Clinical trials registration number: NCT02241122

Status: Patient enrolment ongoing

1 SUMMARY

This prospective multi-institutional study will enroll 400 men with clinical suspicion of prostate cancer due to higher serum level of PSA than 2.5 ng/ml and/or abnormal digital rectal examination. Anatomical magnetic resonance imaging (MRI) and diffusion weighted imaging (DWI) at 1.5/3 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 (1). 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% (2).

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 (3). 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 (4).

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 (5). Transrectal biopsy carries a risk of hemorrhagic and infectious complications (6). 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 (7, 8). Therefore, more accurate noninvasive imaging modalities are needed to improved diagnosis and avoid unnecessary biopsies.

Recently, magnetic resonance imaging had been introduced as a promising imaging tool and it has been proposed e.g. to improve surgical planning and patient selection for active surveillance (9, 10). Only limited data exists of MRI use in pre-biopsy setting. This approach potentially offers i) means of selecting patients for biopsy, ii) improving accuracy of TRUS-guided biopsies, iii) improved treatment planning. In order to be accepted, pre-biopsy MRI has to be safe, cost-effective and accurate.

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 (11, 12), 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 (9, 10). 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 (10) and also correlates with the Gleason score (13-15). 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, fecal, 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 (16, 17).

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 (18). 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 (19). 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.

In the study, a swab specimen of feces at the time of prostate biopsy or a fecal sample prior to prostate biopsy will be obtained to analyze: i) incidence of antibiotic-resistant bacteria (e.g. fluorocinolone resistant E. coli) to investigate risk of biopsy associated adverse infectious events, and ii) association of normal bacterial flora and disease conditions of the prostate.

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 1.5/3T 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 assess the applicability of TRUS guided prostate biopsy based on MRI finding in patient with no previous prostate biopsy

v) To evaluate the role of pre-biopsy MRI (imaging time of 15 minutes) for surgical planning and patients selection for active surveillance.

4 STUDY DESIG

This is a non-randomized prospective multi-institutional study to determine the applicability of 1.5/3T MRI, including DWI and selected biomarkers for the diagnosis of prostate cancer in patients with clinical suspicion of prostate cancer. 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, Helsinki University Hospital, Tampere University Hospital, Kuopio

University Hospital, Pori Central hospital and Seinäjoki Central hospital are potentially eligible. The total population in these hospital districts is approximately 3.165.00.

5.2 Number of patients

This multi-institutional study will include 400 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: 18 to 85 years
- Language spoken: Finnish and Swedish
- Clinical suspicion of prostate cancer, based on: serum level of PSA from 2,5 ng/ml to 20 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 within 6 months
- 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
- hip replacement surgery or other metal in the pelvic area
- 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 university hospital, urologist will confirm need for prostate biopsy and eligibility for the trial. After receiving the signed informed consent the patient is referred to blood and urine tests and MRI examination.

Blood tests will include serum PSA, free-to-total PSA ratio and 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 1.5T or 3T MR scanner. Body array coils will be used for image data acquisition. No endorectal coil will be used. 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 fifteen 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 (16, 17). 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 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 (20, 21) and internal RNA standards as validation of the absolute mRNA levels (22). Fresh prostate tissue specimens are stored in guanidine isothiocyanate

buffer or RNA*later*® (Life Tachnologies 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 (19).

6.4 Transrectal ultrasonography (TRUS)

Transrectal ultrasonography will be performed using Bk Medical Pro Focus Ultraview 2202 system. For the study logistics of patients with suspected prostate cancer, please see Appendix 1. After MRI examination the urologist will perform TRUS-imaging and record TRUS-findings. Subsequently, the MRI report is reviewed. If a suspicious lesion is noted in the MRI report, two biopsies are obtained from the MRI-target. After this, systematic 12-core biopsy is obtained. Finally, two cores for marker research are obtained from areas not suspicious based on MRI-report/TRUS. Additional study biopsy cores will be taken only in case the responsible urologist estimates that no additional risk is associated with the procedure

6.4.1. MRI-TRUS fusion

At the time of study preparation, MRI-TRUS fusion devices are not available in any of the study centers. If such a device is obtained, MRI-TRUS fusion is allowed for obtaining biopsies but these patients are evaluated separately and compared to the patient cohort biopsied without MRI-TRUS fusion device.

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, 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 (23). The use of 14-core TRUS biopsy resulted into higher detection of primary prostate cancer rate compared with 8-core biopsy technique (24). In addition, it was shown that 21-core TRUS biopsy procedure does not increase morbidity compared to sextant (6-core) biopsy approach (25).

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

Dedicated structured reporting system developed in a previous study will be utilized (29). Anonymised imaging data sets will be uploaded to a central server. All imaging data sets will be reported by a local radiologist and the report is confirmed by one designated research fellow will be a guarantor of MR reporting integrity and have final statement in a case of disagreement. 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 (26). Central gland prostate cancers were shown to have higher pathological stage (higher rate of extracapsular extension and seminal vesicle invasion) as well as higher Gleason score (27).

Following completion of the study, anonymised imaging data sets, clinical, and pathology information will be made publically available.

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):

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

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 multi-institutional study which assesses the utility of 1.5T/3T MRI and selected biomarkers for the diagnosis of prostate cancer in patients with clinical suspicion of prostate cancer will enroll 400 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. The sample size is based on the approximation that 50% of patients will be diagnosed with prostate cancer. Of these patients,

50% will undergo subsequent radical prostatectomy, and 50% will have other forms of treatment (e.g. external bean radiation, brachytherapy, and ctive surveillance). This are based on preliminary data of IMPROD clinical trial (NCT01864135).

11 QUALITY ASSURANCE

11.1 Information of study personnel and training

The technical and other supporting personnel in the principal study center (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. All other study centers also have expertise in MRI imaging and prostate biopsies.

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 September 2014 and all mandatory authorizations will be obtained before the beginning of the study. All MRI studies are expected to be performed within 18 to 24 months. Preliminary analysis of all results will be available in October - November 2015 and reports are expected to be written during autumn 2017.

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 and/or abnormal DRE
↓	
Informed consent by patient	
↓	
Referral to blood and urine samples	Serum PSA, serum free-to-total PSA ratio, serum and urine sample
↓	
Referral to MRI examination	
↓	
MRI examination	
↓	
12-core biopsy + 2 cores from MRI suspicious area + 2 core for biomarker analysis All cores will be marked by its location	PSA density, selected tissue biomarkers, TRUS volume measurement
↓	
Final diagnosis	
↓	
Imaging and pathology correlation	
↓	
Clinical follow up	

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