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A Four-kallikrein panel and Beta-microseminoprotein in Predicting High-Grade Prostate Cancer on Biopsy: an Independent Replication from the Finnish Section of the European Randomized Study of Screening for Prostate Cancer.

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ABSTRACT (247 words)

Purpose: A panel of 4 kallikrein markers (total, free and intact prostate-specific antigen, human kallikrein-related peptidase 2) improves predictive accuracy for Gleason score 7 or higher (high-grade) prostate cancer among men biopsied for elevated PSA. A 4-kallikrein panel model was originally developed and validated on the Dutch center of the European Randomized Study of Screening for Prostate Cancer. We assessed whether these findings could be replicated among participants in the Finnish section of ERSPC and whether beta-microseminoprotein, a candidate prostate cancer biomarker, adds predictive value.

Materials and Methods: Among biopsied screening-positive participants from the first 3 screening rounds of the FinRSPC a case-control subset was selected in which 1,632 biopsy-positive cases were matched to biopsy-negative controls based on age at biopsy. Predictive accuracy of pre-specified models were compared with biopsy outcomes.

Results: Among men with PSA 4.0-25 ng/mL, 1,111 had prostate cancer, 318 with high-grade disease. Total PSA and age predicted high-grade cancer with an area under the curve of 0.648 (95% CI 0.614-0.681) and the 4-kallikrein panel increased discrimination to 0.746 (95% CI 0.717-0.774). Adding MSP to the 4-kallikrein panel led to a statistically significant (Wald test; p = 0.015) but small increase (0.003) in discrimination.

Conclusions: These findings provide additional evidence that kallikrein markers can be used to inform biopsy decision-making. Further studies are needed to define the role of MSP.

Prostate cancer screening with prostate-specific antigen is controversial due to a questionable balance between harms (eg, overdiagnosis and overtreatment), and benefits (eg, reduced cancer mortality). The major drawback of PSA as a screening test is its low specificity for aggressive disease. While men with an elevated PSA are at an increased risk of aggressive PC, it can also indicate benign prostatic disease or low risk PC.[1] As a result, many men with elevated PSA levels undergo unnecessary prostate biopsies, which can lead to complications such as infection and bleeding.[2] Prostate biopsy may detect indolent PC that would otherwise never become apparent to the patient during his lifetime (overdiagnosis). Better methods are needed to detect Gleason score ≥7 PC (high-grade) in order to reduce the negative consequences of screening, by decreasing the number of men without aggressive PC unnecessarily referred for biopsy.

Measured PSA is a combination of a number of different molecular subforms: complexed PSA vs free PSA, intact vs nicked PSA, and human kallikrein-related peptidase 2.[3-5] Measuring subforms separately has been shown to improve prediction of biopsy results among men with elevated PSA.[6,7] We developed a prediction model based on the panel of 4 kallikrein markers (total, free and intact PSA, and hK2) using serum samples from the Dutch and Swedish sections of the European Randomized Study of Screening for Prostate Cancer and demonstrated improved discrimination of high-grade PC (Gleason score ≥7) compared to clinical variables and total PSA alone.[8-11] In a large, representative-population-based cohort of unscreened men who gave blood at age 50 or 60, the panel of 4 kallikrein markers significantly enhanced accuracy of predicting future metastatic prostate cancer.[12] The markers are now commercially available as the 4Kscore™. Another abundant prostate-derived protein, beta-microseminoprotein,[13] was shown to be lower in blood among men with PC and a suggested association with high-grade PC in 2 prospective studies, the STHLM3 trial [14] and the Multiethnic Cohort study.[15] We sought to determine whether our previous findings, as well as the addition of MSP to the prediction

model, could be replicated in men participating in the Finnish section of ERSPC, the largest section of ERSPC.

METHODS

Our aim was to assess the predictive accuracy of prediction models comprised of age, the 4 kallikreins, and MSP. A total of 4,861 participants were biopsied in 1996-2008 as a result of an elevated PSA either in the first round of screening (ie, first PSA on study) or in rounds 2 or 3 (ie, with 1 or 2 prior PSA tests on study, respectively) (Fig. 1). Among screen-positive participants, a case-control subset was selected for evaluation of the 4-kallikrein panel and MSP. This included 1,632 biopsy-positive cases that were individually matched based on age at biopsy to 1,632 biopsy negative controls. Biopsy data were missing for 17 of these subjects, leaving 1,615 biopsy-negative controls. There were 156 cases and 174 controls that had missing or inadequate sample to assay the 4-kallikrein panel and MSP, leaving 1,476 cases and 1,441 controls for analysis.

Men randomly allocated to the screening arm in the FinRSPC trial with a screening PSA ≥4.0 ng/mL were referred to a urological clinic for prostate biopsy.[16] Men with PSA levels of 3.0–3.99 ng/mL were referred to an additional test, which in 1996–1998 was DRE and from 1999 determination of the free/total PSA ratio with a cut-off point of <16%. Hence in men with a PSA 3.0 to 3.99 ng/mL, only those with a suspicious DRE or free-to-total PSA ratio <16% were referred for biopsy.[16,17]

Sextant biopsies were performed from the start of the trial in 1996, but a 10-12 biopsy core approach was adopted in 2002.[16] Cryopreserved sample aliquots (serum or plasma) were shipped to the Wallenberg Research Laboratories at Lund University, Malmö, Sweden, for assay of the panel of the 4 kallikrein markers and MSP during 2014-2015. The analysis of total and free PSA,[18] intact PSA,[19] hK2,[20] and MSP[21] were previously reported.[22,23] The assay reagents used in our measurements of intact PSA

and hK2 were the same as those used in the 4Kscore™ test. Total and free PSA concentrations are based on the measurements of cryopreserved samples performed in Malmö. Our study excluded a small number (2%) of screening participants who had a PSA of ≥3.0 ng/mL in the screening trial but whose total PSA was less than 3.0 ng/mL according to the World Health Organization-calibrated measurements performed in Malmö.

We compared the discrimination of a pre-specified model including only total PSA and age, a pre-specified model including age, total and free PSA, and the pre-specified kallikrein model based on age and the 4 kallikrein markers, nonlinear terms were included for free and total PSA. We did not include DRE in these models as it was inconsistent with clinical stage: 36% of patients with a positive DRE and cancer diagnosis had clinical stage T1C and 17% with a negative DRE had clinical stage T2A or greater. These models were developed using serum sample measurements and biopsy data from the Rotterdam screening arm of ERSPC and independently applied to this dataset.[8] This model is calibrated to men undergoing sextant biopsy with pathologic grading by 1990's criteria. By contrast, the proprietary 4Kscore™ model was developed[24] and validated[25] based on 50- to 70-year-old men with PSA levels of 3.0 ng/mL or higher undergoing prostate biopsy with 10 or more cores and with pathology using contemporary Gleason grading approaches.

Our main analyses included men with total PSA of 4.0 to 25 ng/mL. The upper limit was chosen on the grounds that most urologists would hesitate not to biopsy men with a PSA >25 ng/mL, and the panel gives uniformly high risks to this group; therefore a reflex test would not be required among these men.[26] Men with PSA from 3.0-3.99 ng/mL were biopsied depending on their free/total PSA ratio, we did not include these men in the main analysis due to the possibility of verification bias. We performed additional sensitivity analyses for other ranges of total PSA including the range of 3.0-10 ng/mL, often described as the diagnostic "grey zone" for biopsy. Because it is likely that a prior PSA test or prior

negative biopsy affects the accuracy of a predictive model, we assessed all participants who were biopsied as a result of screening round 1 and screening rounds 2-3, separately.[9,11,26]

Analyses of calibration and clinical utility when reporting the results of the 4-kallikrein panel have been typically reported including metrics such as the numbers of biopsies avoided and cancer diagnoses delayed, and decision analyses.[27] The current data set is complicated by the case-control design. Because we have data on the clinical utility of the panel, we did not see the value of such analyses here, as they would involve questions such as the number of men who would have high-grade cancer, as defined in the 1990s, on sextant biopsy.

We investigated whether adding MSP to the prediction model with the 4-kallikrein panel increased predictive value. In order to generate the MSP prediction models for prediction of any cancer and high-grade cancer on biopsy, separately, using constrained logistic regression. Log-transformed MSP was entered into the prediction model along with cubic splines to allow for nonlinearity, kallikrein model risk (estimated based on the 4-kallikrein model) was entered on the inverse-logit scale and the coefficient was constrained to be 1. Ten-fold cross validation was used when assessing the performance of the model including MSP to adjust for statistical overfit.

Individual-level 5-alpha reductase inhibitors (5ARI) purchase data were obtained from a Finnish national prescription database and were available for all study participants. We investigated whether purchasing a 5ARI within 6 months prior to screening affected the relationship between the 4-kallikrein panel prediction model and the risk of high-grade cancer. An interaction between 5ARI and the 4-kallikrein panel model was tested using multivariable logistic regression. Previous studies suggest that total PSA values are reduced by about 50% in men who take 5ARIs.[28] We compared the accuracy defined as the Brier score of the 4-kallikrein prediction model to a model that artificially doubles the values of

total, free and intact PSA among men who purchased a 5ARI within 6 months prior to screening. All analyses were performed using Stata, version 13.0 (StataCorp, College Station, TX).

RESULTS

The characteristics of participants with total PSA between 4.0 and 25 ng/mL included in the analyses are displayed in table 1. Within the first 3 screening rounds of the FinRSPC, a total of 1,111 cancers were diagnosed, of which 318 (29%) were identified as high-grade (Gleason ≥7). Table 1 shows that the concentrations of all 4 kallikrein markers and MSP differed significantly by biopsy status including nicked to total PSA ratio (nicked PSA is free PSA minus intact PSA). An exception is that intact PSA levels were not significantly different in the comparison of high-grade disease vs low-grade or no cancer diagnosis. All prediction models demonstrated a significantly greater predicted risk among those with cancer vs no cancer and high-grade cancer vs low-grade or no cancer (table 1, Wilcoxon rank-sum test all p-values <0.0001).

Because there was little difference in discrimination comparing serum and plasma samples we included both in the primary analysis. Discriminative accuracy increased with addition of each marker (table 2). Discrimination based on age and total PSA was low (0.595 and 0.648 in predicting any- and high-grade disease, respectively; table 2). The highest increase in discrimination occurred with the addition of free PSA to the model with age and total PSA for the prediction of both any- and high-grade with AUC gains of 0.126 and 0.051, respectively. Although MSP was found to be predictive of any- and high-grade disease after adjusting for the kallikrein panel (Wald Test; p-values <0.0001 and 0.015, respectively), it represented the smallest increase in AUC with gains of 0.012 and 0.003, respectively. The prespecified 4-kallikrein model displayed moderately strong discriminative ability with AUCs of 0.743 and 0.746 in predicting any- and high-grade disease, respectively.

Although the discriminative ability of all prediction models varied slightly across the PSA ranges, the increment in predictive accuracy associated with the markers was similar in all analyses. The exceptions were in discrimination by screening round and when MSP was added to the 4-kallikrein model: discrimination improved among men without a prior screening but not for those with a previous PSA test. Conversely, intact PSA and hK2 added discrimination among previously screened men (table 3). The lowest increase in discrimination from the model based on age and total PSA compared to the 4-kallikrein model occurred when we analyzed measurements based on serum samples only (0.071; table 3), which could suggest degradation of the decay-prone components, free and intact PSA, in serum.

We did not find evidence of an interaction between 5ARI status and 4-kallikrein model (p=0.4). Additionally, we found no evidence that predictive accuracy improved when the levels of PSA isoforms were doubled in patients on 5ARIs, with a poorer Brier score (0.199) for the adjusted marker levels than for the unadjusted levels (0.170).

DISCUSSION

In this study of FinRSPC participants with an elevated PSA, we found that a pre-specified model based on the 4-kallikrein panel improved predictive discrimination for any- and high-grade (Gleason ≥7) PC compared to a prediction model based on age and total PSA, and compared to a model that also included free PSA. These results support the earlier findings that the 4Kscore™ is strongly predictive of the risk of high-grade cancer at biopsy such that use of the model could guide biopsy decision making, which would reduce the harms of PSA screening. Further empirical research is necessary to ascertain how the 4-kallikrein panel should be amended for patients on 5ARIs.

We found that the addition of MSP to the kallikrein panel provides a small improvement in diagnostic performance. In one study, MSP provided no added discriminatory value to age and the 4-

kallikrein panel. However, this was observed among men in a community-based setting, who were indicated for biopsy due to an elevated PSA (≥3.0 ng/mL) and <20% free-to-total PSA ratio, or suspicious DRE.[23] In another large, prospective, screening trial (STHLM3), MSP improved discrimination when added to a model including PSA, age, family history, prior biopsy and a genomic risk score. However, these authors did not report whether MSP was added to a model already incorporating free PSA, intact PSA and hK2.[14] In the Multiethnic Cohort, MSP levels in blood were inversely correlated with the risk of subsequent PC, but not add to PSA in terms of risk prediction.[15] Further well-defined studies on MSP are warranted to determine its role in PC prediction.

Our findings add to the growing body of evidence that the 4-kallikrein panel can be used as a reflex test among those with an elevated PSA in order to aid biopsy decision-making. Many studies have shown the kallikrein panel improves discrimination compared to a model with only age and total PSA including 3 other sections of the ERSPC: Tarn, Göteborg, and Rotterdam.[8-11,23-26,29] We previously developed a prediction model among previously unscreened men in the Göteborg arm including age and the 4-kallikrein panel and showed that it increased discrimination on top of age and total PSA dramatically from 0.68 to 0.87 among men with a total PSA of ≥ 3 ng/mL.[10] Among Rotterdam participants there was a statistically significant increase with the addition of free PSA, intact PSA and hK2 to the model with age and total PSA increasing from 0.776 to 0.825 for the detection of high-grade prostate cancer.[8] The 4-kallikrein panel demonstrated a high discriminative capability (AUC 0.82) in a large prospective setting among US men and superior net benefit compared to the widely used Prostate Cancer Prevention Trial Risk Calculator 2.0, which incorporates standard clinical variables (AUC 0.74).[25] The AUC for high-grade cancer we report is slightly lower than these prior studies, but the added benefit of the markers was very similar. For instance, in the US validation study, the kallikrein panel had an AUC of 0.08 units greater than a model including PSA only, compared to an increase of 0.10 here.

We found that the addition of intact PSA and hK2 improved discrimination in all sensitivity analyses except among first screening round participants, where most men had not had a prior PSA test whereas other studies demonstrate the added value of intact PSA and hK2 is independent of screening history. In particular, the statistical model used in the 4Kscore™ test was built on unscreened participants in the ProtecT trial [24], but had excellent discrimination and calibration when applied to a US clinical cohort,[25] where almost all patients had prior screening. We believe our finding was likely a chance result attributable to multiple testing: just as an ineffective marker might by chance show predictiveness in at least one of multiple subgroups tested, an effective marker might by chance fail to show predictiveness in at least one subgroup.

This study is an independent external validation of prespecified predictive models based on a large, prospective, screening cohort, and as such the results of the study are generalizable to the Finnish and likely other northern European populations. A notable aspect of our study is that participants were biopsied based on a scheme unique to the Finnish arm of ERSPC. Here, a PSA of ≥4.0 ng/mL was the cutoff for biopsy, with men in the range of 3.0–3.9 ng/mL subject to followup testing to determine biopsy as previously described. Since the panel incorporates free PSA, there is a risk of verification bias when assessing the value of the panel in men with PSA 3.0–3.9 ng/mL. Therefore, our primary analysis was performed in men with a PSA from 4.0 to 25 ng/mL. Another limitation is that we did not incorporate DRE results into our prediction model. Although this may slightly underestimate the value of the panel, several other studies, including Braun et al.[23] and Bryant et al.[24] demonstrate that the inclusion of intact PSA and hK2 increases the prediction of any- and high-grade cancer detection when DRE is not incorporated.

CONCLUSION

We have replicated in a large, prospective independent cohort the finding that a panel of 4 kallikreins can improve the predictive accuracy of prostate cancer and high-grade PC. We also found that adding MSP marginally improves prediction, although further studies are needed to define the role of this marker. Our data provides further evidence that kallikrein models can be used as a reflex test to determine which men with elevated PSA can avoid biopsy, reducing unnecessary biopsies and overdiagnosis of low-grade disease.

Abbreviations

AUC = area under the receiver operating characteristic curve

DRE = digital rectal examination

ERSPC = European Randomized Study of Screening for Prostate Cancer

FinRSPC = Finnish section of ERSPC

hK2 = human kallikrein-related peptidase 2MSP = beta-microseminoprotein

PC = prostate cancer

PSA = prostate-specific antigen

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CONFLICT OF INTEREST

Hans Lilja holds patents for free PSA, hK2, and intact PSA assays, and is named, along with Andrew J. Vickers, on a patent for a statistical method to detect prostate cancer. The marker assay patents and the patent for the statistical model have been licensed and commercialized as the 4Kscore™ by OPKO Diagnostics. Drs. Vickers and Lilja receive royalties from sales of this test. Additionally, Dr. Lilja owns stock and Dr. Vickers owns stock options in OPKO.

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Table 1. Characteristics of participants with total PSA between 4 and 25 ng/mL by biopsy result. P values are reported for the comparison of participants with no diagnosis vs participants with a cancer diagnosis and participants with no or low-grade cancer diagnosis vs participants with a high-grade cancer diagnosis. Estimates are given as median (interquartile range) or frequency (percentage).

Characteristic	No Cancer (N=1,019; 48%)	Cancer (N=1,111; 52%)	p-value*	No or Low-grade Cancer (N=1,810; 85%)	High-grade Cancer (N=318; 15%)	p-value*
Age at Biopsy	67 (63, 68)	67 (63, 68)	0.2	67 (63, 68)	67 (63, 70)	<0.0001
Total PSA (ng/mL)	5.2 (4.5, 6.6)	5.9 (4.7, 8.2)	<0.0001	5.3 (4.6, 7.0)	6.5 (5.0, 10.0)	<0.0001
Free PSA (ng/mL)	1.25 (0.94, 1.71)	1.02 (0.73, 1.43)	<0.0001	1.16 (0.84, 1.58)	1.05 (0.78, 1.42)	0.005
Free to Total PSA Ratio	0.23 (0.18, 0.29)	0.16 (0.12, 0.22)	<0.0001	0.20 (0.15, 0.27)	0.15 (0.11, 0.21)	<0.0001
Intact PSA (ng/mL)	0.73 (0.55, 0.99)	0.64 (0.46, 0.88)	<0.0001	0.69 (0.50, 0.94)	0.69 (0.51, 0.94)	0.9
Nicked to Total PSA Ratio	0.093 (0.071, 0.127)	0.058 (0.039, 0.085)	<0.0001	0.081 (0.055, 0.113)	0.050 (0.033, 0.073)	<0.0001
hK2 (ng/mL)	0.064 (0.041, 0.097)	0.075 (0.049, 0.116)	<0.0001	0.067 (0.044, 0.103)	0.079 (0.053, 0.117)	<0.0001
MSP (ng/mL)	28 (19, 38)	23 (15, 31)	<0.0001	26 (18, 36)	23 (15, 31)	0.001
Gleason Score at Biopsy						
≤6		791 (71%)		791 (44%)		
7		231 (21%)			231 (73%)	
≥8		87 (7.8%)			87 (27%)	
Unknown		2 (0.2%)				

*p-values determined by Wilcoxon Rank Sum for continuous variables and Fisher's exact test for categorical variables.

PSA = prostate-specific antigen

hK2 = human kallikrein-related peptidase 2

MSP = beta-microseminoprotein

Table 2. AUCs and 95% confidence intervals and differences in AUCs for predicting prostate cancer status among participants with a total PSA of 4 – 25 ng/mL.

Outcome	PSA + Age	Age + PSA + free PSA	Increase with free PSA	Kallikrein model	Kallikrein model vs Age + PSA	Kallikrein model vs Age + PSA + free PSA	Kallikrein model + MSP	Increase with MSP
Cancer 1,111 Cases 1,019 Controls	0.595 (0.571, 0.619)	0.721 (0.700, 0.743)	0.126	0.743 (0.722, 0.763)	0.148	0.022	0.755 (0.734, 0.775)	0.012
High grade 318 Cases 1,810 Controls	0.648 (0.614, 0.681)	0.699 (0.668, 0.731)	0.051	0.746 (0.717, 0.774)	0.098	0.047	0.749 (0.721, 0.778)	0.003

PSA = prostate-specific antigen
MSP = beta-microseminoprotein

Table 3. Sensitivity analyses. Discrimination (AUCs) for high-grade cancer.

Analysis	PSA + Age	Age + PSA + free PSA	Increase with free PSA	Kallikrein model	Kallikrein model vs. Age + PSA	Kallikrein model vs. Age + PSA + free PSA	Kallikrein model + MSP	Increase with MSP
Main analysis: PSA 4 – 25 318 Cases 1,810 Controls	0.648 (0.614, 0.681)	0.699 (0.668, 0.731)	0.051	0.746 (0.717, 0.774)	0.098	0.047	0.749 (0.721, 0.778)	0.003
PSA ≥ 4 349 Cases 1,843 Controls	0.668 (0.635, 0.700)	0.715 (0.685, 0.745)	0.047	0.756 (0.729, 0.783)	0.088	0.041	0.759 (0.733, 0.786)	0.003
PSA 3 – 10 287 Cases 2,234 Controls	0.615 (0.579, 0.651)	0.698 (0.666, 0.729)	0.083	0.744 (0.715, 0.772)	0.129	0.046	0.746 (0.718, 0.775)	0.002
PSA 3 - 25 365 Cases 2417 Controls	0.655 (0.623, 0.687)	0.709 (0.680, 0.738)	0.054	0.758 (0.733, 0.783)	0.103	0.049	0.761 (0.736, 0.786)	0.003
Serum only 160 Cases 1,273 Controls	0.671 (0.624, 0.718)	0.699 (0.655, 0.744)	0.028	0.742 (0.703, 0.782)	0.071	0.043	0.749 (0.711, 0.788)	0.007
Screening Round 1 43 Cases 516 Controls	0.682 (0.593, 0.770)	0.779 (0.709, 0.850)	0.097	0.774 (0.710, 0.838)	0.092	-0.005	0.787 (0.726, 0.848)	0.013
Screening Rounds 2-3 275 Cases 1,294 Controls	0.644 (0.606, 0.681)	0.706 (0.671, 0.741)	0.062	0.759 (0.728, 0.790)	0.115	0.053	0.757 (0.725, 0.788)	-0.002

PSA = prostate-specific antigen
MSP = beta-microseminoprotein

Fig. 1. Flow of participants.