biology of these markers as more cancer-specific than PSA and highlight their promise in reducing the morbidity associated with PSA-based screening.

Patient summary: In a group of men with no evidence of prostate cancer, we found that each of three urine-based markers of cancer—PCA3, T2:ERG, and the commercially available MyProstateScore test—showed less variability with noncancer factors (age and prostate volume) than serum PSA (prostate-specific antigen) did. These findings support their proposed use as noninvasive markers of prostate cancer that could improve the accuracy of early detection.

1. Introduction

The emergence of prostate-specific antigen (PSA)-based screening in the early 1990s led to a dramatic rise in the incidence of prostate cancer (PCa) [1]. Although PSA is specific for prostate tissue, it is not specific for cancer, leading to frequent PSA elevation in the absence of cancer [2]. This has resulted in high incidence of negative, unnecessary prostate biopsies [3]. Furthermore, PSA testing was adopted with a universal threshold of 4.0 ng/ml for biopsy [4], despite expected variability by age, race, and prostate volume [5–8]. The at-risk population would benefit greatly from thoughtful adoption of newer cancer-specific biologic markers.

PCA3 (a noncoding RNA) is one of the most widely studied molecular markers of PCa, with a body of literature supporting its association with clinically significant cancer and other oncologic endpoints. More recently, the TMPRSS2:ERG gene fusion (T2:ERG) has emerged as the most PCa-specific marker reported to date, with >99.99% specificity for cancer on immunohistochemistry studies. As both PCA3 and T2:ERG are detectable in urine, they have been combined with serum PSA as the clinically available MyProstateScore (MPS) test (LynxDx, Ann Arbor, MI, USA; previously called MiPS high-grade). Validation studies have demonstrated that MPS significantly improves diagnostic accuracy for Gleason grade group (GG) ≥2 cancer relative to PSA-based risk calculators [9–12], thereby limiting the use of unnecessary biopsy while preserving detection of clinically significant cancers.

While the associations of PCA3, T2:ERG, and MPS with cancer are well established, there are limited data describing their variation due to factors other than cancer. Using a unique cohort of men who underwent longitudinal screening with no evidence of PCa, we sought to describe the variability of these markers with age and prostate volume.

2. Patients and methods

2.1. Study cohort

The Olmsted County Study of Urinary Symptoms and Health Status Among Men was a population-based, prospective cohort established in 1990 to evaluate the natural history of lower urinary tract symptoms in white male residents of Olmsted County, Minnesota. The study protocol and patient selection have previously been described in detail [13]. In brief, 2115 of 3874 men (55%) aged 40–79 yr without a history of PCa or other conditions known to interfere with voiding completed a questionnaire concerning urinary symptoms, quality of life, medical history, and risk factors. A 25% subsample ($n = 537$) was randomly selected to undergo a detailed urologic examination, including uroflowmetry, digital rectal examination (DRE), transrectal ultrasound (TRUS), abdominal ultrasound, serum PSA measurement, anthropometric measurement, and cryopreservation of serum for subsequent hormone assays. All men were followed biennially from 1990 to 2010, with PSA testing and DRE carried out at each visit. Men with PSA ≥4.0 ng/ml or abnormal DRE underwent TRUS-guided prostate biopsy, and those diagnosed with PCa were excluded from the cohort and treated with standard of care. At the final clinic visit (2009–2010), remaining participants ($n = 373$) provided post-DRE urinary specimens.

2.2. Specimen processing

Serum PSA levels were measured at the Mayo Clinic Chemical Core Laboratory on a Beckman Coulter Access system. Post-DRE urine specimens were mixed with stabilization buffer and stored at −70 °C. Urinary *PCA3*, *T2:ERG*, and *PSA* mRNA copy numbers were calculated using transcription-mediated nucleic acid amplification [14]. Results for participants with insufficient *PSA* mRNA were considered inconclusive. The PCA3 score was calculated as *PCA3*/*PSA* mRNA ratio × 1000, and the T2:ERG score as T2:ERG/PSA mRNA ratio × 100 000. MPS was calculated

using the validated, locked regression model incorporating serum PSA, urinary PCA3, and urinary T2:ERG scores for detection of GG ≥2 cancer [11]. MPS values are reported on a continuous scale from 0 (very unlikely to detect GG ≥2 cancer on prostate biopsy) to 100 (very likely to detect GG ≥2 cancer on prostate biopsy).

2.3. Statistical analysis

Patient-level factors included age, serum PSA, prostate volume, family history, and previous biopsy status. Data were assessed in accordance with the Clinical and Laboratory Standards Institute guideline [15,16]. Specifically, emphasis was placed on retaining outlying observations, and conservative thresholds for removal were used according to the methods of Dixon [17,18]. As determined a priori, data normality was visually assessed, and data that did not follow a normal distribution were natural log (ln)-transformed for subsequent analyses. The Pearson correlation coefficient (r) was used to quantify correlation of age and prostate volume with each marker, and these associations were assessed via multivariable linear regression. The t statistic was used to quantify the strength of associations independent of the unit of measurement, and R^2 values were used to describe the proportion of biomarker variance explained by age and prostate volume. Percentile values for MPS for limited subgroups based on age and prostate volume were calculated (Supplementary Table 1). All statistical analyses were performed using Stata v13.1 (Stata Corp., College Station, TX, USA).

3. Results

3.1. Longitudinal study cohort

Of 373 subjects at final study follow-up, 356 (95%) provided a urine sample. Thirty-five men (9.8%) had noninformative specimens and five (1.4%) were diagnosed with PCa on final assessment. Two subjects were excluded on the basis of extreme outlier analysis [15,18]. This yielded the final study cohort of 314 men. After biennial screening with serum PSA and DRE, no men in the final cohort had a diagnosis of PCa. The median age was 64.6 yr (interquartile range [IQR] 59.9–71.1), median prostate volume was 30.4 cm^3 (IQR 24.4–42.0), and median PSA was 0.97 ng/ml (IQR 0.62–2.08). The median PCA3 score was 16.7 (IQR 6.78–36.1), the median T2:ERG score was 3.1 (IQR 0.17–27.1), and the median MPS was 7.5 (IQR 3.3–16.7; Table 1).

Table 1 – Demographics of the study cohort

Variable	Result
Subjects (n)	314
Median age, yr (interquartile range)	64.6 (59.9–71.1)
Median prostate volume, cm^3 (interquartile range)	30.4 (24.4–42.0)
Positive family history, n (%)	56 (17.8)
History of previous biopsy, n (%)	62 (19.8)
Median prostate-specific antigen, ng/ml (interquartile range)	0.97 (0.62–2.08)
Median PCA3 score (interquartile range)	16.7 (6.78–36.1)
Median T2:ERG score (interquartile range)	3.1 (0.17–27.1)
Median MyProstateScore (interquartile range)	7.5 (3.3–16.7)

Table 2 – Pearson coefficients for correlation of the study biomarkers with age and volume

	Correlation coefficient	
	Age	ln(prostate volume)
ln(PSA)	0.41 *	0.67 *
ln(PCA3 score)	0.45 *	0.23 *
ln(T2:ERG score)	0.11	0.06
ln(MyProstateScore)	0.54 *	0.43 *

ln = natural logarithm ($\log_e$); PSA = prostate-specific antigen.
* $p < 0.001$.

3.2. Correlation and multivariable analysis

Pearson coefficients for correlation of PCA3, T2:ERG, and MPS with age and prostate volume are listed in Table 2. PSA, PCA3 score, and MPS were each significantly correlated with both age and prostate volume. By contrast, T2:ERG score was not significantly correlated with either factor.

We constructed multivariable regression models for each marker with age and prostate volume as the input variables (Table 3) and the t statistic was used to quantify the strength of associations. PSA was significantly associated with prostate volume ($t = 12.5$; $p < 0.001$) but not with age. By contrast, the PCA3 score was strongly associated with age ($t = 7.51$; $p < 0.001$) but not prostate volume. The T2:ERG score was not significantly associated with either factor. The composite MPS was significantly associated with both age ($t = 7.45$; $p < 0.001$) and prostate volume ($t = 3.56$; $p < 0.001$).

According to the models including both age and prostate volume, PSA was the marker with the highest variability attributable to these factors. Age and prostate volume accounted for 45% of the PSA variability observed ($R^2 = 0.45$). Notably, the model including only prostate volume accounted for the same proportion of PSA variability ($R^2 = 0.45$), suggesting age-based PSA variability is secondary to changes in volume. For both the PCA3 score and MPS, the age-only model explained the variability observed to a similar extent as the model with both age and volume. Consistent with correlation analysis, the T2:ERG score was not associated with age or prostate volume; these factors explained only 1% of the variability observed for the T2:ERG score ($R^2 = 0.01$).

4. Discussion

Serum PSA testing was introduced to clinical practice using a threshold value of 4.0 ng/ml to prompt prostate biopsy [4]. According to this approach, PSA-based screening conferred specificity of only 25–30%, resulting in excess unnecessary prostate biopsies [19,20]. Moreover, negative PSA tests were falsely reassuring for 15–25% of men, some of whom harbored high-grade cancers [21,22]. While PSA is inherently limited as a marker of cancer, a better understanding of its association with demographic and clinical factors could have mitigated the impact of its limitations [5–7].

Thus, we sought to characterize the association of patient-level factors (age and prostate volume) with

Table 3 – Multivariable linear regression of age and prostate volume with PSA, PCA3 score, T2:ERG score, and MPS

		Coefficient (95% CI)	*t*	*p* value	Model R^2
ln(PSA)	Age	0.005 (−0.003 to 0.01)	1.11	0.3	0.17
	ln(PV)	1.28 (1.08 to 1.49)	12.5	<0.001	0.45
	Age + ln(PV)				0.45
ln(PCA3 score)	Age	0.05 (0.04 to 0.06)	7.51	<0.001	0.21
	ln(PV)	−0.05 (−0.38 to 0.28)	−0.29	0.8	0.05
	Age + ln(PV)				0.20
ln(T2:ERG score)	Age	0.02 (−0.01 to 0.06)	1.25	0.2	0.01
	ln(PV)	0.06 (−0.8 to 0.9)	0.14	0.9	0.004
	Age + ln(PV)				0.01
ln(MPS)	Age	0.04 (0.03 to 0.05)	7.45	<0.001	0.29
	ln(PV)	0.50 (0.22 to 0.77)	3.56	<0.001	0.18
	Age + ln(PV)				0.31

CI = confidence interval; ln = natural logarithm ($\log_e$); MPS = MyProstateScore; PSA = prostate-specific antigen; PV = prostate volume (cm^3).

emerging biomarkers among men without PCa. PCA3 and T2:ERG are two urine-based cancer-specific markers commercially available through the MPS test [23]. Combining PCA3 and T2:ERG with serum PSA, the MPS model has been validated for improved prediction of GG ≥2 cancer relative to PSA-based risk calculators [24]. In a unique, longitudinally screened cohort with no evidence of cancer, we found that PCA3, T2:ERG, and MPS each demonstrated less variability with these noncancer characteristics than PSA did, as age and prostate volume accounted for 45% of the PSA variability observed. By contrast, only 20% of PCA3 variability was explained by age and volume, while T2:ERG was independent of these factors ($R^2 = 0.01$). Notably, 31% of MPS variability was explained by age and volume, but accounting for age alone ($R^2 = 0.29$) explained the variability due to both age and volume.

In 1993, Oesterling and colleagues [6] described 471 men who underwent evaluation with PSA, DRE, and TRUS with no evidence of PCa. The current cohort included the subset of men with no evidence of cancer after a 20-yr screening interval. As expected, our PSA-based findings are highly consistent with those previously described. Both analyses found that PSA was correlated with prostate volume ($r = 0.55$; $r = 0.67$ in the current analysis) and age ($r = 0.43$; $r = 0.41$ in the current analysis). Considering the known association of age and prostate volume [5], Oesterling et al concluded that increasing prostate volume was most likely to be responsible for age-related increases in PSA. Our analysis further supports this conclusion, as we found that accounting for prostate volume alone explains PSA variability to the same extent as accounting for age and prostate volume ($R^2 = 0.45$ for both models).

While previous studies have explored the impact of age and prostate volume on PSA [5,6], there are limited data describing their associations with PCA3, T2:ERG, and MPS. Furthermore, these relationships are generally explored in at-risk populations referred for prostate biopsy, in which the presence or absence of cancer strongly drives observations, potentially confounding the true relationship between biomarkers and noncancer factors. Klatte et al [25] previously assessed the association of PCA3 with age among 205 men referred for 14-core biopsy because of PSA >3.0 ng/ml or suspicious DRE. On biopsy, 76 men (37%) had cancer. In a multivariable regression model, the authors found that age ($t = 4.77$; $p < 0.001$) and cancer ($t = 4.31$; $p < 0.001$) were independently associated with PCA3. Interestingly, the R^2 value for their multivariable model with age was 0.212, nearly identical to the $R^2 = 0.21$ observed for our age-based model. Consistent with the current study, Klatte et al found no association between PCA3 and prostate volume ($t = 0.48$; $p = 0.632$). These corroborating data further support the validity of our findings.

Importantly, we found that neither age ($r = 0.11$) nor prostate volume ($r = 0.06$) was significantly correlated with the T2:ERG score. The independent nature of the T2:ERG gene fusion from these common confounders further supports its use for cancer detection as a truly cancer-specific marker. Given that more than three-quarters of men with cancer harbor at least one ERG-positive tumor [26], elevated urinary T2:ERG would appear to provide a highly reliable rule-in test for patients considering biopsy [27]. Moreover, T2:ERG appears to be associated with tumor aggressiveness [28], a highly important factor given the indolent nature and disparate management strategies for low-grade PCa.

Given that the validated MPS test incorporates serum PSA, it is not surprising that we observed variability due to noncancer factors. Importantly, the MPS variability observed was limited and appears to be explained by age. Previous authors have hypothesized that the age-related increase in PCA3 could be due to inflammation or interaction with prostatic stroma [29]. The finding that PCA3 is higher in men with high-grade prostatic intraepithelial neoplasia than in men with benign prostatic hyperplasia is thought to be due to early molecular changes in this precursor lesion [30,31]. Acknowledging the limited sensitivity of PSA and TRUS-guided biopsy for cancer [21,22,32], it is also possible that a limited proportion of patients “negative” for cancer in this cohort actually harbored cancer. Interestingly, on clinical validation, addition of age and other factors to the MPS model did not impact the predictive accuracy of the biomarker-only model for GG ≥2 cancer [11]. This could be explained by the strong

association of age with cancer prevalence, such that a modest increase in MPS with age reflects a real, underlying increase in risk.

There are limitations to the current study. As described, the limited sensitivity of the PSA threshold of 4 ng/ml and standard TRUS-guided biopsy suggest that a low proportion of study patients probably harbored cancer. Still, the longitudinal nature of our follow-up confers an even lower likelihood of cancer than in previous study populations considered cancer-free on the basis of a single evaluation. Second, these data were obtained in an era before magnetic resonance imaging (MRI). While the use of MRI could have detected cancer in some men with low PSA, current practice is that MRI is considered following elevated PSA, whereas patients in the current cohort were referred directly for biopsy. The incremental increase in diagnoses afforded by MRI is unlikely to have a major impact on our findings, but is notable nonetheless. Furthermore, the noninformative testing rate of 9.8% is higher than in previous studies and contemporary practice. The MPS test is noninformative when insufficient *PSA* mRNA is detected to calculate PCA3 and T2:ERG scores. This is often attributable to inadequate DRE, but could also be due to an extended urine storage interval in this population. Data were not available to ensure that no systematic differences existed between patients with informative and noninformative urine, although this has been assessed in previous studies. In addition, it is possible that a broader range of PSA levels and prostate volumes could have been more informative, but the data are presented for the patients participating in this observational cohort study. Finally, data were not available for patients diagnosed with PCa during the course of the study. Thus, this cohort is not well suited for validating test performance, which has previously been described in pertinent populations [11,12,23]. Instead, these data describe the expected variability of MPS and its component markers among men without clinically detected PCa, with the aim of facilitating thoughtful adoption of these tests moving forward.

5. Conclusions

In a longitudinal cohort of screened men without evidence of PCa, we found that MPS demonstrated less variability due to noncancer factors (age, prostate volume) than PSA did, as MPS values increased modestly with age. These findings support the biology of this marker as more cancer-specific than PSA. Combined with data from clinical screening populations, these findings will help to establish the optimal clinical applications of MPS.

Author contributions: Jeffrey J. Tosoian had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Tomlins, Chinnaiyan, Sarma.
Acquisition of data: St. Sauver, Jacobson, McGree, Groskopf, Jacobsen, Kunju, Sarma.
Analysis and interpretation of data: All authors.
Drafting of the manuscript: Tosoian, Niknafs, Vince.
Critical revision of the manuscript for important intellectual content: All authors.
Statistical analysis: Tosoian, Dunn.
Obtaining funding: Chinnaiyan, Sarma.
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Other: None.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.euros.2021.04.009.

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