Danish Prostate Cancer Consortium Study 1 (DPCC-1) protocol

Multicentre prospective validation of the urine-based three-microRNA biomarker model uCaP

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ABSTRACT

Introduction The primary objective of the Danish Prostate Cancer Consortium Study 1 (DPCC-1) is to provide validation for a novel urine-based microRNA biomarker, called uCaP, for a diagnosis of prostate cancer.

Methods and analysis Eligible participants are biopsy naïve men aged ≥18 years with prostate-specific antigen (PSA) levels ≥3 ng/mL, who are referred to prostate MRI due to suspicion of PC at one of the following three major urology/uroradiology centers: Aarhus University Hospital, Herlev & Gentofte University Hospital, or Odense University Hospital, where MRI and targeted biopsy are implemented in clinical use. Exclusion criteria include previous diagnosis of urogenital cancer, contraindication to MRI, gender reassignment treatment or PSA level >20 ng/mL. The participants will be asked to donate a urine sample in connection with their MRI. The study is observational, uses a diagnostic accuracy testing setup and will integrate into the current diagnostic pathway.
We will measure the levels of the three microRNAs in the uCaP model (miR-222–3 p, miR-24–3 p and miR-30c-5p) in extracellular vesicle-enriched cell-free urine samples, to assess if uCaP can improve specificity and retain sensitivity for International Society of Urological Pathology Grade Group ≥2 PC, when used as a reflex test to PSA ≥3 ng/mL. We hypothesise that uCaP can improve selection for prostate MRI and reduce the number of unnecessary scans and biopsies.

Ethics and dissemination This study is approved by the Central Denmark Region Committee on Health Research Ethics (reference number: 1-10-72-85-22). All participants will provide written informed consent. Study results will be published in peer-reviewed journals and presented in scientific meetings.

Trial registration number NCT05767307 at clinicaltrials.gov.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ Due to recent changes in guidelines for a diagnosis of prostate cancer, which now recommend the use of MRI before biopsy, few studies have investigated biomarkers that may improve pre-selection of men to MRI.
⇒ Patients will be recruited at multiple centres in Denmark, facilitating generalisability of the results.
⇒ The study is observational and follows current clinical practice, as recommended in the guidelines used in most developed countries without population-wide screening for prostate cancer.
⇒ The investigated urine microRNA biomarker model, uCaP, was developed in the pre-MRI era and may require post analytic adjustment of cutoffs/coefficients, when transferred to the current MRI-first setting.

INTRODUCTION

Prostate cancer (PC) accounts for approximately 20% of all cancers diagnosed among males in Denmark, with 4500 new cases each year, and numbers are expected to rise in the future due to the ageing population.[1 2]

Historically, detection of PC has been based on symptoms, an elevated prostate-specific antigen (PSA) test and/or a suspect digital rectal examination (DRE) followed by a confirmation test by transrectal ultrasound guided biopsies. However, as of 2020, clinical guidelines in both Europe and USA recommend MRI prior to any biopsy in cases where PC is suspected.[3–5] This guideline shift to 'MRI first' has resulted in a significant increase in demand for MRI and targeted/fusion biopsy at radiology and urology centres. The usage of MRI has been demonstrated to reduce overdiagnosis of clinically insignificant PC, typically defined as International Society of Urological Pathology (ISUP) Grade Group 1 (GG1). However, only approximately 35% of positive MRI cases will have clinically significant PC, often defined as

ISUP Grade Group ≥2 (GG ≥2).[6–8] Hence, development of a more effective pre-selection tool for prostate MRI is needed to reduce the number of men being overdiagnosed with PC and/or subjected to overtreatment by surgery as well as to reduce demands for already scarce healthcare resources such as MRIs, biopsies and medical consultations. These problems are expected to increase in the near future due to the ageing population. As a result, there is an immediate requirement for a biomarker test that is more specific than the current standard test, PSA, for identifying men who have clinically significant PC and consequently are candidates for MRI and targeted biopsy. A new biomarker in combination with PSA, but with higher specificity than PSA alone while maintaining the sensitivity, would help free up valuable healthcare resources by better pre-selecting those men who truly need a prostate MRI.

Our research group has recently identified and validated a urine-based biomarker model for PC, named uCaP, which is composed of three microRNAs (calculated as a ratio model: miR-222–3 p*miR-24–3 p/2*miR-30c-5p) that are present in extracellular vesicles (EV) in cell-free urine samples.[9] While several urine-based microRNA biomarker candidates have been suggested for detection of PC based on earlier biomarker discovery studies, the vast majority of prior studies are limited to low patient sample numbers and/or lack large-scale independent clinical validation.[10–12] In contrast, our retrospective studies that used urine samples from a total of 1280 PC patients and healthy controls in multiple international cohorts found that uCaP had a significantly higher specificity than PSA, at the same sensitivity, for distinguishing between men with and without PC.[9 13 14] We will now test uCaP in a large-scale prospective clinical study that involves 2500 patients and three major urology/uroradiology centres in Denmark. The study is a diagnostic accuracy study with uCaP as a reflex test to elevated PSA (≥3 ng/mL), where we expect that uCaP is a more specific pre-selection test for MRI indication (ie, able to reduce MRI usage), while maintaining the same sensitivity for clinically significant PC (defined as GG ≥2) as PSA ≥3 ng/mL.

METHODS AND ANALYSIS

Study objectives

The primary objective of the study is to test if uCaP is more specific than the current 'gold standard' test (PSA) at identifying men who have clinically significant PC, as detectable by an MRI of the prostate. To this end, we will collect urine samples (~10 mL) from 2500 men who receive an MRI due to suspicion of PC at one of the three participating centres in Denmark. We will measure uCaP in all urine samples and collect blood samples (~60 mL) from all participants for bio-banking and to be used in future biomarker development studies. We have used the Standard Protocol Items: Recommendations for Interventional Trials reporting guidelines[15] in writing this protocol.

Patient and public involvement

Patients and the public were not involved in the design, conduct, reporting or dissemination plans of our research.

Setting

In Denmark, general practitioners (GPs) act as gatekeepers for the rest of the healthcare system and are usually the first point of contact for most patients.[16] Here, men who visit their GP with health concerns or urological symptoms that may indicate PC can undergo PSA testing and a DRE as part of the diagnostic process. If either PSA or DRE findings are concerning, the man will be referred to a urology/radiology department at a hospital for further examination.[17] At the hospital, the man is offered an MRI scan (bi- or multiparametric) and any lesions with PI-RADS score ≥4 (or PI-RADS ≥3 if PSA-density ≥0.15 ng/mL^2) undergo targeted biopsy with two to four needle cores from each lesion. Patients with PI-RADS <3 but PSA-density ≥0.15 ng/mL^2 may undergo systematic biopsies at the urologists' discretion. Biopsy tissues will be handled in accordance with clinical routine at a department of pathology and classified by an expert pathologist according to ISUP 2014 Grade Group.[18] We define clinically significant PC as ISUP GG2 or higher and clinically insignificant PC as ISUP GG1. Any further investigations and/or treatments will be handled according to the clinical guidelines.[17]

This study will integrate into, but not alter, this existing diagnostic workflow.

Study population

The participants will be men referred to prostate MRI at one of the three participating urology/uroradiology centres: AUH, HGH or OUH.

Inclusion criteria:

- Age ≥18 years.
- Understand oral and written Danish.
- Blood PSA level ≥3 ng/mL.

Exclusion criteria:

- Previously diagnosed with urogenital cancer.
- Previous prostate biopsy.
- Contraindication to MRI.
- Gender reassignment treatment.
- Blood PSA level >20 ng/mL.

Recruitment and sample collection

The study is a collaboration between the Department of Urology, Department of Molecular Medicine (MOMA) and Department of Radiology at AUH, as well as the Department of Urology at OUH and the Department of Urology and Department of Radiology at HGH. This is the first study within the Danish Prostate Cancer Consortium (DPCC). Together, the corresponding uptake regions for these three hospitals account for approximately 60% of all newly diagnosed cases of PC each year in Denmark.[19]

Men referred to an MRI due to suspicion of PC will be scheduled an appointment at a department of urology, either before the MRI, at the day of the MRI, or when

they receive the result of the MRI. At this consultation, project personnel will inform the participant in a private conversation (along with any attendant of the participant) about the project, hand out project materials and collect a signed consent. The participant will also be informed that consent can be withdrawn at any time and provided with instructions on how to do so. If the participant requires more time to decide on the study invitation, he can request a new appointment scheduled at least 24 hours later. The participant will also be encouraged to seek more information on the DPCC website www.dpcc.dk.

The study opened for recruitment on 1 August 2022 and is expected to be concluded by 31 December 2025. From non-fasting participants, we will collect approximately 10 mL urine and 60 mL blood. If possible, samples will be collected prior to MRI. If this is not possible, samples will instead be collected within 14 days after the MRI but always before any biopsy. Urine samples will be centrifuged and split into supernatant and pellet. Blood samples will be centrifuged and split into plasma/serum as well as buffy coat. All samples will be processed within 3 hours after collection and stored at the recruitment centre at −80°C and shipped periodically on dry ice to MOMA, AUH, for long-term storage at −80°C.

uCaP measurement

From~3.5 mL frozen urine supernatant samples, we will first enrich for EVs using the miRCURY Exosome Cell/Urine/CSF Kit (QIAGEN). Next, RNA will be extracted from EVs using the miRNeasy Kit and reverse transcribed using miRCURY LNA RT Kit (QIAGEN). This kit includes the spike-in UniSp6 (resembles a miRNA in structure but lacks close sequence similarities to known miRNAs), which is added to the template RNA as a positive control for reverse transcription, as previously described.[9 13 14] Expression levels of the three microRNAs included in the uCaP model (miR-222–3 p, miR-24–3 p and miR-30c-5p) will be determined by reverse transcriptase quantitative polymerase chain reaction using miRCURY SYBR Green kit (QIAGEN) and miRCURY LNA miRNA Detection Probes (QIAGEN) according to the manufacturer's protocol and as previously described.[9 13 14] All uCaP analyses will be performed at MOMA, AUH.

Patient flow

Out of the 2500 men who will have an MRI performed during the study period (figure 1), we expect 70% (n=1750) to show no indication for targeted biopsy (PI-RADS score ≤3, or <3 if PSA-density ≥0.15 ng/mL^2). The remaining 30% (n=750) of men are expected to show suspicious MRI lesion(s) (PI-RADS score ≥4, or PI-RADS ≥3 if PSA-density ≥0.15 ng/mL^2) and will be offered targeted biopsy of the lesion(s). Of the patients having a biopsy, we expect that 25% (n=188) will have benign biopsies only (no cancer), whereas 75% (n=562) will get a PC diagnosis. Of these, we expect 50% (n=281) to be GG ≥2 PC, while the other 50% (n=281) will be GG1 PC.

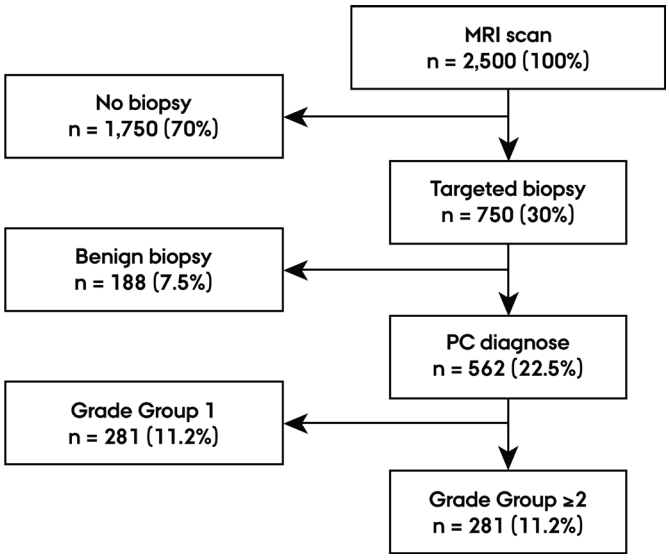

Figure 1 Patient flow from recruitment of 2500 men in the current MRI-first diagnostic pathway.

MRI scan

All MRIs will be performed at the radiologists' discretion, as either multiparametric MRI (mpMRI) or bi-parametric MRI (bpMRI) according to PI-RADS v2.1 guidelines[20] if possible, but will allow for patient-specific deviations. In brief, bi-parametric MRI (bpMRI) will consist of high-resolution T2W sequences of the prostate in two to three planes and an axial diffusion-weighted imaging (DWI) sequence with subsequent quantification of diffusion restriction by apparent diffusion coefficient mapping. A dynamic contrast enhancement sequence will be added to the T2W and DWI sequences in case of mpMRI.

All scans will be evaluated by an experienced radiologist in MRI of the prostate, as part of routine clinical work-up. Each identified lesion will be assigned a score from 1 to 5 in accordance with the PIRADS classification.[20]

Endpoints and statistical power

All patient data required, such as MRI results, PC diagnoses and treatment selection, will be collected from electronic patient files and stored in a REDCap database[21] hosted by Aarhus University. As the primary endpoint, we will compare the total number of MRIs required to diagnose the same number of GG ≥2 PC in this study population for the combination of PSA ≥3 ng/mL + uCaP vs PSA ≥3 ng/mL alone. In this diagnostic accuracy study setup, with uCaP as a reflex test to PSA ≥3 ng/mL, we expect that uCaP will result in higher specificity as a pre-selection test for MRI indication than PSA ≥3 ng/mL alone, while maintaining the same sensitivity for GG ≥2 PC. We expect that uCaP will be able to reduce MRI expenditure by at least 20%, while detecting the same number of GG ≥2 PC as the current diagnostic strategy based on elevated PSA (≥ 3 ng/mL). Similarly, we expect that PSA ≥3 ng/mL + uCaP will be able to reduce the number of biopsies by at least 20% compared with PSA ≥3 ng/mL. Hence, future inclusion of uCaP in the novel 'MRI-first' diagnostic

pathway could help to solve a bottleneck caused by limited MRI capacity and trained personnel by saving unnecessary MRIs and targeted biopsies.

As secondary endpoints, we will compare the total number of biopsies, the number of men diagnosed with GG1 PC and the number of benign biopsies (no cancer) for PSA ≥3 ng/mL + uCaP vs PSA ≥3 ng/mL alone. We expect that the addition of uCaP can reduce the total number of biopsies, the number of biopsies with GG1 PC and the number of benign biopsies.

The study sample size (n=2500), with 281 men expected to have GG ≥2 PC, gives us >90% power (to a significance level of 0.005) to validate uCaP with an effect size of >0.3 (small effect size[22]), corresponding to an approximate reduction of 20% in the number of MRIs required to find the same number of GG ≥2 PCs.

ETHICS AND DISSEMINATION

This study conforms to the principles of the Helsinki Declaration[23] and the protocol (version 1.0) is approved (7 June 2022) by The Central Denmark Region Committee on Health Research Ethics (reference number: 1-10-72-85-22). All participants will receive written and oral information about the study and give written consent. The study has been registered at clinicaltrials.gov (reference number: NCT05767307). The participants are free to withdraw their consent at any time and to have their data and samples destroyed.

A Data Monitoring Committee (DMC) will not be established as the study is purely observational with no interventions and cannot be discontinued due to the collected data.

After publication of study results in peer-reviewed journals, uCaP scores as well as clinical data will be uploaded to the European Genome-phenome Archive.

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Contributors JF, KDS, BGP and MB designed the study. LPB, VL, MHP, BGP and MB will conduct patient data collection. All authors will have access to the dataset, but JF and ENG will conduct uCaP measurements and perform statistical analysis. JF and KDS wrote the manuscript. All authors read, provided edits and approved the final version of the manuscript.

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Competing interests JF and KDS are co-inventors on an issued patent ("A microRNA-based method for early detection of prostate cancer in urine samples" #US10400288B2, #EP3256602B1, #ES2749651T3) licensed to Qiagen. KDS is co-inventor on an issued patent ("Biomarkers for prostate cancer" #US10106854B2, #AU2013275761B2, #JP6242388B2) licensed to Qiagen and on an issued patent ("A microRNA-based method for assessing the prognosis of a prostate cancer patient" #US10358681B2, #EP3262186B1, #ES2724404T3, #JP6769979B2), licensed to Qiagen.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

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