Assessment of high-grade prostate cancer risk using prostate cancer biomarkers

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Introduction: *To identify patients at risk of high-grade prostate cancer using prostate cancer biomarkers.*

Materials and methods: *A total of 601 men were screened for prostate cancer in 2012, 2015, and 2016 using prostate cancer biomarkers: prostate health index (phi), 4KScore, and SelectMDx. The first two are blood tests that incorporate several PSA isoforms; SelectMDx measures mRNA levels of homeobox C6 and distal-less homeobox 1 in post-digital rectal examination urine samples. The performance of each biomarker was evaluated using cut off values based on published literature. Gleason Grade Group (GG) ≥ 2 is considered as high-grade prostate cancer.*

Results: *For patients with PSA < 1.5 ng/mL, none were at risk for GG ≥ 2 cancer based on SelectMDx > 0%, whereas 17.1% were at intermediate to high risk of finding GG ≥ 2 cancer with 4KScore ≥ 7.5%, and 3.5% were at risk of finding any prostate cancer with phi ≥ 36 at biopsy. For cut offs revised for finding men at high risk for GG ≥ 2 cancer at biopsy, only one patient with PSA < 1.5 ng/mL would be at risk with 4KScore ≥ 20% and none with phi ≥ 52.7. For patients with PSA 1.5 to 3.99 ng/mL, 2%, 8%, and 1% were at high risk for finding GG ≥ 2 cancer at biopsy based on phi, 4KScore, and SelectMDx, respectively.*

Conclusions: *Men with PSA < 1.5 ng/mL are at very low risk of finding high-grade prostate cancer at biopsy. However, some men with PSA between 1.5 to 3.99 ng/mL may be at intermediate to high risk for high-grade prostate cancer. Thus, primary care physicians could run biomarkers test and refer those with positive biomarker results to a specialist for further evaluation.*

Key Words: prostate cancer, screening, biomarkers, PSA, *phi*, 4Kscore, SelectMDx

Introduction

Prostate cancer remains a major health concern for men of North America and Europe. In 2019, an estimated 174,650 men will be diagnosed with prostate cancer and 31,620 men will die from this disease in the United States alone.[1] For decades, assessment of prostate cancer risk in men relied upon demographical and clinical factors including age, race, family history, serum prostate-specific antigen (PSA) levels, and digital rectal examination (DRE). PSA is not prostate cancer specific since elevated levels can be due to benign prostatic hyperplasia or acute prostatitis and cannot accurately assess a man's risk for high-grade prostate cancer (Gleason score ≥ 7). Thus, additional tools are needed to accurately identify men with aggressive disease.

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National Comprehensive Cancer Network (NCCN) guidelines recommends transrectal ultrasound (TRUS) guided prostate biopsies for men 45-75 years of age with PSA > 3 ng/mL and/or very suspicious DRE.[2] The Prostate Cancer Prevention Trial results show that ~15% of men with PSA < 4.0 had high-grade prostate cancer.[3] On the other hand, men with baseline PSA between 1.5-4.0 have 15-fold increase in risk of prostate cancer compared with those who have an initial PSA < 1.5 over subsequent 4 year period.[4] Hence, there remains a lack of consensus as to the correct PSA threshold for screening for men specifically at risk for high-grade prostate cancer, with suggested values ranging between 1.0-4.0 ng/mL. We postulate that combining PSA levels with well-validated prostate cancer markers (PCMs) may hold the key to improving risk assessment and selection of patients at risk for high-grade prostate cancer. The addition of PCMs can identify men at risk who may benefit from earlier intervention while reducing unnecessary biopsies for those at low risk. The [–2] proPSA (p2PSA) is a new serum-based PCM used in the prostate health index (*phi*), which is associated with prostate cancer risk and disease aggressiveness.[5] The median *phi* was significantly higher in men with prostate cancer than in those with negative TRUS biopsies.[6] Another promising serum-based PCM is the kallikrein panel used in 4KScore that consists of total PSA, free PSA, intact PSA, and human kallikrein 2 (hK2).[7] The 4KScore has been shown to increase predictive capability of high-grade prostate cancer.[8]

The SelectMDx test measures mRNA levels of the homeobox C6 (HOXC6) and distal-less homeobox 1 (DLX1) biomarkers in post-DRE urine samples where higher expression levels of HOXC6 and DLX1 are associated with an increased probability of high-grade prostate cancer.[9] SelectMDx test is independent from PSA isoforms. The results reported here focus on the performance of three PCMs in a screening population based on different PSA values.

Materials and methods

This study was approved by the Colorado Multiple Institutional Review Board (COMIRB) Protocol # 00-8400. During the 2012, 2015, and 2016 Prostate Cancer Awareness Week (PCAW) at the University of Colorado Hospital, a total of 601 men received free screening using PSA, DRE, and several PCMs. The cohort was reduced to 540 men after excluding men with previous prostate biopsy and treatment history such as transurethral resection of the prostate, brachytherapy, radiation, or surgery to pelvis area. In 2012, 216 men were screened with *phi*; in 2015, 152 men were screened with 4KScore; and in 2016, 172 men were screened with SelectMDx. Their collected urine and blood samples were subsequently studied. Beckman & Coulter, OPKO, and MDx Health provided test results free of charge for *phi*, 4KScore, and SelectMDx, respectively. Gleason Grade Group (GG) = 1 is considered as low-grade prostate cancer and GG ≥ 2 is considered as high-grade cancer.

The *phi* value is calculated using the formula *phi* = ([-2]proPSA/free PSA) × √PSA and is based on p2PSA, total PSA (tPSA), free PSA (fPSA) in serum.[5] A recent study found patients with PSA between 2-10 ng/mL have an 8.4% chance of finding any cancer at biopsy when *phi* < 21 , 21.0% chance when *phi* is between 21-40, and 44.0% chance when *phi* > 40.[10] Patients had biopsies when the *phi* ≥ 36 which indicated an intermediate to high probability of any prostate cancer. In a cohort of template-guided transperineal mapping biopsy patients, the median *phi* was significantly higher in patients with GG ≥ 2 versus GG = 1 cancer or benign disease (52.7 versus 39.7; p = 0.04).[11]

The 4KScore is a blood test that incorporates a panel of four kallikrein protein biomarkers: tPSA, fPSA, intact PSA, human kallikrein protein, and clinical information. Based on 4KScore, patients are stratified into low risk (< 7.5%), intermediate risk (7.5%-19.9%), and high risk (≥ 20%) for aggressive prostate cancer with GG ≥ 2.[12] Using 4KScore ≥ 20% as the threshold for biopsy would have reduced the number of biopsies by 57% and missed only 20% GG = 1 and 7.5% GG ≥ 2 cancers.[8]

The SelectMDx post-DRE urine test measures the mRNA levels of the homeobox C6 and distal-less homeobox 1 biomarkers. Patients with SelectMDx

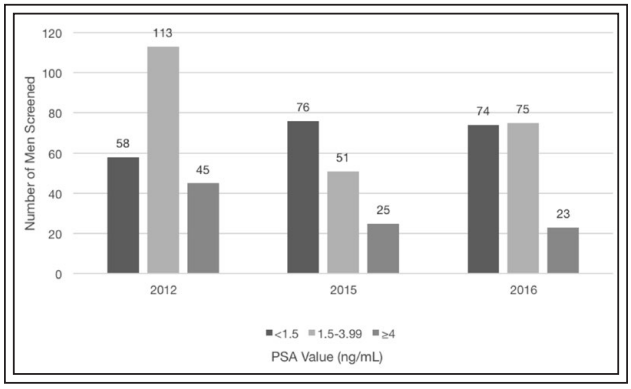

Figure 1. Distribution of PCAW participants based on different PSA ranges during 2012, 2015, and 2016.

TABLE 1. **Patient demographics of each year of prostate cancer marker test**

	2012 (*Phi*)	2015 (4KScore)	2016 (SelectMDx)
Number of patients	216	152	172
Median age (range) years	65.5 (36-98)	65.2 (35-87)	66.2 (20-88)
Mean PSA (range) ng/mL	2.77 (1.01-8.54)	2.32 (0.07-15.53)	2.19 (0.04-11.57)
Median PSA (95% CI) ng/mL	2.32 (2.04-2.6)	1.51 (1.3-1.88)*	1.81 (1.47-2.04)*
Abnormal DRE	16%	7%	12%
Mean testosterone (range) ng/dL	371 (121-808)	400 (98-1115)	325 (102-1492)
Family history of prostate cancer	26%	32%	37%
Race/ethnicity			
White	82%	77%	75%
Black	11%	14%	5%
Asian	3%	3%	-
Hispanic	3%	4%	1%
Hawaiian or Pacific Islander	0.5%	-	-
Native Alaskan	-	-	1%
Native American	-	1%	16%
Unknown	0.5%	1%	2%

*median PSA in 2015 ($p < 0.0001$) and 2016 ($p < 0.0001$) were lower than in 2012

score at very low risk may avoid prostate biopsy with a negative predictive value of 98% for GG ≥ 2 prostate cancer.[9] However, patients with SelectMDx score > 0% may be at risk of high-grade prostate cancer. We evaluated the performance of three PCMs for several of the above-mentioned cut off values using standard statistical measures. Wilcoxon Rank Sum used for comparison with statistical significance at $p \leq 0.05$.

Results

PCAW participant demographics and baseline information are given in the Table 1. The median PSA of PCAW participants in 2015 ($p < 0.0001$) and 2016 ($p < 0.0001$) were significantly lower than those in 2012. Distribution of the PCAW participants based on different PSA ranges are given in the

TABLE 2. **Prostate cancer marker (PCM) test results based on PSA values**

PSA ng/mL	Year	PCM performed	Number of patients	Age median (range)	Abnormal test results (*phi* ≥ 36, 4KScore ≥ 7.5%, SelectMDx > 0%)	Abnormal test results (*phi* ≥ 52.7, 4KScore ≥ 20%)
< 1.5	2012	*phi*	58	62 (40-86)	2/58* (3.5%)	0/58** (0%)
	2015	4KScore	76	63 (35-85)	13/76 (17%)	1/76** (1%)
	2016	SelectMDx	74	66 (27-80)	0/74** (0%)	-
1.5-3.99	2012	*phi*	113	67 (36-98)	30/113* (27%)	2/113** (2%)
	2015	4KScore	51	68 (47-79)	18/51 (35%)	4/51** (8%)
	2016	SelectMDx	75	68 (20-88)	1/74** (1%)	-
≥ 4	2012	*phi*	45	66 (52-82)	34/45* (76%)	13/45** (28%)
	2015	4KScore	25	70 (35-87)	15/25 (60%)	8/25** (32%)
	2016	SelectMDx	23	67.5 (56-77)	6/23** (26%)	-

*at intermediate to high risk for any prostate cancer (Gleason Grade Group ≥ 1)
**at risk for high grade prostate cancer (Gleason Grade Group ≥ 2)

TABLE 3. **Baseline data of men with PSA ≥ 4 ng/mL and positive SelectMDx test**

	Diagnostic measure			
	Age	**PSA**	**DRE**	**SelectMDx (Prostate cancer%/high-grade prostate cancer% risks)**
Patient 1	71	4.44	Positive	43%/17%
Patient 2	74	4.74	Positive	50%/23%
Patient 3	70	6.33	Negative	35%/11%
Patient 4	69	7.66	Negative	44%/18%
Patient 5	77	8.35	Negative	60%/32%
Patient 6	56	11.57	Negative	39%/14%

histogram of Figure 1. Performance of PCMs are summarized in the Table 2. No participants with PSA < 1.5 ng/mL were indicated at risk for GG ≥ 2 cancer by SelectMDx > 0%. For the same PSA range, however, 17% men were at intermediate to high risk for high-grade prostate cancer with 4KScore ≥ 7.5% at biopsy and 3.5 % at risk for any prostate cancer with *phi* ≥ 36 at biopsy. With cut offs revised to identify men at high risk of finding GG ≥ 2 cancer at biopsy, only 1/76 (1%) would be at risk with 4KScore ≥ 20 and none with *phi* ≥ 52.7, Table 2. For men with PSA between 1.5 to 3.99 ng/mL, only one participant (1%) was indicated at risk for GG ≥ 2 cancer by SelectMDx > 0%, 4/51 (8%) with 4KScore ≥ 20%, and 2/113 (2%) with *phi* ≥ 52.7. The baseline characteristics of 6 men with PSA ≥ 4 ng/mL at risk for high-grade prostate cancer based on SelectMDx > 0% are summarized in the Table 3.

Discussion

PCAW participants are a heavily screened population based on PSA, DRE, family history, and PCMs without any information on prostate biopsy. A large proportion of PCAW participants had PSA < 4.0 (79%, 84%, and 87%, in 2012, 2015, 2016, respectively) and hence did not need prostate biopsies. However, those men with PSA ≥ 4 may have had biopsies, but that information was not available during PCAW. Secondly, men with PSA ≥ 4 follow up with their outside providers and we do not have any records of their biopsies unless they report that in a follow up year of screening. Hence, it was not possible to determine the accuracy of these PCMs compared to histopathology data of prostate biopsies. Instead, we attempted to evaluate their performances for different PSA ranges.

Crawford et al found that prostate cancer rates were 15-fold higher in patients with PSA ≥ 1.5 ng/mL versus patients with PSA < 1.5 ng/mL (7.85% versus 0.51%).[4] African American patients with PSA between 1.5-4.0 ng/mL had a 19-fold increase in prostate cancer. The PSA 1.5 threshold gave the maximum sensitivity and specificity for ROC curve with an estimated area of 0.87. Thus, PSA of < 1.5 ng/mL (~70% of men who have a screening PSA) constitutes a very low risk category for developing prostate cancer (particularly high-risk disease) and recommendations were made to screen again in 5 years.[13] Goldberg reviewed 199 men less than 50 years of age undergoing prostate biopsies and no one below 1.5 ng/mL PSA had a Gleason score ≥ 7.[14] This supports the PSA cut off of 1.5 ng/mL as a threshold to repeat testing at 2-4 years intervals unless DRE is very suspicious for men 45-75 years of age.

Ryan et al, however, found a substantial proportion of men (26%) with Gleason score ≥ 8 cancer had a PSA ≤ 1.0 ng/mL at least 3 years prior to their diagnosis and were associated with worse overall and prostate cancer specific survival.[15] Their results do not support discontinuing PSA screening among men with a single PSA measurement less than 1 ng/mL. Hence, PCMs have a definitive role screening for patients at risk of aggressive prostate cancer.

If the PSA is ≥ 1.5, or the primary care physician identifies an abnormality on DRE, refer to a specialist or consider a PCM to assess risk more precisely.[13] Other options include: following up with the patient in 6 months or 1 year or using new techniques such as MRI to determine whether the patient is at risk of high-grade prostate cancer.[16] As supported by our findings, men with a PSA between 1.5-3.99 ng/mL with positive PCM results may be referred for further evaluation. However, we do not recommend prostate

biopsy be performed unless the risk of high-grade prostate cancer indicated by PCM is high, and following a thorough discussion of benefits and risks with the patient.[13]

As a genomic test, SelectMDx test has the advantage of being unaffected by PSA isoforms whereas *phi* and 4KScore may be subsequently impacted. Thus, SelectMDx test may be carried out for any PSA value, but *phi* and 4KScore tests may be affected by stability of PSA isoforms at low PSA values. *Phi* was previously validated for men with PSA ≥ 4.[17] The study showed that men are at 9.8%, 16.8%, 33.3%, and 50.1% risk for prostate cancer when *phi* was ≤ 26.9, 27.0-35.9, 36.0-54.9, and ≥ 55.0, respectively. In our study, 21% (45/216) patients met this condition. Thus, 7% (3/45), 18% (8/45), 51% (23/45), and 24% (11/45) men were in the above risk categories, respectively, with PSA ≥ 4.

Phi has been tested for men with PSA > 2[10,18] and in our cohort 51% (127/216) patients met this condition. Accordingly, 5.5% (7/127) patients with *phi* < 21 were at 8.4% risk, 60% (76/127) with *phi* between 21-40 were at 21.0% risk, and 34.5% (44/127) with *phi* > 40 were at 44.0% risk for prostate cancer. Forty-one percent (89/216) men had a PSA ≤ 2 ng/mL for which *phi* testing is not validated. Previous study also found that prostate biopsies diagnosed 33% of men with GG ≥ 2 cancer and only 27% with GG = 1 cancer when decision to biopsy was based on *phi* ≥ 36.[10] In comparison, the control group that did not use *phi* diagnosed 32% GG ≥ 2 and 31% with GG =1 cancer. In our study, 45% (57/127) of participants had *phi* ≥ 36 with PSA > 2.

Men with 4Kscore < 7.5% have a 99% chance to be free of prostate cancer metastases within 15 years of long term follow up and may be safely monitored less frequently.[12] In our study, only one man was at high risk for high-grade prostate cancer with PSA < 1.5 and 4KScore at 27%. He is a Caucasian with a normal DRE and no family history of prostate cancer. Conversely, prostate cancer risk is increased by 15-fold in men with PSA ≥ 1.5.[4] SelectMDx test at 10% indicated one 75-year-old participant with PSA 2.39 and positive DRE may be at risk for high-grade prostate cancer. He is eligible for prostate biopsy due to abnormal DRE per NCCN guidelines or could be referred to a specialist. There were six men at risk for high-grade prostate cancer based on SelectMDx test results with PSA ≥ 4 ng/mL, Table 3. All of these men are eligible for prostate biopsy per NCCN guidelines or could be referred to a specialist for further evaluation including a discussion of the value of PCMs or MRI prior to a prostate biopsy.

Primary care physicians order 90% of the PSA tests and they need clear guidelines on patient management based on PSA and its derivatives.[18] However, in 2012, the US Preventive Services Task Force (USPSTF) recommended against annual PSA screening for asymptomatic men under 55 years of age to reduce unnecessary biopsies and diagnosis of indolent tumors.[19] Nevertheless, the USPSTF did acknowledge strong evidence that treatment of localized prostate cancer reduced mortality compared with observation alone, citing a Scandinavian randomized, controlled trial with 15 years of follow up showing that radical prostatectomy resulted in a sustained 38% decrease in prostate cancer–specific mortality and 25% reduction in all-cause mortality.[20]

There is new evidence that previous USPSTF recommendation has had a negative side effect; a growing number of men with lethal prostate cancer is now being diagnosed too late.[21] Thus, USPSTF finally upgraded PSA screening in 2017 realigning with American Urological Association (AUA) recommendations.[22] Yet, PSA has poor specificity for this disease and decisions to biopsy patients need to be supplemented by additional PCM tests or MRI with referrals to specialist for men with positive test results with PSA between 1.5 to 3.99 ng/mL

One of the limitations of the study is small sample size. Since this study included men at a voluntary screening event, there is no confirmatory of biopsy data to verify the accuracy of these tests. Furthermore, this study also lacks follow up data as most men seek advice or further care from providers outside of our hospital. Each year, different men came to the PCAW screening events and hence it is not possible to compare the performance of different biomarkers on same individual from year-to-year. Our data shows that median PSA levels of the PCAW participants in 2015 and 2016 were significantly lower than in 2012 which may be attributed to the USPSTF recommendations. This may also hinder a direct comparison of the performance of three PCMs.

Conclusions

Our study confirmed that men with PSA < 1.5 ng/mL are at very low risk of being diagnosed with high-grade prostate cancer based on the findings of three PCMs in patient's screening. This study also found that some men with PSA between 1.5 to 3.99 ng/mL with a positive PCM result may be at high risk of finding high-grade prostate cancer at subsequent biopsy and men in this range should be further evaluated. □

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