Clinical trials registration number: NCT01864135

Status: Patient enrolment completed

1 SUMMARY

Prostate cancer has been the most common neoplastic disease in men in Finland over the last ten years. Prostate-specific antigen (PSA) plays an important role in screening of prostate cancer. However, PSA has a limited sensitivity and specificity for prostate cancer detection (1). Commonly, the diagnosis of prostate cancer is done by transrectal ultrasonography (TRUS) guided biopsy. Because of the low accuracy of TRUS a systematic biopsy is usually performed instead of targeted TRUS biopsy (2) (3). As biopsy carries a risk of increase in complications, there is an increasing interest in developing more accurate non-invasive imaging modalities.

This study will enroll 150 men with clinical suspicion of prostate cancer due to higher serum level of PSA than 2.5 ng/ml or abnormal digital rectal examination. Anatomical magnetic resonance imaging (MRI) and diffusion weighted imaging (DWI) at 3 Tesla (T) magnetic field using surface coils will be used to non-invasively predict the presence or absence of prostate cancer. Targeted TRUS guided biopsy based on MRI findings will be performed in addition to routine twelve core TRUS biopsy. Moreover, selected serum and urine biomarkers as well as biomarkers extracted from fresh biopsy sample will be collected and correlated with the presence or absence of prostate cancer.

2 INTRODUCTION

Prostate cancer continues to be the most common cancer in elderly men and the second leading cause of cancer death in men (4). The incidence of prostate cancer in Finland has increased dramatically over the last few years. In 2010 the number of new PCa cases diagnosed in Finland was 4709 (www.cancerregistry.fi). Prostate cancer incidence continues to increase worldwide, both as a result of population aging and because of better diagnostic methods. As the result of common PSA screening, most prostate cancers are currently being diagnosed at an early stage. At detection most of prostate cancers are still localized within the gland with an incidence of local lymph node metastasis <10% (5).

Despite the commonness of the disease, there is currently no routine method of choice for treatment within the individual patient. Several clinical nomograms exist which are based mainly on the differentiation between the indolent and aggressive disease, with the Gleason score and serum level of PSA as the major indicator of tumor aggressiveness (6). Knowledge of tumor volume and extent is an important determinant in choice of treatment. Although clinical nomograms partly facilitate the choice of management, there is a great demand for further individualization in order to limit the current practice of over-treatment of men with an intrinsic good prognosis (7).

Traditionally the diagnosis of prostate cancer is mostly based on the result of random transrectal ultrasonography (TRUS) guided biopsies. Systematic sextant biopsy has a higher cancer detection rate compared to the targeted biopsy performed on the basis of TRUS findings (8). Transrectal biopsy carries a risk of hemorrhagic and infectious complications (9). The accuracy for prostate cancer detection is limited because in more than 85% of cases the cancer is multifocal and intermingled with normal tissue and about 30% of tumors are localized in central and transitional zone (10, 11). Therefore, more accurate noninvasive imaging modalities are needed to improved diagnosis and avoid unnecessary biopsies.

2.1 Magnetic resonance imaging

Anatomical magnetic resonance imaging (MRI) at 1.5T compared with transrectal US has demonstrated a higher sensitivity for tumor detection but almost the same specificity (12, 13), stressing the need for additional metabolic MR imaging. Several different types of sequences have been proposed as an addition to the anatomical MR imaging. Diffusion weighted imaging (DWI) has been shown to be particularly valuable in prostate cancer detection and characterisation (14, 15). The apparent diffusion coefficient (ADC), which is calculated from DWI data assuming a mono-exponential signal decay with increasing b-values, improves the accuracy of prostate cancer detection (15) and also correlates with the Gleason score (16-18). Most studies determining the accuracy of anatomical MRI and DWI at 1.5T or 3T magnetic field involved patients treated with radical prostatectomy. However, this group of patients does not represent the most common type of patients with generally non-aggressive disease (low Gleason score and PSA). Thus, at current no reliable prospective validation has been performed for anatomical MRI and DWI at 3T in patients with clinical suspicion of prostate cancer. As a result the clinical role of anatomical MRI and DWI at 3T in patients with clinical suspicion of prostate cancer is currently unclear.

2.2 Serum, urine and tissue biomarkers

Total serum PSA level is commonly elevated due to benign conditions such as prostatitis or benign prostatic hyperplasia. Due to low specificity of serum PSA for prostate cancer, it has been proposed that combination of different biomarkers instead of total PSA or free-to-total PSA ratio could potentially aid in the estimation of prostate cancer risk. A panel of immunoassays has been developed at the Division of Biotechnology, Department of Biotechnology and Food Chemistry, University of Turku, Finland. This so-called kallikrein panel includes serum total, free and intact PSA and kallikrein-related peptidase 2 (hK2). It has been shown (in collaboration with Memorial Sloan Kettering Cancer Center, New York, USA) that by using the panel the number of biopsies could be reduced by approximately 50 % without missing a significant number of aggressive prostate cancers (19, 20).

In addition to serum markers, potential urine markers are of utmost interest due to the easy accessibility of urine samples. At the Division of Biotechnology, University of Turku a promising assay method has been developed for studying PSA glycosylation patterns in healthy men and prostate cancer patients. It has been shown that the glycosylation pattern of proteins is changed in cancer cells (21). Based on preliminary experiments, these changes in PSA glycosylation might be measurable from the urine samples of prostate cancer patients.

Changes related to neoplastic characteristics of the prostate tissue have been detected in the expression of suggested prostate cancer marker genes in studies of the Division of Biotechnology (manuscript in preparation), University of Turku. These changes are seen in mRNA expression on a molecular level and can be quantified by reverse-transcription (RT) - PCR assays developed at the same project (22). The preliminary cohort of prostate tissues presented differences in for example *TMPRSS2-ERG* gene fusion transcript expression both between histologically defined cancer and benign tissue of a prostate cancer patient, and between histologically benign tissue of a prostate cancer patient and an individual without clinical evidence of prostate cancer. The latter case is of particular interest for further studies, as it would indicate a possibility to apply these RT-PCR assays for detecting a potentially increased risk of presence of cancer from histologically negative biopsies.

3 OBJECTIVES AND PURPOSE

Specific aims of the current study are as follows:

i) To determine the sensitivity, specificity and accuracy of anatomical MRI and DWI at 3T magnetic field alone and their combinations for detection of prostate cancer in correlation with systematic TRUS guided biopsy

ii) To determine the sensitivity, specificity and accuracy of selected serum, urine and tissue biomarkers for detection of prostate cancer

iii) To develop statistical model for diagnosis of prostate cancer incorporating findings of MRI and selected biomarkers

iv) To asses the applicability of TRUS guided prostate biopsy based on MRI finding in patient with no previous prostate biopsy

v) To develop and validate an imaging protocol which will become the standard protocol for prostate imaging at Medical Imaging Centre of Southwest Finland (VSKK) / TYKS, Turku, Finland.

4 STUDY DESIGN

This is a non-randomized prospective study to determine the applicability of 3T MRI, including DWI and selected biomarkers for the diagnosis of prostate cancer in patients with clinical suspicion of prostate cancer. We hypothesized that the increased signal-to-noise ratio at 3T magnetic field compared to 1.5T magnetic field shows more accurately heterogeneous distribution of primary prostate. In addition to routine twelve core biopsy, patients with suspicious cancer lesions at MRI will have target biopsy potentially leading to more accurate diagnosis. Additional tissue samples will be taken for mRNA analysis. The proposed study would allow the collection of preliminary data for the comparison and development of a prediction model for prostate cancer risk based on the biomarkers and imaging findings. In addition, information on the PSA glycosylation patterns in different group of patients will be correlated with other biomarkers and MRI findings.

If the hypothesis is proven, the use of 3T MRI, including DWI, and specific biomarkers would allow selecting patients who need prostate biopsy. This would result into substantially decreased number of biopsies and biopsy-related complications.

5 PATIENT SELECTION

5.1 Source population

All patients with clinical suspicion of prostate cancer living in referral areas to Turku University Hospital living either in the Hospital Districts of Southwest Finland, Satakunta, or Åland islands are potentially eligible. The total population in these three hospital districts is approximately 724000.

5.2 Number of patients

This study will include 150 patients with clinical suspicion of prostate cancer. All imaging datasets will be analyzed after each imaging session and before TRUS biopsy in order to benefit from MR findings concerning possible cancer localization.

5.3 Inclusion criteria

- Age: 40 to 85 years
- Language spoken: Finnish
- Clinical suspicion of prostate cancer, based on: serum level of PSA from 2,5 ng/ml to 25 ng/ml in two following measurements and/or abnormal digital rectal examination
- Mental status: Patients must be able to understand the meaning of the study
- Informed consent: The patient must sign the appropriate Ethics Committee (EC) approved informed consent documents in the presence of the designated staff

5.4 Exclusion criteria

- previous prostate biopsies
- previous diagnosis of prostate carcinoma
- previous prostate surgeries, e.g. TURP (transurethral prostatic resection)
- symptomatic of acute prostatitis
- contraindications for MRI (cardiac pacemaker, intracranial clips etc)

- uncontrolled serious infection
- claustrophobia
- any other conditions that might compromise patients safety, based on the clinical judgment of the responsible urologist

6 SCREENING MODALITIES

6.1 Pre-study evaluation

After patient referral to Turku University hospital, all patients will be seen by an urologist who perform DRE and after receiving the signed informed consent the patient is referred to blood and urine tests (TYKSLAB) and MRI examination.

Blood tests will include serum PSA, free-to-total PSA ratio and standard blood counts. In addition, selected biomarkers will be analyzed from the anticoagulated EDTA plasma (10 ml) and urine (min. 10 ml). Patient scheduled for the MRI examination (as described below) will receive natriumpikosulphate drops (Laxoberon, Boehringer Ingelheim GmbH) for bowel preparation**.**

6.2 MRI

MR imaging of the prostate will be performed using a 3T Siemens system (Magnetom Verio 3T [76x18] Q-engine, Erlangen, Germany). The body matrix coil in combination with a spinal coil will be used for image data acquisition. Glucagon (0.2 mg - 0.5 mg, GlucaGen, Novo Nordisk A/S) will be injected subcutaneously into lower abdomen immediately prior to the beginning of the MR imaging examination to reduce peristalsis as a part of the normal clinical routine. T2-weighted anatomic imaging will be performed in axial and sagittal plane. Single-shot spin-echo echo-planar imaging will be used for DWI. The total scan time will be approximately twenty minutes.

6.3 Serum, urine and tissue biomarkers

Measurements for the kallikrein panel from the EDTA plasma samples (volume of 10 ml) and the statistical evaluation will be done as previously published (19, 20). In addition, other potential biomarkers could be measured from the samples.

The measurement of PSA glycosylation patterns in urine (minimum volume of 10 ml) will be done with unpublished research assays developed at the Division of Biotechnology, University of Turku. The assays utilize lectins, which bind to the specific carbohydrate structures on the PSA molecule.

Quantitative reverse-transcription PCR methods are used to study the mRNA expression of novel prostate cancer marker candidate genes (including but not limited to: PCA3, TMPRSS2-ERG, SPINK1, AR, AMACR) in both benign and cancerous prostate tissue.

Specific assays are available for several different target genes. The assay method is based on a closed-tube concept, using time-resolved fluorescence in the detection of amplification products (23, 24) and internal RNA standards as validation of the absolute mRNA levels (25). Fresh prostate tissue specimens are stored in guanidine isothiocyanate buffer at -80 C for stabilization of RNA. RNA extraction is performed by a commercial kit and followed by cDNA synthesis and real-time PCR analysis (22).

6.4 Transrectal ultrasonography (TRUS)

Transrectal ultrasonography will be performed using Bk Medical Pro Focus Ultraview 2202 system. The time period between the MRI examination and TRUS guided biopsy will be a maximum of 4 weeks. For the study logistics of patients with suspected prostate cancer, please see Appendix 1. After MRI examination the urologist will perform systematic 12-core biopsy and also obtain samples from suspicious areas based on MRI findings. The maximum of two cores will be obtained from MRI suspicious lesion. In addition, two tissues core will be obtained for mRNA analysis. Additional study biopsy cores will be taken only in case the responsible urologist estimates that no additional risk is associated with the procedure.

7 ADVERSE EVENTS

Since anatomical MRI and DWI are not based on ionizing radiation, the risk for adverse events in properly selected patients is considered minimal if any. Claustrofobic patients will be excluded from the study. Commonly no side-effects are associated with administration of

Glucagon (0.2 mg - 0.5 mg, GlucaGen, Novo Nordisk A/S) but it is recommended for patients to eat (sugar containing food) after MRI examination to prevent mild nausea. Commonly no side-effects or only mild side-effects are associated with taking of natriumpikosulphate drops (Laxoberon, Boehringer Ingelheim GmbH) for bowel preparation but it is recommended for patients to maintain their water balance with increased water intake. No MRI contrast agents will be given to the patients.

The TRUS guided biopsy procedure is done by following routine clinical standards, i.e. prophylactic antibiotic treatment (levofloxaxin), standard ultrasound device and 12-core routine biopsy. In addition to 12-core routine TRUS guided biopsy, two tissue cores for mRNA analysis and in presence of MRI suspicious lesion up to 2 addition cores will be taken. The addition of up to 4 biopsy cores to the 12-core routine TRUS biopsy does not significantly increase the risk of complications associated with TRUS guided prostate biopsy. Extended biopsy techniques including more than 12-cores have already been used in the detection of primary prostate cancer (26). The use of 14-core TRUS biopsy resulted into higher detection of primary prostate cancer rate compared with 8-core biopsy technique (27). In addition, it was shown that 21-core TRUS biopsy procedure does not increase morbidity compared to sextant (6-core) biopsy approach (28).

8 ETHICS

8.1 Ethical considerations

The study will be conducted in compliance with the current revision of Declaration of Helsinki guiding physicians and medical research involving human subjects (59nd World Medical Association General Assembly, Soul, Korea, 2008).

8.2 Ethical Review

Prior to commencement of this investigation, the study protocol, patient information sheet and informed consent form will be submitted for approval to ethics committee of the Hospital District of Southwest Finland. The Principal Investigator (PI) is responsible for obtaining approval of the ethics committee for the study protocol including its appendices. The PI shall file all correspondence with the ethics committee in the Investigator`s Study File.

8.3 Potential risks and benefits to study subjects

The risks for the patients inflicted by participation in study are deemed minimal. Anatomical MRI and DWI are considered as safe techniques. Participating patients potentially benefit of a more exact diagnosis and may be prevented from unnecessary repeated biopsies. There is no evidence that the extra 4 biopsy cores taken would increase the risk of complications. Clearly benefits of participation outweigh risks for patients eligible for study**.**

9 DATA ANALYSIS

9.1 Qualitative analysis of MRI data

A modified version of a dedicated structured reporting system developed in a previous study will be utilized (29). The prostate gland will be divided according to zonal anatomy into 6 regions of interests (ROIs) (covering the whole organ) in the same fashion as biopsy samples are taken. The base is defined as the upper third, which extended from the vesical margin of the prostate; the mid-region is defined as the central third; the apex is defined as the inferior remaining third. Each third will be further divide into right and left side. Prostate cancer in the peripheral zone appears as round or ill-defined, low-signal-intensity foci on T2-weighted images while central gland tumors appear as homogeneous low signal intensity lesions with irregular margins and without a capsule. Invasion of the pseudocapsule with lenticular extension into the urethra or anterior fibromuscular zone is commonly seen on T2-weighted images of central gland tumors (30). The central zone prostate cancers tend to have higher Gleason scores compared with cancers located in peripheral zone (31). Moreover, the central zone prostate cancers were shown to have higher pathological stage (higher rate of extracapsular extension and seminal vesicle invasion) as well higher Gleason score (31).

9.2 Quantitative analysis of DWI

The signal intensity of DWI will be fitting using monoexponetial fit.

Monoexponential calculation of apparent diffusion coefficient (ADC) is described by the following equation (eq.1):

$$ADC = -\ln\left(\frac{SI(b_2)}{SI(b_1)}\right) \quad \text{(eq. 1)}$$

where $SI(b_2)$ and $SI(b_1)$ denotes the signal intensity at higher (b_2) and at b = 0 mm^2/s (b_1).

10 SAMPLE SIZE

This prospective feasibility study which assesses the utility of 3T MRI and selected biomarkers for the diagnosis of prostate cancer in patients with clinical suspicion of prostate cancer will enroll 150 patients. Imaging data analysis will be performed after every patient with emphasis on reporting the suspected location of possible tumor within the prostate gland. The study may be interrupted at the discretion of principal investigator after consulting other chief investigators.

11 QUALITY ASSURANCE

11.1 Information of study personnel and training

The technical and other supporting personnel of Medical Imaging Centre of Southwest Finland, Department of Urology and Department of Radiology, University of Turku, Finland is well experienced. In the beginning of the study all investigators will be informed on the practical implementation of the protocol in a separate institutional meeting. They will be informed on the rationale of the study and possible clinical implications as well.

11.2 Protocol amendments

According to Finnish national regulations, protocol amendments can be made if all investigators agree. They are presented in a written form and dated as applicable. They include the original chapter of the study protocol and the amended chapter, with an explanation to this change. Important protocol amendments are reviewed by the local ethics committee.

12 STUDY SCHEDULE

The study will start in February 2013 and all mandatory authorizations will be obtained before the beginning of the study. All MRI studies are expected to be performed within 12 to 18 months. Preliminary analysis of all results will be available in October - November 2014 and reports are expected to be written during summer 2015.

13 FINANCING

The study will be financed by Finnish Governmental Special Funding (In Finnish: ’Erityisvaltionosuus, EVO’) and Sigrid Jusélius Foundation.

14 Appendix

14.1 Appendix 1

SEQUENCE OF EVENTS

Event	*Measures*
clinical suspicion of prostate	Serum PSA
↓	
Inform consent by patient	
↓	
Referral to blood and urine samples	Serum PSA, serum free-to-total PSA ratio, kallikrein panel and other biomarkers
↓	
Referral to MRI examination	
↓	
MRI examination	
↓	
12-core biopsy + up to 2 cores from MRI suspicious area + 2 core for mRNA analysis All cores will be marked by its location	PSA density, selected tissue biomarkers, TRUS volume measurement
↓	
Final diagnosis	
↓	
Imaging and pathology correlation	

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