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Detection of High-grade Prostate Cancer Using a Urinary Molecular Biomarker–Based Risk Score

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Abstract

Background: To reduce overdiagnosis and overtreatment, a test is urgently needed to detect clinically significant prostate cancer (PCa).
Objective: To develop a multimodal model, incorporating previously identified messenger RNA (mRNA) biomarkers and traditional risk factors that could be used to identify patients with high-grade PCa (Gleason score $\geq$7) on prostate biopsy.
Design, setting, and participants: In two prospective multicenter studies, urine was collected for mRNA profiling after digital rectal examination (DRE) and prior to prostate biopsy. The multimodal risk score was developed on a first cohort (n = 519) and subsequently validated clinically in an independent cohort (n = 386).
Outcome measurements and statistical analysis: The mRNA levels were measured using reverse transcription quantitative polymerase chain reaction. Logistic regression was used to model patient risk and combine risk factors. Models were compared using the area under the curve (AUC) of the receiver operating characteristic, and clinical utility was evaluated with a decision curve analysis (DCA).
Results and limitations: HOXC6 and DLX1 mRNA levels were shown to be good predictors for the detection of high-grade PCa. The multimodal approach reached an overall AUC of 0.90 (95% confidence interval [CI], 0.85–0.95) in the validation cohort (AUC 0.86 in the training cohort), with the mRNA signature, prostate-specific antigen (PSA) density, and previous cancer-negative prostate biopsies as the strongest, most significant components, in addition to nonsignificant model contributions of PSA, age, and family history. For another model, which included DRE as an additional risk factor, an AUC of 0.86 (95% CI, 0.80–0.92) was obtained (AUC 0.90 in the training cohort). Both models were successfully validated, with no significant change in AUC in the validation cohort, and DCA indicated a strong net benefit and the best reduction in unnecessary

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biopsies compared with other clinical decision-making tools, such as the Prostate Cancer Prevention Trial risk calculator and the PCA3 assay.
Conclusions: The risk score based on the mRNA liquid biopsy assay combined with traditional clinical risk factors identified men at risk of harboring high-grade PCa and resulted in a better patient risk stratification compared with current methods in clinical practice. Therefore, the risk score could reduce the number of unnecessary prostate biopsies.
Patient summary: This study evaluated a novel urine-based assay that could be used as a noninvasive diagnostic aid for high-grade prostate cancer (PCa). When results of this assay are combined with traditional clinical risk factors, risk stratification for high-grade PCa and biopsy decision making are improved.

1. Introduction

Prostate cancer (PCa) is the second most frequently diagnosed cancer among men worldwide, with an estimated 1.1 million new cases and 307 500 deaths in 2012 [1]. With the introduction of serum prostate-specific antigen (PSA) testing in the 1990s, the incidence of PCa has increased. PSA testing has also led to an increased number of unnecessary biopsies and the diagnosis of clinically insignificant tumors that would not have been life threatening (ie, potential overtreatment). This is particularly the case in a PSA gray zone <10.0 ng/ml, at which 65–70% of men have a negative biopsy result [2]. Men with indolent disease who undergo treatment may experience complications without reducing their risk of dying from PCa [3]. Albertsen et al showed that men with Gleason score (GS) 8–10 PCa have a relatively high probability of dying from PCa within 10 yr (12.1%), whereas this risk is minimal for men with low-grade disease [4].

The major challenge is to improve the detection of clinically significant or high-grade PCa in an early stage. Both overdiagnosis and overtreatment could be reduced if PCa-specific biomarkers could accurately distinguish indolent from aggressive tumors. Ideally the biomarkers could be measured in a sample that could be obtained noninvasively (eg, in urine). The urinary test based on the prostate cancer antigen 3 (*PCA3*) gene (Progensa PCA3; Hologic Inc, Marlborough, MA, USA) is the only molecular diagnostic test approved by the US Food and Drug Administration for the detection of PCa in urine [5,6]. *PCA3* was identified as a gene encoding a long noncoding RNA that is consistently upregulated in PCa [7,8]. PCA3 was shown to be of value in PCa detection; however, the relation with tumor aggressiveness and thus prognostic value remains controversial [9–11]. New biomarkers for PCa detection are the blood-based Prostate Health Index (PHI) and the four-kallikrein panel [12–17]. Studies describing head-to-head comparison of these markers showed that PHI outperforms PCA3 in the prediction of significant PCa [14].

Previous studies have shown the potential of noninvasive urinary biomarkers to accurately predict the presence of high-grade disease and thus aid in decision making regarding further diagnostic evaluations (eg, prostate biopsies or imaging) and treatment while avoiding unnecessary biopsies. Leyten et al described a stepwise approach for the identification and selection of new biomarkers using messenger RNA (mRNA) expression profiling [18]. A panel measured in urinary sediments predicted a GS ≥7 on prostate biopsy. The test, based on detecting increased mRNA levels of homeobox C6 (HOXC6), distal-less homeobox 1 (DLX1), and tudor domain containing 1 (TDRD1), was shown to have independent additional value to PSA for predicting high-grade PCa on biopsy. HOXC6, DLX1, and TDRD1 may be involved in the onset of PCa and are associated with high-grade PCa [18]. In this study, homeobox C4 (HOXC4) was also included because it was shown to be overexpressed in urine sediments and is transcribed from the same transcription unit as HOXC6 [18].

The aim of this study was to validate the gene panel–based mRNA test performed on whole urine and to develop a model combining molecular profiling with traditional clinical risk factors that could be used to identify patients accurately with high-grade PCa (GS ≥7) on prostate biopsy. To optimize patient management and clinical utility, all relevant information to make the most accurate assessment for each patient should be taken into account. Multimodal risk assessment approaches have been developed that combine multiple information sources into an overall optimal risk prediction for each individual patient. One such score is the Prostate Cancer Prevention Trial risk calculator (PCPTRC), which combines PSA with digital rectal examination (DRE), race, family history, age, and whether a patient had a previous biopsy [19]. Patient management and risk assessment benefit from combining different complementary information sources into one coherent risk score because no single marker can obtain a similar performance on its own [19,20]. The optimal diagnostic model was validated in urine samples from a second, independent cohort to ensure robustness of the proposed risk score.

2. Materials and methods

2.1. Study population

In two prospective multicenter studies, men who were scheduled for (initial or repeat) prostate biopsies, based on elevated PSA levels (≥3 ng/ml), abnormal DRE, or a family history of PCa, were consecutively included. Urine samples were collected after a standardized DRE consisting of three strokes per lobe [5]. Subjects were enrolled from six urology clinics in the Netherlands (Radboud University Medical Center Nijmegen, ZGT Hospital Hengelo, AMC University Medical Centre Amsterdam, CWZ Hospital

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