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Prostate Cancer

A Four-kallikrein Panel Predicts High-grade Cancer on Biopsy: Independent Validation in a Community Cohort

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Abstract

Background: A statistical model based on four kallikrein markers (total prostate-specific antigen [tPSA], free PSA [fPSA], intact PSA, and human kallikrein-related peptidase 2) in blood can predict risk of Gleason score ≥7 (high-grade) cancer at prostate biopsy.
Objective: To determine the value of this model in predicting high-grade cancer at biopsy in a community-based setting in which referral criteria included percentage of fPSA to tPSA (%fPSA).
Design, setting, and participants: We evaluated the model, with or without adding blood levels of microseminoprotein-β (MSMB) in a cohort of 749 men referred for prostate biopsy due to elevated PSA (≥3 ng/ml), low %fPSA (<20%), or suspicious digital rectal examination at Skåne University Hospital, Malmö, Sweden.
Outcome measurements and statistical analysis: The kallikrein markers, with or without MSMB levels, measured in cryopreserved anticoagulated blood were combined with age in a published statistical model (Prostate Testing for Cancer and Treatment [ProtecT]) to predict high-grade cancer at biopsy. Predictive accuracy was compared with a base model.
Results and limitations: The %fPSA was low (median: 17; interquartile range: 13–22) in this cohort because this marker was used as a referral criterion. The ProtecT model improved discrimination over age and PSA for high-grade cancer (0.777 vs 0.720; $p = 0.002$). At one illustrative cut point, use of the panel would reduce the number of biopsies by 236 per 1000 and detect 195 of 208 (94%) but delay diagnosis of 13 of 208 high-grade cancers. MSMB levels in blood did not improve the accuracy of the panel ($p = 0.2$).
Conclusions: The kallikrein model is predictive of high-grade cancer if criteria for biopsy referral also include %fPSA, and it can reduce unnecessary biopsies without missing an undue number of tumors.
Patient summary: We evaluated a published model to predict biopsy outcome in men biopsied due to low percentage of free to total prostate-specific antigen. The model helps reduce unnecessary biopsies without missing an undue number of high-grade cancers.

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1. Introduction

In unscreened men aged 45–60 yr, the risk of metastasis and death from prostate cancer (PCa) is strongly associated with the prostate-specific antigen (PSA) concentration in the blood [1]. Data from large randomized trials in Europe show that PSA-based screening for PCa significantly reduces death from PCa among men not otherwise screened for this disease [2–4]. PSA is widely used for the early detection of PCa [5]. However, PSA has only moderate specificity, with only a minority of men with elevated PSA harboring high-grade PCa.

Previous studies showed that a statistical model based on a panel of four kallikrein markers (total PSA [tPSA], free PSA [fPSA], intact PSA, and human kallikrein-related peptidase 2 [hK2]) can predict the outcome of prostate biopsy in a variety of different settings including unscreened men, men with a prior PSA test, and those with a prior negative biopsy [6–11].

These studies involved cohorts from the European Randomized Study of Screening for Prostate Cancer (ERSPC) in which decisions to biopsy men were based on findings of an elevated PSA (≥3 ng/ml). In routine clinical practice, urologists often take into account percentage of fPSA to tPSA (%fPSA) before deciding to biopsy. Because fPSA is part of the kallikrein panel, it is plausible that the panel would lose its value if evaluated in this context. We evaluated the four-kallikrein panel in a large community-based referral cohort at Skåne University Hospital, Malmö, Sweden, in which referral criteria explicitly included low %fPSA.

As a secondary aim, we assessed whether another abundant secretory protein in the prostate suggested as an additional blood marker, microseminoprotein-β (MSMB) [12], would contribute additional discriminatory accuracy above and beyond that of the kallikrein-based model alone. The common single nucleotide polymorphism (rs10993994) in the 5′ region of the *MSMB* gene encoding β-microseminoprotein (MSP) is associated with both circulating levels of MSP in serum and with PCa risk [13,14]. MSP levels in serum were shown to be significantly lower in men with PCa and even lower in men with aggressive disease [15,16].

2. Patients and methods

2.1. Patient population

The study cohort included 749 men referred to Skåne University Hospital for prostate biopsy between 2004 and 2010. Decisions to perform prostate biopsies were based on tPSA levels in serum ≥3.0 ng/ml, %fPSA ≤20%, or a suspicious digital rectal examination (DRE). Patients underwent an eight-core prostate biopsy. All participants gave written informed consent at the time of recruitment, and the project was approved by the research ethics board at Lund University (research authorization number 367/2003). Histopathologic assessment of biopsies was undertaken by urologic pathologists blinded to marker levels using standardized protocols [17].

2.2. Immunoassay measurements of four kallikrein markers and MSMB

Ethylenediaminetetraacetic acid anticoagulated blood was collected by venipuncture, centrifuged at 3000 g for 10 min within 30–90 min of the blood draw, and frozen at −80 °C as anticoagulated plasma to minimize the risk of biomarker degradation in the cryopreserved samples [18,19]. Immunoassay measurements for tPSA, fPSA [18], intact PSA, hK2 [20,21], and MSMB [22,23] were conducted on the AutoDelfia 1235 automatic immunoassay system in H.L.'s laboratory at the Wallenberg Research Laboratories, Department of Translational Medicine, Lund University, Skåne University Hospital, as reported previously [9,12].

The fPSA and tPSA were measured using the dual-label DELFIA Prostatus tPSA/fPSA assay (Perkin-Elmer, Turku, Finland) [18] calibrated against the World Health Organization (WHO) 96/670 (PSA-WHO) and WHO 68/668 (fPSA-WHO) standards. The measurements of intact PSA and hK2 were performed with F(ab′)2 fragments of the monoclonal capture antibodies, as previously reported [8]. The intact PSA assay measures only single-chain intact forms of fPSA (uncomplexed) because the monoclonal 4D4 immunoglobulin G used in this assay does not bind multichain forms of fPSA cleaved at Lys_{145} or Lys_{146} [24]. Production and purification of the polyclonal rabbit anti-MSMB antibody, protocols for biotinylation and Europium labeling of the anti-MSMB antibody, and performance of the MSMB immunoassay were carried out as previously reported [12,23]. All assay measurements were conducted blind to biopsy result.

2.3. Statistical analysis

Our primary aim was to validate the accuracy of the four-kallikrein model for predicting high-grade cancer on biopsy in a large referral cohort at Skåne University Hospital. The kallikrein model was built utilizing data from 4765 men enrolled in the prospective randomized trial Prostate Testing for Cancer and Treatment (ProtecT) [25]. In brief, the ProtecT study enrolled men aged 50–69 yr and invited them between 2001 and 2008 to receive PSA testing. Men with a PSA measurement ≥3.0 ng/ml were invited to undergo a 10-core prostate biopsy [26]. Although the primary aim was to assess the performance of the kallikrein markers to predict high-grade cancer (Gleason score ≥7) on biopsy, because these men will likely undergo immediate treatment, we repeated all analyses evaluating the performance of a model predicting any-grade cancer using the four-kallikrein markers and patient age. Models incorporating the DRE result (normal vs abnormal), patient age, and the four kallikrein markers were also assessed. The DRE result was not available in the ProtecT cohort at the time of the model development, and so the coefficient for DRE was taken from a separate model [7].

We began by comparing the performance of the kallikrein model with two base models consisting of tPSA and patient age and tPSA, patient age, and DRE. The base models were built on the Skåne University Hospital cohort using logistic regression. The tPSA was entered into the model using restricted cubic splines to account for any nonlinearity. The predictions from the resulting model were corrected for overfit using 10-fold cross validation. Separate models were created for predicting high-grade PCa and any-grade cancer on biopsy.

Analysis of area under the curve (AUC) was utilized to assess the ability of each model to discriminate between patients with and without evidence of high-grade cancer at biopsy. To compare the discrimination of the kallikrein model with the discrimination of the base model, the DeLong, DeLong, and Clarke-Pearson method was used [27]. Calibration plots were used to assess calibration.

To determine the clinical value of the four-kallikrein model in this cohort, we used decision curve analysis [28]. This method estimates a net benefit for prediction models by summing the benefits (true positives) and subtracting the harms (false positives). Because the harms of missing a true positive generally differ from those resulting from a false positive, the net benefit calculation weights true and false positives differently. The weighting is derived from the threshold probability of a disease at which a patient would opt for intervention, in this case the