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Original Article

Seminal plasma enables selection and monitoring of active surveillance candidates using nuclear magnetic resonance-based metabolomics: A preliminary investigation

Matthew J. Roberts [a, b, c, d], Renee S. Richards [b], Clement W.K. Chow [b], Marion Buck [e], John Yaxley [d], Martin F. Lavin [b], Horst Joachim Schirra [c, *], Robert A. Gardiner [b, d, f, *]

[a] School of Medicine, The University of Queensland, Brisbane, Australia
[b] Centre for Clinical Research, The University of Queensland, Brisbane, Australia
[c] Centre for Advanced Imaging, The University of Queensland, Brisbane, Australia
[d] Department of Urology, Royal Brisbane and Women's Hospital, Brisbane, Australia
[e] Department of Environmental Health Sciences, University Medical Centre Freiburg, Freiburg, Germany
[f] Exercise Medicine Research Institute, Edith Cowan University, Joondalup, Australia

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ABSTRACT

Background: Diagnosis and monitoring of localized prostate cancer requires discovery and validation of noninvasive biomarkers. Nuclear magnetic resonance (NMR)-based metabolomics of seminal plasma reportedly improves diagnostic accuracy, but requires validation in a high-risk clinical cohort.
Materials and methods: Seminal plasma samples of 151 men being investigated for prostate cancer were analyzed with ^{1}H-NMR spectroscopy. After adjustment for buffer (add-to-subtract) and endogenous enzyme influence on metabolites, metabolite profiling was performed with multivariate statistical analysis (principal components analysis, partial least squares) and targeted quantitation.
Results: Seminal plasma metabolites best predicted low- and intermediate-risk prostate cancer with differences observed between these groups and benign samples. Lipids/lipoproteins dominated spectra of high grade samples with less metabolite contributions. Overall prostate cancer prediction using previously described metabolites was not validated.
Conclusion: Metabolomics of seminal plasma *in vitro* may assist urologists with diagnosis and monitoring of either low or intermediate grade prostate cancer. Less clinical benefit may be observed for high-risk patients. Further investigation in active surveillance cohorts, and/or in combination with *in vivo* magnetic resonance spectroscopic imaging may further optimize localized prostate cancer outcomes.

1. Introduction

Accurate prostate cancer (CaP) diagnosis to prolong life with minimal morbidity is a daily challenge for urologists. Although early treatment of localized clinically significant CaP (csCaP) with curative intent reduces mortality and metastases,[1] harms associated with overdetection and treatment of indolent CaP driven by injudicious use of serum prostate specific antigen (PSA) and prostate biopsy have reduced overall CaP detection.[2] Limitations of serum PSA have driven advancements in multiparametric magnetic resonance imaging and biomarkers in serum (e.g., Prostate Health Index) and urine [prostate cancer antigen 3 (*PCA3*), *TMPRSS2:ERG* fusion gene].[3–6] However, due to cost-effectiveness concerns, these are used as adjunctive tests rather than as standalone detection tests despite their improved diagnostic accuracy.[5,6]

Prostatic fluid, produced as seminal plasma (SP) after physiological prostatic smooth muscle contraction, contains the clinical biomarkers PSA and prostatic acid phosphatase (PAP).[7,8] Malignant prostatic cells in ejaculates of men with CaP have been shown to express genes (*PCA3*, Hepsin) and microRNAs that improve detection compared with serum PSA.[9–11] Metabolomics is a modern biomarker approach that quantifies small metabolites, most commonly using nuclear magnetic resonance (NMR) spectroscopy

* Corresponding authors. University of Queensland Centre for Clinical Research (UQCCR), Level 6, Building 71/918 Royal Brisbane Hospital, Herston, QLD 4006, Australia (R.A. Gardiner); The University of Queensland, Centre for Advanced Imaging, Building 57, Research Road, Brisbane, QLD 4072, Australia (H.J. Schirra).
E-mail addresses: h.schirra@uq.edu.au (HJ Schirra), f.gardiner@uq.edu.au (RA Gardiner).

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or mass spectrometry.[12,13] NMR-based metabolomics is highly sensitive and reproducible with affordable sample-to-sample costs.[12] SP metabolite profiles improve PSA-based diagnosis,[14,15] but require clinical validation.

This study investigates the feasibility of SP analysis using NMR-based metabolomics for the prediction of csCaP in a high-risk clinical cohort and compares metabolite profile CaP diagnosis against prostate biopsy and radical prostatectomy (RP) histology.

2. Materials and methods

Ethical approval was obtained from the University of Queensland Medical Research Ethics Committee (Project no. 2006000262) and the Royal Brisbane and Women's Hospital Human Research Ethics Committee (HREC/09/QRBW/320, HREC/09/QRBW/305 and 1995/088B).

2.1. Patients and clinical data

Male patients ($n = 154$) attending either the Royal Brisbane and Women's Hospital Urology outpatient department or local private consulting rooms for investigation of elevated PSA and/or abnormal digital rectal examination between January 2007 and February 2013 were enrolled in this prospective cohort study. Following informed consent, patients provided ejaculate specimens on site or at home prior to or at least 1 month after prostate biopsy, prior to commencement of any treatment. No specifications to time of day, relation to voiding, urethral meatus sterilization, or other parameters were provided to patients to simplify the sample collection process. Patients denied surgical treatment for benign prostatic hyperplasia and subsequent retrograde ejaculation prohibiting sample collection.

Patient data collected included age, serum PSA and detailed prostate biopsy, and radical prostatectomy histology records. Biopsy and RP specimens were reported by uropathologists according to the 2005 International Society of Urological Pathology classification.[16] Patients were monitored for biopsy progression, such as CaP detection following initial false negative biopsy or upgraded Gleason score with further biopsy or RP ($n = 60$).

Risk stratification (low, intermediate, high risk) was performed according to the D'Amico criteria recommended in the American Urological Association Guidelines[17] and used to determine csCaP presence (intermediate, high risk requiring treatment; Table 1). Given established disparity between biopsy and RP histopathology, risk classification accuracy was optimized using whichever histopathology best described tumor characteristics.

2.2. Specimen processing

Ejaculate specimens were deposited directly into sterile microurine jars containing 20 mL Hanks Balanced Salt Solution (HBSS; Gibco, Life Technologies Australia, Mulgrave, Australia) for the first 117 patients used initially for cytology and RNA analyses, which was thereafter replaced by phosphate buffered saline (PBS) (in-house preparation) because glucose in HBSS interfered with preliminary metabolomics analysis. All specimens were provided to the laboratory without cooling as soon as logistically possible by the patients and were processed in the laboratory within 2 hours of production. Specimens were combined with 20 mL HBSS or PBS, layered over 10 mL isotonic Percoll (GE Healthcare-Pharmacia, Rydalmere, Australia) and centrifuged at 974*g* for 30–60 minutes at 4°C. Isolated supernatants, referred to as SP, were snap-frozen on dry ice in 1-mL aliquots and stored at −80°C.

2.3. Sample preparation

SP samples were thawed on wet ice and distributed in 100-μL aliquots. Eighty μL of PBS solution were added along with 20 μL D_2O as lock substance that contained 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS) as internal chemical shift standard and 1,1-difluoro-1-trimethylsilanyl methylphosphonic acid as internal pH indicator leading to final concentrations of 100μM, resulting in 200 μL total sample volume. Samples were transferred to sterile 3-mm NMR tubes (Bruker Biospin, Rheinstetten, Germany).

2.4. NMR spectroscopy

NMR spectra of SP samples were measured on a Bruker Avance 900 spectrometer operating at a ^{1}H frequency of 900.13 MHz (Bruker Biospin), equipped with a 5-mm self-shielded z-gradient triple resonance cryoprobe and SampleJet sample changer. One-dimensional (1D) nuclear Overhauser effect spectroscopy (NOESY) spectra were acquired at 298 K with the "*noesypr1d*" pulse sequence, accumulating 200 transients (following 8 dummy scans) at 32,738 data points with a spectral width of 14 ppm.[18] The transmitter frequency was set to the water resonance, which was

Table 1
Demographic information for patients based on biopsy and radical prostatectomy (RP) histology.

		Age (y)	Serum PSA (ng/mL)	Pathological stage, *n*		
				pT2	pT3a	pT3b
Biopsy	***n* = 151**					
Overall		61 (55–66)	6.5 (4.3–9.2)			
CaP status	Positive ($n = 98$)	60.5 (55–65)	6.4 (4.5–11)			
	Negative ($n = 53$)	62 (55.75–68.25)^{NS}	6.5 (3.6–7.9)^{NS}			
csCaP status	Present ($n = 82$)	61 (55–66)	6.75 (4.5–11.9)			
	Absent ($n = 69$)	61 (55–67)^{NS}	6.0 (3.6–8.1)*			
RP	***n* = 60**					
Overall		57 (54–64)	6.15 (4.1–9.1)			
ISUP group	1 ($n = 2$)	56 (54–57)	6.5 (4–9)	2		
	2 ($n = 30$)	57.5 (53–64)	5.5 (4–7)	26	3	1
	3 ($n = 20$)	57 (55–61)	7.3 (5–12)	15	5	
	4 ($n = 1$)	55	19	1		
	5 ($n = 7$)	60 (55–68)	10 (6–12)	3	1	3
Primary/ tertiary pattern	≥ 4 ($n = 33$)	57 (55–64)	7.3 (5–12)	23	7	3
	3 ($n = 27$)	57 (51–63)^{NS}	5.4 (4–7)**	25	2	1

Median and interquartile range are shown for age and serum PSA. All comparisons were made using the Mann–Whitney *U* test (two-tailed).
* $P < 0.05$, ** $P < 0.01$.
CaP, prostate cancer; csCaP, clinically significant prostate cancer; ISUP, International Society of Urological Pathology; NS, not significant; PSA, prostate specific antigen.

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