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The Effect of Digital Rectal Exam on the 4Kscore for Aggressive Prostate Cancer

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Abstract

Background: The 4Kscore is a new commercially available blood-based diagnostic test which predicts risk for aggressive, clinically significant prostate cancer on prostate biopsy. The 4Kscore is currently restricted to patients who have not had a digital rectal exam (DRE) in the previous 96 hours, owing to prior mixed data suggesting that prostate specific antigen (PSA) isoforms may increase by a statistically significant—if not necessarily clinically significant—amount shortly after DRE. Our primary objective was to determine if 4Kscore test results are affected by a preceding DRE.

Methods: Participants at a Prostate Cancer Awareness Week screening event sponsored by the Prostate Conditions Education Council filled out clinical history questionnaires and had blood samples for 4Kscore testing drawn prior to DRE, then 15–45 minutes following DRE. Patients with prior cancer diagnosis, 5-alpha reductase inhibitor medication use, or lower urinary tract procedures in the prior six months were excluded, resulting in a population of 162 participants for analysis. Values were then compared to determine if there was a significant difference in 4Kscore following DRE.

Results: A statistically significant increase was seen in levels of 3 kallikreins measured (total PSA, free PSA, and intact PSA; median <0.03 ng/mL for all). This resulted in a small but statistically significant decrease in post-DRE 4Kscore (median absolute score decrease 0.43%). Using a 4Kscore cutoff of 7.5% resulted in reclassification of 10 patients (6.2%), nine of whom were “downgraded” from above the cutoff to below.

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Note: Results of this study in abstract form have been presented during 2016 American Urological Association (AUA) meeting in San Diego, CA and 2016 AUA South Central Section meeting in Colorado Springs, CO.

Conclusions: If the blood draw for the 4K score is performed after a screening DRE, there is a statistically significant difference in the 4K score results, but in the vast majority of cases it would not affect clinical decision making.

Introduction

The 4Kscore® Test (OPKO BioReference Lab, Elmwood Park, NJ) is a new blood-based diagnostic test used to predict risk of Gleason score 7 or greater prostate cancer on prostate needle biopsy. The 4Kscore is assessed by a computer algorithm using levels of four serum human kallikrein biomarkers including total PSA (tPSA), free PSA (fPSA), intact PSA (iPSA), and human kallikrein 2 (hK2) along with clinical information including age, digital rectal exam (DRE) findings, and prior biopsy status. The 4Kscore has shown high specificity for Gleason score 7 prostate cancer in both retrospective¹⁻⁵ and prospective⁶ studies and has also demonstrated potential for reducing unnecessary prostate needle biopsies^{1, 7} making it a valuable decision-making tool for urologists.

Currently OPKO recommends against 4Kscore testing in patients who have undergone a DRE within the previous 96 hours⁸. This is presumably based on mixed past data examining the effect of DRE and other prostatic manipulations (cystoscopic procedures and prostate biopsies) on total and/or free PSA levels and serum kinetics studies demonstrating a half-life of 14–18 hours for free PSA^{9, 10}. In a study of 82 patients, Collins *et al.* found that DRE increased total PSA levels on average by 0.3 ng/mL and free PSA by 0.25 ng/mL, resulting in an average absolute increase in free/total PSA ratio of 0.07¹¹. Based on this, the authors recommended phlebotomy precedes any prostatic manipulation. Lechevallier, *et al.* reported significant increases in PSA and free PSA of 50% and 20%, respectively, and thus also recommended blood draw prior to DRE¹². Conversely, there have been multiple other studies showing either no statistically significant change in total PSA and/or free PSA, or statistically significant but not clinically significant changes after DRE and other prostatic manipulations^{13–18}.

In most cases, it is feasible and only a minor inconvenience to wait a few days for a blood draw to obtain a 4Kscore. However, in a tertiary care setting or other practices with a large referral area, it can be a significant burden for patients from far away to wait a few days and return for a blood draw. As there are no studies in the literature examining the effect of DRE prior to 4Kscore blood draw, we investigated whether performing a DRE prior to a blood draw results in statistically and/or clinically significant changes in the 4Kscore.

Materials and Methods

Study Population

This study was approved by the Colorado Multiple Institutional Review Board (COMIRB) Protocol #00–840. We obtained consent from participants at the annual Prostate Cancer Awareness Week (PCAW) event sponsored by the Prostate Conditions Education Council on September 21, 2015. We excluded patients with 5-alpha reductase inhibitor use, prior cancer diagnosis, has received a DRE in the previous 96 hours before phlebotomy, or therapy to treat symptomatic benign prostatic hyperplasia (BPH) or any invasive urologic procedure

within the prior 6 months from this study but they were eligible to participate in the other screening and educational activities of the event.

Data Collection

Patients filled out a clinical history questionnaire including demographic and clinical information, then underwent a blood draw prior to DRE and screening discussion with one of seven physicians volunteering at the event. Fifteen to 45 minutes after the DRE, the patients underwent a second blood draw. Time interval from DRE to this second blood draw was recorded.

Sample Preparation

Venous blood samples were collected in a K2EDTA tube (Lavender top) with approximately 7.5 mL of blood (at least 5 mL of blood). To ensure good mixing with the EDTA, the tube of blood was inverted eight times shortly after sample collection. These quantities of blood ensure that enough plasma can be obtained. The single determination of the four kallikrein markers requires approximately 1 mL of plasma, and the Laboratory requires 2 mL of plasma to allow re-run of the sample, as needed. Blood samples were stored at 2 to 8°C and centrifuged within 60 hours of collection at 1,000 x g for 10 minutes. Plasma samples were sent to OPKO Lab for analysis.

Hypothesis

Our hypothesis was that there would not be a difference between pre- and post-DRE 4Kscore values.

Data Analysis

Pre- and post-DRE 4Kscore values were compared using Wilcoxon signed-rank test and Passing-Bablok regression analysis. Multivariate regression analysis was also performed to examine potential association between patient clinical factors (age, DRE findings, and prior biopsy status) and significant changes in 4Kscore. We further examined changes in a subgroup of patients with initial 4Kscore between 5% and 10%, as these values are close to the 7.5% threshold for proceeding with prostate biopsy described in the literature and would potentially be most affected by changes after DRE. We used McNemar's test to compare proportions of men upgraded/downgraded based on pre- and post-DRE 4Kscore. Results were considered statistically significant at $P = 0.05$.

Results

A total of 162 PCAW patients met inclusion criteria and consented to participate in our study. Study population baseline characteristics are reported in Table 1. A small but statistically significant increase ($P = 0.05$) in the medians and interquartile ranges (IQR) were seen in levels of three kallikreins after DRE – tPSA (median = +0.027 ng/mL, IQR = -0.03–0.10), fPSA (median = +0.024, IQR = -0.001–0.066), and iPSA (median = +0.012, IQR = -0.003–0.033) as reported in the Table 2. Variations in the levels of hk2 were not significantly different (median change = 0 ng/mL, IQR = -0.002–0.001). The net effect of

these changes was a median absolute decrease in 4Kscore of 0.43% (IQR = -0.91% to -0.04%). Figure 1 illustrates changes in the four kallikreins and 4Kscore pre- and post-DRE.

Passing-Bablok analysis demonstrated generally good agreement between pre- and post-DRE 4Kscore values (Figure 2). The slope of this comparison fit line was 0.92, while a comparison line showing no effect of DRE would have an expected slope of 1.0.

Multivariate logistic regression analysis demonstrated that positive DRE and increasing age were independently associated with increase in post-DRE fPSA ($p = 0.025$ and $p = 0.017$, respectively) and decrease in post-DRE 4Kscore ($p = 0.017$ and $p = 0.006$, respectively). Other factors examined in the multivariate regression which did not demonstrate any independent effect on the degree of change in 4Kscore included patient race (Caucasian versus African American), prior negative biopsy history, and time to second blood draw.

Using a 4Kscore of 7.5% as a theoretical trigger for prostate biopsy, we found that 10 patients (6.2% of participants) would be reclassified based on change in 4Kscore; 9 of these patients had a 4Kscore greater than 7.5% prior to DRE and 4Kscore less than 7.5% after DRE while one patient had an initial 4Kscore less than 7.5% prior to DRE and greater than 7.5% after DRE. Thus, a statistically significant proportion of men at risk for high grade prostate cancer with pre-DRE 4Kscore $\geq 7.5\%$ were downgraded by post-DRE 4Kscore $< 7.5\%$ compared to those upgraded (McNemar's test $P = 0.022$). These results are reported in Table 3. All ten of these patients had an initial 4Kscore between 5% and 10% and post-DRE 4Kscores remained between 5% and 10%. The absolute change in results from pre- to post-DRE 4Kscore among patients with initial 4Kscore between 5% and 10% ranged from -3.7% to +1.2%.

Discussion

Overall changes in kallikrein levels measured in our study were small, with median change less than 0.03 ng/mL for all four measured. The majority of change was seen in free PSA levels. These changes resulted in a median decrease in the 4Kscore of less than 0.5%. With a median pre-DRE 4Kscore of 4.8%, this represents a small change. Our data demonstrate that while a DRE prior to blood draw appears to result in a statistically significant change in 4Kscore, this small change is clinically insignificant in the overwhelming majority of cases (about 94%). As a result, a significantly higher proportion of men will be downgraded from high-risk to low-risk for high-grade prostate cancer than those upgraded. Due to lack of biopsy information for this cohort (limitations discussed further below), we are unable to assess the clinical impact. However, the overall range of change in 4Kscore was -9.5% to +1.5%, which is enough to potentially impact clinical decision-making, particularly in patients with 4Kscore values near the biopsy threshold of 7.5%.

Our study does have several limitations. First, the study population is at risk of selection bias, given that these are men who voluntarily chose to seek annual prostate cancer screening and education at our PCAW event. Second, DRE technique could potentially have some effect on the degree of change in serum kallikreins. Having multiple providers performing DREs should hopefully have limited this effect to some degree. We did

retrospectively look at the providers who performed DREs on patients with absolute change in 4Kscore greater than 1.5%, and all providers were represented in this group in similar numbers. Third, although we did this study in a prospective fashion, one could argue that including controls undergoing two 4Kscore blood draws without an intervening DRE could help by providing comparison to the effect of variation between sequential blood draws alone. Finally, the nature of this study at an informational event certainly introduces a limitation due to the fact that while patients undergoing screening receive advice to seek follow up to discuss abnormal test results, we do not have access to information about how many patients actually proceeded to biopsy nor to pathology results from those who subsequently did so, as patients who did seek further evaluation likely did so across multiple locations and providers, many outside of our health system. In an ideal study all patients undergoing blood testing would also have undergone prostate biopsy, but this would have exposed many patients to potential unnecessary harm and thus was ethically not feasible.

Conclusions

We conclude that while a DRE does appear to have a minor effect on 4Kscore results, the impact of the change is small and is unlikely to affect clinical decision making in the overwhelming majority of cases. Ideally, it is preferable for a blood draw to be performed prior to DRE, however a DRE should not necessarily preclude a patient from undergoing a subsequent 4Kscore blood draw at the same visit. If the 4Kscore result is less than 5% or greater than 10%, we feel the clinician can be confident that the result is reliable even after a recent DRE. However, physicians and their patients with 4Kscore results between 5% and 10% should consider the possibility that DRE caused the result to change relative to the 7.5% threshold and consider if such a change would alter management decisions. It is important to also keep in mind that while a value of 7.5% is often used as a trigger for prostate biopsy, this is not an absolute cutoff; rather, the 4Kscore is meant to be considered in conjunction with other patient information to aid the physician and patient in making management decisions.

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FIGURE 1.

Changes in 4 individual Kallikreins and 4Kscore After Digital Rectal Exam. p-value <0.01 for all except hk2, which was >0.05

FIGURE 2.
Passing-Bablok Regression Graph for Pre- and Post-DRE 4Kscore Values.

Table 1:

Study Population Characteristics (N = 162)

Characteristic	Value
Median Age (IQR)	67.13 yrs (60.93 - 72.51 yrs)
Median Time between DRE and 2 nd Blood Draw (IQR)	20 mins (18 – 26 min)
Abnormal DRE	18 patients (11%)
Prior Negative Biopsy	28 patients (17%)
Mean tPSA before DRE	2.37 ng/mL
Mean tPSA after DRE	2.41 ng/mL
Caucasian race	123 (75.9%)
African American race	24 (14.8%)

Table 2:

4Kscore and Kallikrein values before and after DRE

	Pre-DRE	Post-DRE	Delta, Median (IQR)	p value
4Kscore (%)			−0.43% (−0.91%, −0.04%)	<0.01
10 th percentile	1.4	1.2		
25 th percentile	2.9	2.7		
50 th percentile	4.8	4.5		
75 th percentile	8.7	7.6		
90 th percentile	16.8	15.3		
tPSA (ng/ml)			0.027 (−0.03, 0.10)	<0.01
10 th percentile	0.51	0.52		
25 th percentile	0.93	0.90		
50 th percentile	1.61	1.72		
75 th percentile	3.19	3.38		
90 th percentile	4.84	4.92		
fPSA (ng/ml)			0.024 (−0.001, 0.066)	<0.01
10 th percentile	0.16	0.17		
25 th percentile	0.26	0.26		
50 th percentile	0.43	0.47		
75 th percentile	0.73	0.81		
90 th percentile	1.24	1.26		
iPSA (ng/ml)			0.012 (−0.003, 0.033)	<0.01
10 th percentile	0.11	0.13		
25 th percentile	0.17	0.17		
50 th percentile	0.28	0.29		
75 th percentile	0.44	0.50		
90 th percentile	0.70	0.72		
hK2 (ng/ml)			0 (−0.002, 0.001)	NS
10 th percentile	0.013	0.014		
25 th percentile	0.025	0.025		
50 th percentile	0.048	0.049		
75 th percentile	0.074	0.074		
90 th percentile	0.114	0.115		
f/tPSA ratio			0.009 (0.003, 0.026)	<0.01
10 th percentile	0.16	0.17		

	Pre-DRE	Post-DRE	Delta, Median (IQR)	p value
25 th percentile	0.20	0.23		
50 th percentile	0.26	0.27		
75 th percentile	0.35	0.37		
90 th percentile	0.42	0.44		

Table 3:

Reclassification of 4Kscore Above and Below DRE Cutoff of 7.5%

		Post-DRE 4Kscore < 7.5%	Post-DRE 4Kscore ≥ 7.5%	Total
Pre-DRE 4Kscore ≥ 7.5%	112	1	113	
Pre-DRE 4Kscore < 7.5%	9	40	49	
Total	121	41	162	
Reclassified patients*	9	1	10	

* McNemar's test P = 0.022