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Prostate Cancer

Twenty-year Risk of Prostate Cancer Death by Midlife Prostate-specific Antigen and a Panel of Four Kallikrein Markers in a Large Population-based Cohort of Healthy Men

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Abstract

Background: Prostate-specific antigen (PSA) screening reduces prostate cancer deaths but leads to harm from overdiagnosis and overtreatment.
Objective: To determine the long-term risk of prostate cancer mortality using kallikrein blood markers measured at baseline in a large population of healthy men to identify men with low risk for prostate cancer death.
Design, setting, participants: Study based on the Malmö Diet and Cancer cohort enrolling 11 506 unscreened men aged 45–73 yr during 1991–1996, providing cryopreserved blood at enrollment and followed without PSA screening to December 31, 2014. We measured four kallikrein markers in the blood of 1223 prostate cancer cases and 3028 controls.
Outcome measurements and statistical analysis: Prostate cancer death (n = 317) by PSA and a prespecified statistical model based on the levels of four kallikrein markers.
Results and limitations: Baseline PSA predicted prostate cancer death with a concordance index of 0.86. In men with elevated PSA ($\geq$2.0 ng/ml), predictive accuracy was enhanced by the four-kallikrein panel compared with PSA (0.80 vs 0.73; improvement 0.07; 95% confidence interval 0.04, 0.10). Nearly half of men aged 60+ yr with elevated PSA had a four-kallikrein panel score of $<$7.5%, translating into 1.7% risk of prostate cancer death at 15 yr—a similar estimate to that of a man with a PSA of 1.6 ng/ml. Men with a four-kallikrein panel score of $\geq$7.5% had a 13% risk of prostate cancer death at 15 yr.
Conclusions: A prespecified statistical model based on four kallikrein markers (commercially available as the 4Kscore) reclassified many men with modestly elevated PSA, to have a low long-term risk of prostate cancer death. Men with elevated PSA but low scores from the four-kallikrein panel can be monitored rather than being subject to biopsy.
Patient summary: Men with elevated prostate-specific antigen (PSA) are often referred for prostate biopsy. However, men with elevated PSA but low scores from the four-kallikrein panel can be monitored rather than being subject to biopsy.

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1. Introduction

There is clear randomized trial evidence that prostate-specific antigen (PSA) screening reduces prostate cancer mortality [1]. However, PSA has modest specificity for aggressive prostate cancer [2–4], with the result that many men must be screened, biopsied, and diagnosed to prevent one death [1].

There have been extensive efforts to improve the specificity of PSA, including longitudinal change in PSA levels ("PSA velocity" [5]), the ratio of free-to-total PSA [6], and more recently developed markers such as PHI [7], *PCA3* [8], or *TMPRSS2-ERG* [9]. Based on the theory that the enzymatic action of PSA is critical to its function as a biomarker in the blood, we developed a panel of four kallikrein markers in the blood. We have demonstrated in multiple studies that a statistical model based on this panel enhances the specificity of PSA for Gleason score 7 (GGG2) or higher prostate cancer on prostate biopsy [10–13]. Following a prospective, independent, multicenter validation study in the USA [14], the biomarker panel has been made commercially available by OPKO Inc. as the 4Kscore.

One limitation of our prior studies, similar to most other studies of markers for prostate cancer detection, is the use of biopsy outcome as the end point. Gleason grade on biopsy is a surrogate end point and does not have a 1:1 association with prostate cancer morbidity or mortality: a biopsy may miss a GGG2 or higher (high-grade) cancer or detect a cancer that, while seemingly aggressive, would never become apparent to the patient during the course of his natural life. As such, we have an interest in determining whether the four-kallikrein panel can predict the true clinical outcome of prostate cancer morbidity and mortality. We have previously shown that in a largely unscreened population, the four-kallikrein panel can discriminate men with elevated PSA who later developed distant prostate cancer metastases from those who had no evidence of metastases by 10–15 yr [15]. Here, we attempt to replicate the findings of this prior study in a large independent population-based cohort of unscreened men with longer follow-up so that the performance of the four-kallikrein panel for the critical outcome of prostate cancer death can be determined.

2. Patients and methods

2.1. Patient population

The Malmö Diet and Cancer (MDC) project is a large, prospective, observational, population-based study of the relationship between diet and incident cancer that has been described previously [16,17]. In brief, 11 506 men living in Malmö, Sweden, born between 1923 and 1945, participated and provided an anticoagulated blood plasma sample at enrolment during 1991–1996. The Swedish Cancer Registry, the National Prostate Cancer Registry of the Southern Region [18,19], and Swedish National Cause of Death Registry, previously shown to provide highly accurate data, were used to identify men subsequently diagnosed with prostate cancer, along with diagnostic data, as of December 31, 2014. Cause of death was obtained from the Causes of Death Registry at the National Board of Health and Welfare in Sweden, a reliable source of the cause of death from prostate cancer [20]. A personal identity number, unique for every Swedish citizen, was used to track and merge data from the different registries. Rates of PSA screening in this cohort were initially very low, but have increased during the last years of follow-up [21].

Data on follow-up for prostate cancer diagnosis and death from prostate cancer in the MDC cohort were collected until December 31, 2014. Overall, 1476 men were diagnosed with and 317 died from prostate cancer. The kallikrein panel markers were measured for 291 of the prostate cancer deaths and 1223 diagnoses. There were 3028 men without prostate cancer whose kallikrein panel markers were also measured. Details on the selection of men for marker measurement has been reported previously [22]; additional details can also be found in the Supplemental material.

2.2. Laboratory methods

We measured four kallikrein markers—human kallikrein-related peptidase 2 (hK2) and total, free, and intact PSA—in cryopreserved (below −70 °C) heparin anticoagulated blood plasma from cases and controls. All laboratory analyses were conducted blind to outcome and case-control status. We measured total and free PSA with the dual-label DELFIA ProStatus assay (PerkinElmer, Turku, Finland) [23], calibrated against the World Health Organization (WHO) 96/670 (PSA-WHO) and WHO 68/668 (free PSA-WHO) standards, in previously unthawed cryopreserved heparin anticoagulated blood plasma. Intact PSA and hK2 were measured using F(ab′)2 fragments of the monoclonal capture antibodies to reduce the frequency of nonspecific assay interference [24].

2.3. Statistical methods

Our primary aim was to assess the ability of a prespecified model using the four kallikrein markers to predict long-term risk of prostate cancer mortality. Total PSA, intact PSA, free PSA, and hK2 from controls who were not selected as a part of the case-control subset were imputed using predictive mean matching across 10 multiple imputation sets. Analytic results were combined from these 10 sets using Rubin's methods [25].

The four-kallikrein panel of markers were combined as previously described into a prespecified prediction model that provides the risk of Gleason score 7 (GGG2) or higher (high-grade) cancer on prostate biopsy [11]. The model was developed utilizing data from 4765 men who underwent a 10-core biopsy without a prior negative prostate biopsy and had a PSA of >3 ng/ml, who were enrolled in the Prostate Testing for Cancer and Treatment (ProtecT) study. The model included total PSA, free PSA, intact PSA, hK2, and age.

Discrimination of the four-kallikrein panel was assessed in the MDC cohort with Harrell's concordance index (c-index). The kallikrein panel has previously been shown to be useful among men with elevated PSA and is designed as a reflex test to improve the specificity of PSA [11,12,14,15,26]. Our primary focus is the performance of the panel among men with elevated PSA levels. As the markers are subfractions of PSA occurring at about 1–10% of the level of total PSA, the precision at which these markers can be measured in men with total PSAs below median (≈1 ng/ml) is impaired compared with those with higher PSAs, and, therefore, our primary focus is the performance of the panel among men with elevated PSA levels. The discrimination of the panel of four kallikrein markers was compared with total PSA alone, and with another model using total PSA, free PSA, and patient age. This second prespecified model was generated using the same cohort as that used for the full four-kallikrein panel model [11]. Bootstrap resampling was utilized to estimate the 95% confidence intervals (CIs) for the difference in discrimination between the base models and the full four-kallikrein panel model. To assess whether the discrimination of PSA, the four-

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