A multicenter randomized diagnostic study assessing whether a diagnostic pathway using the Stockholm3 test and MRI followed by targeted biopsies outperforms PSA and systematic biopsies for men invited to prostate cancer testing.

Acronym: STHLM3 MRI Phase 2

SPIRIT 2013-compliant

STUDY PROTOCOL

V 2.3 2020-01-15

1.1. Table of contents

2. Trial identifier

ClinicalTrials.gov Identifier: NCT03377881

3. Study organization

The co-investigators comprise the principal investigator, the trial steering committee and the trial working group. Decisions on planning or execution of the study, including closure of the study, is to be made by the Principal Investigator alone. The trial steering committee and the trial working group advices the principal investigator.

3.1. Principal investigator

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3.2. Trial steering committee

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3.3. Trial working group

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4. Trial Sponsor

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5. Date and version identifier

Revision version, date, author	Reason	Main Change
V2.1 2018-05-14; TN	After DSMB initial meet	Clarifications regarding working group, and hypotheses
V2.3 2020-01-15	Pre-analysis clarificartions	See separate document

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7. Abstract

Prostate cancer is a leading cause of cancer death among men in the Western world. Early detection of prostate cancer has been shown to decrease mortality, but has limitations with low specificity leading to unnecessary biopsies and over-diagnosis of low-risk cancers. The STHLM3 trial has paved the way for improved specificity in early detection of prostate cancer using the blood-based STHLM3 test for identifying men at increased risk of harbouring significant prostate cancer [1].

Targeted prostate biopsies based on MRI images have been shown non-inferior sensitivity to detect significant prostate cancer and decrease the number of biopsies and non-significant cancers among men referred for prostate biopsy in clinical practice [2]. Evidence is limited to study populations including men in current clinical practice.

The overarching strategy of the STHLM3-MR/Fusion projects is to study an improved diagnostic pathway including an improved blood-based test for identification of men with increased risk of prostate cancer and use of MRI to select men for diagnostic workup with targeted prostate biopsies. The aim is to increase the specificity in early detection of prostate cancer without decreasing the sensitivity of aggressive prostate cancers.

Endpoints include the number of detected prostate cancers, number of performed biopsy procedures and number of performed MRIs. Additional aims include to assess the health economic consequences and development of automated image-analysis.

The STHLM3-MR project is performed in two separate phases, analyzed separately. STHLM3-MR Phase 1 is a paired design study which closed inclusion 2017-06-01 and includes 533 men planned for prostate biopsies. All participants underwent target and systematic biopsies together with STHLM3 test analysis. The study constitutes a current practice cohort and levels of the STHLM3 test were not used for selecting participants.

STHLM3-MR Phase 2 is a study comparing traditional prostate cancer detection using PSA and systematic biopsies with the improved pathway for prostate cancer detection using the STHLM3 test and targeted biopsies in a screening context. The study will recruit 10,000 participants during September 2017-April 2018 combining a paired and randomized design. The STHLM3 MRI studies were described previously {NordstromSTHLM3MR} and this protocol follows SPIRIT guidelines [3].

8. Introduction

8.1. Public health significance of prostate cancer

Prostate cancer is the most common cancer and the leading cause of cancer death among men in Sweden. In year 2011 over 10,000 men were diagnosed with prostate cancer and more than 2,500 died due to the disease, approximately 20% of these in the Stockholm region. Prostate cancer incidence rates in Sweden are now comparable to rates in countries that had an early introduction of PSA testing, while prostate cancer mortality rates in Sweden are higher than in most other countries[4]. With over 90,000 prevalent cases, the health burden and the costs on the health care system are substantial. While a number of risk factors have been proposed for prevention of prostate cancer, including diet and occupational exposures, the only factors conclusively shown to increase risk of the disease are age, ethnicity and family history. Given the high prevalence of the cancer and limited opportunities for primary prevention, improved detection would reduce both procedure-related harm to men and economical cost in the healthcare system.

8.2. Early detection and treatment of prostate cancer: benefits and harms

The PSA test was first used to monitor disease progression in prostate cancer patients. The PSA test was taken up as a *de facto* screening test for prostate cancer in many countries, leading to rapid rises in prostate cancer incidence. The test characteristics for the PSA test in detecting prostate cancer are comparable to those for mammography for breast cancer screening, with a sensitivity of 72% and a specificity of 30-35% at a test threshold of 4 ng/ml[5]. However, a lower threshold of 3 ng/ml adopted in Sweden recently has led to increased sensitivity at the expense of reduced specificity. Recent analyses of PSA testing in the Stockholm area confirms these results showing that 46%, 68% and 77% of men 50-59, 60-69 and 70-79 years respectively have had at least one PSA test during a 9 years period[6].

Recent results from the large European Randomized Study of Screening for Prostate Cancer (ERSPC) including over 180,000 men provide increasing evidence that PSA screening has led to reduced mortality[7]. This report showed that PSA screening without digital rectal examination was associated with a 21% relative reduction in the death rate from prostate cancer at a median follow-up of 11 years, with an absolute reduction of about 7 prostate cancer deaths per 10,000 men screened. Estimations from the ERSPC trial (men aged 55-69) show that 1,048 men would need to be offered screening and an additional 37 would need to be managed to prevent one prostate-cancer death during a 10-year period, leading to a significant overtreatment of indolent disease. The effectiveness of PSA testing was more

marked at the Göteborg site of the ERSPC trial, with a risk reduction of 44% over 14 years in men aged 50-64[8]. This effect size is larger than that observed for mammographic screening for breast cancer and fecal occult blood testing for colorectal cancer.

However, using traditional systematic biopsies for diagnosis, approximately half of diagnosed cancers are low-risk tumors using the same main cutoff for biopsy as the ERSPC trial (PSA=3ng/ml) [9,10]. It has been shown that men with low-risk tumors treated without curative intent have the same survival as men in the background population[11], illustrating the large proportion of over-diagnosed cancers[12].

The STHLM3 study has shown a way to improve identification of men at increased risk of significant prostate cancer. Using the STHLM3 test, 32% of the prostate biopsies may be saved while not decreasing the sensitivity to high-grade disease (defined as Gleason Score ≥7) and simultaneously decreasing the number of low-grade tumors (Gleason Score ≤6) by 17%, thus decreasing overdiagnosis[10].

8.3. Traditional evaluation of men with increased risk of prostate cancer

Men at increased risk of prostate cancer - commonly estimated using PSA and palpatory findings - are traditionally assessed using systematic prostate biopsies. The procedure is performed under local anesthesia using antibiotic prophylaxis and includes 10-12 cores taken from predefined areas of the peripheral zone of the gland as visualized by endorectal ultrasound. While the biopsies systematically covers the prostatic gland rather than targeting a lesion, and non-lethal tumors are common, the risk of over-diagnosis (i.e. detection of non-significant tumors) is high [12]. The risk of non-representative biopsy findings result in underestimation of tumor grade compared with subsequent prostatectomy in up to 40% of men undergoing surgery[13]. The risk of severe post-biopsy infection has increased to 1-2% with increasing frequency of antibiotic resistance, further illustrating the need both to increase precision and decrease the number of performed biopsies[14].

8.4. Multi-parametric Magnetic Resonance Imaging (mpMRI) for detection of prostate cancer

Multi-parametric magnetic resonance imaging (mpMRI) incorporating anatomical and functional imaging has now been validated as a means of detecting and characterizing prostate tumors and can aid in risk stratification and treatment selection. The European Society of Urogenital Radiology (ESUR) in 2012 established the Prostate Imaging Reporting

and Data System (PI-RADS) guidelines aimed at standardizing the acquisition, interpretation and reporting of prostate mpMRI. Consensus on an updated version (PI-RADS v2) have recently been published, outlining aspects of both interpretation and the technical execution[15-17]. Use of the revised PI-RADS provides moderately reproducible MR imaging scores for detection of clinically relevant disease[18]. Using MP-MRI to triage men might allow 27% of patients avoid a primary biopsy and diagnosis fewer clinically insignificant cancers. If subsequent TRUS-biopsies were directed by MP-MRI findings, up to 18% more cases of clinically significant cancer might be detected compared with the standard pathway of TRUS-biopsy for all[19].

In summary, PI-RADS recommends to use 3T or 1.5T machines, including T2- and T1-weighted sequences together with diffusion weighted images (DWI). Currently, the added value of dynamic contrast is not firmly established regarding tumor detection. At this time, there is no consensus among experts concerning the potential benefits of the use of endorectal coils for cancer detection. It has been suggested that the prevalence of suspicious lesions on MRI in men with clinical suspicion of prostate cancer is approximately 60% [20].

8.5. Targeted prostate biopsies guided by fusion technology

Targeted biopsies of the prostate consist of imaging (MRI) detecting significant tumors and a biopsy procedure where biopsies are targeted to the tumor using various devices for guidance [21]. While traditional endorectal ultrasound poorly identifies tumors, direction of biopsy needles can be performed in various ways. Cognitive or soft fusion is based on skilled urologists/radiologists interpreting the MRI images and directing needles solely based on the ultrasound images. The disadvantages of cognitive fusion lie in the potential for human error when attempting to mentally fuse the MRI with TRUS while aiming for cancers that are often <1 cm in diameter and the inability to track the location of each biopsy site. Hard fusion enables proper fusion of MRI information on the ultrasound image, possibly increasing precision.

Despite methodological flaws, a number of studies have investigated the value of fusion biopsies, primarily using non-randomized designs and non-screening populations (see **Error! Reference source not found.**) [22]. In 2018, Kasivisvanathan et al provided high quality evidence for men referred for prostate biopsy and showed that MRI/target biopsies are non-

inferior for detection of significant cancer and decreases the number of in-significant cancers and number of biopsies as compared with systematic biopsies [2].

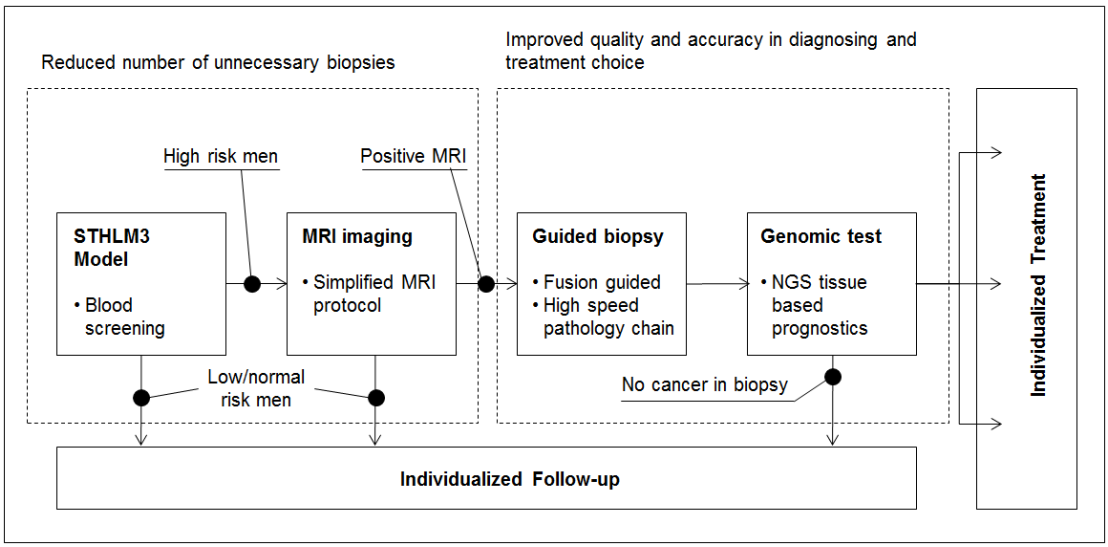
The proportion of men upgraded when comparing specimen from targeted biopsies and subsequent prostatectomy have been shown to be very low (<5%) when using targeted biopsies[23], increasing the proportion of men where treatment decisions are based on valid risk estimations.

8.6. Improving the diagnostic pathway for prostate cancer detection

The current diagnostic pathway for prostate cancer detection is characterized by several challenging hallmarks. First, testing with PSA is frequent also in men not benefitting from testing due to low PSA levels or high age[6]. Second, the currently used test for detection (PSA) lacks in specificity, resulting in frequent over-diagnosis[24,25]. Third, systematic biopsies shows high frequencies of benign tests, over-diagnosis, up-grading at prostatectomy, and risk of infectious complications[10,26]. Further, PSA testing increases with educational length and men with long education are more likely to have a prostate biopsy after an increased PSA value. These differences may contribute to the worse prostate cancer outcomes observed among men with lower socioeconomic status[27].

The STHLM3 test offers improved disease detection[10]. To further decrease over-detection, improve disease classification and spare men of test-related harm, prostate biopsy practice need to be improved. We hypothesize that an improved pathway for prostate cancer detection including a better blood-based screening test, improved selection to biopsy based on MRI findings and targeted biopsies guided by MRI/ultrasound fusion would dramatically decrease the number of biopsy procedures, overdiagnosis and improve treatment decisions (see Figure 1).

Figure 1: Improved pathway for prostate cancer detection.



9. Objectives

9.1. Primary hypothesis

The overarching primary hypothesis of the STHLM3MRI trial is that a diagnostic pathway using the Stockholm3 test to select men for further workup using MRI followed by targeted biopsies and systematic biopsies (S3M+MRI+TBx+SBx) has non-inferior sensitivity for detecting clinically significant cancer (ISUP grade ≥2) and shows superior specificity (reduction in number of performed biopsy procedures and detected ISUP 1 tumours) compared with the diagnostic pathway using systematic biopsies in men with PSA ≥ 3 ng/mL (PSA+SBx).

9.2. Additional hypotheses

1. When compared with performing systematic biopsies for men with elevated risk of prostate cancer in prostate cancer screening, targeted and systematic prostate biopsies performed on MRI positive men has non-inferior sensitivity for detecting clinically significant cancer (ISUP grade group ≥2) and reduces the number of performed biopsy procedures, which also translates to lower proportion of men with elevated risk who experience severe post-biopsy infections. Elevated risk can here be defined using PSA or S3M – we will clarify the exact contrasts for testing this hypothesis below.
2. When compared with performing systematic biopsies for men with elevated risk of prostate cancer in prostate cancer screening, targeted biopsies only

performed on MRI positive men has non-inferior sensitivity for detecting clinically significant cancer (ISUP grade group ≥2) and reduces the number of performed biopsy procedures, which also translates to lower proportion of men with elevated risk who experience severe post-biopsy infections. Elevated risk can here be defined using PSA or S3M – we will clarify the exact contrasts for testing this hypothesis below.

3. A diagnostic chain consisting of Stockholm3 followed by MRI and targeted+systematic biopsies (S3M+MRI+TBx+SBx) versus a diagnostic chain based on PSA ≥3 ng/ml followed by MRI and targeted+systematic biopsies (PSA+MRI+TBx+SBx) will lead to: non-inferior sensitivity for detecting clinically significant cancer (ISUP grade group ≥ 2); an inferior sensitivity for ISUP1 cancers (i.e. reduced overdiagnosis); and a reduction in the number of MRI examinations and performed biopsies.
4. A diagnostic pathway using the Stockholm3 test to select men for further workup using MRI followed by ONLY targeted biopsies (S3M+MRI+TBx) has non-inferior sensitivity for detecting clinically significant cancer (ISUP grade group ≥2) and reduces the number of performed biopsy procedures compared with a diagnostic pathway using systematic biopsies in men with PSA ≥3 ng/mL (PSA+SBx).
5. Biopsy compliance is higher after biopsy is recommended based on MRI compared to recommended without MRI.
6. SBx in the MRI arm has superior sensitivity than SBx in the non-MRI arm (due to cognitive fusion).
7. A diagnostic chain consisting of Stockholm3 followed by MRI and targeted+systematic biopsies (S3M+MRI+TBx+SBx) is cost-effective (ICER < 750 000 SEK per QALY gained) compared to a diagnostic chain based on PSA ≥3 ng/ml followed by MRI and targeted+systematic biopsies (PSA+MRI+TBx+SBx).
8. A diagnostic chain using the Stockholm3 test to select men for further workup using MRI and targeted+systematic biopsies (S3M+MRI+TBx+SBx) is cost-effective compared to a diagnostic chain using systematic biopsies in men with PSA ≥3 ng/ml (PSA+SBx).
9. Adding prostate volume as a variable in the diagnostic chain with Stockholm3 test (i.e. using the full Stockholm3 model described in Ström et al.[5]) and MRI/Fusion biopsies improves model precision, leading to further improvements

in specificity compared to the use of the Stockholm3 test without the inclusion of prostate volume.

10.Primary aim

To compare a diagnostic pathway using the Stockholm3 test to select men for further workup using MRI (PI-RADS ≥ 3) and targeted biopsies (S3M+TBx) to a diagnostic pathway using systematic biopsies in men with PSA ≥3 ng/ml (PSA+SBx) with respect to number of diagnosed clinically significant cancer (ISUP grade group ≥ 2) and number of performed biopsies.

11.Additional aims

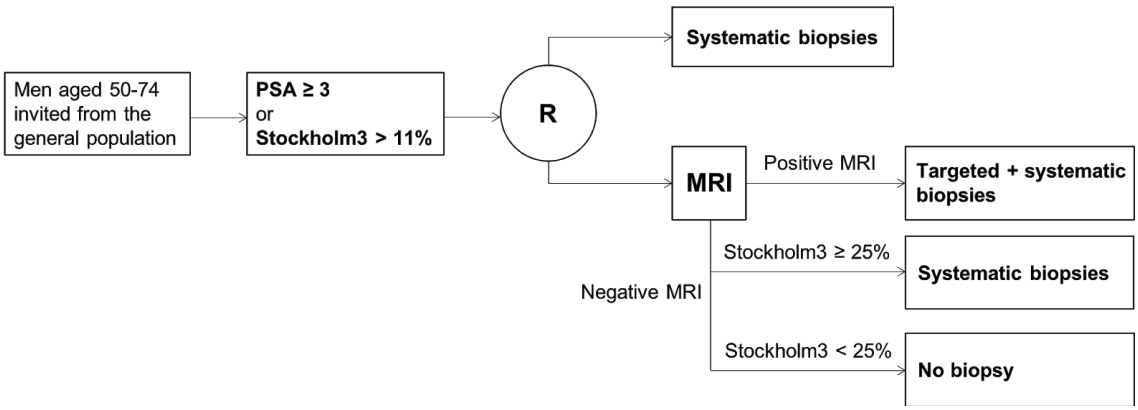
Additional aims corresponding to hypotheses 2-9 above will be assessed.

12. Study design

12.1. Design overview

STHLM3-MR Phase 2 is a study combining a paired and a randomized design (see below). The study will follow the following outline: Participants will be invited by mail. All participants will undergo a blood-test, including PSA and the STHLM3 test. Men with an elevated PSA ≥3 ng/ml *or* PSA ≥1.5ng/ml and S3M>11% will be randomized to either traditional prostate biopsies or MR with targeted biopsies on MR lesions.

Figure 2: Design overview



13. Methods: Participants, interventions and outcomes

13.1. Study setting

This is a screening-by-invitation study including one study administrative center, two radiological sites and three urological sites where data will be collected.

Participating urological centras

Department of Urology, Capio St Görans Hospital: dr Henrik Grönberg

Uroclinic, Sophiahemmet, Stockholm; dr Olof Jansson

Odenplans läkarhus; dr Magnus Annerstedt

Urologifocus; dr Gunnar Trygg

13.2. Eligibility criteria

Inclusion criterias

Men age 50-74 years without prior diagnosis of prostate cancer (ICD-9 C61).

Permanent postal address in Stockholm

Not a previous participant in the Stockholm3 study (2012-2014)

Exclusion criterias

Severe illnesses such as metastatic cancers, severe cardio-vascular disease or dementia

Contraindications for magnetic resonance imaging (MRI) eg pacemaker, magnetic cerebral clips, cochlear implants or severe claustrophobia.

Men with a previous prostate biopsy the preceding 60 days before invitation.

13.3. Randomization

Randomization is performed 2:3 between control arm and experimental arm. Randomisation will be performed using stratification on disease risk (three strata). Disease risk is assessed using the Stockholm3 test.

Six allocation lists have been created, specifying the sequence of study arm allocation. Participants are first allocated to corresponding list, and then allocated to study arm

according to the order in which they participate. The allocation sequence is blinded from the study investigators and handled by the study database administrator (SDA, A Björklund).

In order to enhance resource usage, men are allocated to the study sites according to local availability of biopsy procedure slots.

13.4. Interventions

Blood sampling

Participating men undergo blood-sampling with analysis of PSA and the Stockholm3 test at any of Unilabs blood-testing site .

For the main analysis, the Stockholm3 test include clinical data as answered when consenting participation (previous biopsy, age, finasteride medication, relatives with prostate cancer); single nucleotide polymorphisms and measurements of protein levels (MSMB, MIC1, PSA, fPSA, hK2). For secondary analyses, clinical information on DRE and prostate volume is included. The algorithm for calculation of the Stockholm3 test result has been described (Ström et al, European Urology 2018).

Definition of EXPERIMENTAL ARM

Men randomized to the experimental arm undergoes MRI. If suspicious lesions are found, the participant undergoes targeted biopsies using Fusion technology *followed by systematic biopsies.*

Men without lesions are excepted from further intervention and receives notification on recommendation for follow-up. Technology and process are described below.

Men with a Stockholm3 risk ≥25% and no suspicious lesion on MRI will undergo systematic biopsies.

Definition of CONTROL ARM

Men randomized to the control arm undergoes systematic biopsies as defined below.

Technology

Cut-offs for performing the STHLM3 test

The STHLM3 test will be performed for men with a PSA ≥ 1.5 ng/ml

Cut-offs for entering randomization

Participants with PSA ≥ 3.0 ng/ml or STHLM3-test ≥ 11% risk of Gleason Score ≥7 cancer will be randomized and offered to undergo either MR or systematic biopsies (See Process description).

MRI technology

Location and MRI equipment

Capio St Görans Hospital: General Electric 3T

Globen Healthcare: Siemens Magnetom Aera 1.5T

Patient preparations

Refraining from sexual activity with ejaculation 3 days prior to examination

Fasting patient 6 h

Minimal preparation enema prior to examination

Antispasmodic agent (Glucagon) just before the examination

MRI Protocol

A short (16 minutes) MRI protocol developed through STHLM3MR Phase 1 will be used. A detailed description of protocols used below. Briefly, the protocol includes: 3D T2 alt T2 ; Diffusion for ADC B100 , B450, B800, B1500 limited to the prostate location; T1 or FS limited to the prostate location; No endorectal coil will be used.

Image processing the ADC map is fusioned together with the T2 series using Nordic Ice software. The Color scale for ADC maps is the inverted rainbow color scale. Color adjustment is set to min. 50 and max. 220.

Definition of MRI protocol:

MRI Protocol, 1,5 T Siemens Magnetom Aera						
Sequence, plane of acquisition	Pulse sequence	Repetition time/Echo time (ms)	Acquired voxel size (mm)	Field of view (mm)	Slices (n)	Time of acquisition (min:sec)
3 Plane Localizer	FSE	1500/102	1.8x1.8x8	460 x 460		00:20
T2w 2D ax	TSE	3200/134	0.6x0.6x3	200 x 200	30	03:20
T2w 2D, sag	TSE	3630/117	0.6x0.6x4	265 x 215	13	1:40
T2w 2D, cor	TSE	3250/134	0.6x0.6x3	200 x 200	16	01:42
T1w 2D, ax	Vibe	6.86/2.38/4.75	0.6x0.6x3	380 x 297		00:20
DWI (focus), ax, b=0,800	EPI DWI	4140/57	2x2x4	200 x 200	22	03.41

MRI protocol, 3T Signa Architect, GE Healthcare						
Sequence, plane of acquisition	Pulse sequence	Repetition time/Echo time (ms)	Acquired voxel size (mm)	Field of view (mm)	Slices (n)	Time of acquisition (min:sec)
3 Plane Localizer	SE	Minimum /80		420 x 420		00:17
T2w 2D ax	FSE	3000/120	0.6x0.6x3.0	200 X 200	32	04:36
T2w 2D, sag	FSE	2500/120	0.6x0.6x3.0	180 X 180	24	02:05
T2w 2D, cor	FSE	2500/120	0.6x0.7x3.5	200 x 200	24	02:05
T1w 2D, ax	FSE	767/Minimum Full	0.9x1.1x4.0	250 x 250	20	00:35
DWI (focus), ax, b=0,1000*	DWI	4996 / Minimum	1.7x1.7x4.1	200 x 100	17	03:45

MRI Interpretation

MRI interpretation is centralized to Capio St Görans hospital. Assessments are based on "Assessment Without Adequate DCE" from PI-RADS v2 and v2.1.

Dr Fredrik Jäderling is responsible for MRI interpretation. Dr Jäderling or 1-2 other, experienced radiogists at his department performs all MRI interpretations.

Fusion biopsy technology

Brand/models

BK Medical (BK Ultrasound ; www.bkultrasound.com/bk-medical/fusion)

The BK Medical fusion system is the only fusion device compatible with BK Medicals ultrasound devices, used by the urology departments participating in the study. The system represents a second generation ultrasound system with integrated MRI Fusion. MRI data is imported through HIPAA-compliant PACS connection with the local radiology department.

Definition of targeted biopsies

Using MRI data with pre-marked borders of the prostate and tumor, fusion of MRI images and ultrasound images are performed bedside. Using local anesthetic and antibiotic prophylaxis, lesions are according to below. Targeted biopsies are always combined with systematic biopsies.

Biopsy procedure for targeted biopsies

PI-RADS≥3: 3-4 targeted biopsies on marked lesions + systematic biopsies

Large diffuse lesions or poor image quality: Systematic biopsies including lesion

No PI-RADS≥3, diffuse lesions and at least acceptable image quality: No biopsies are performed.

Definition of systematic biopsies

10-12 systematic biopsies are taken from the peripheral zone as previously described in STLHLM3 and the National Guidelines. Extra biopsies are allowed from additional sites visible on ultrasound or according to palpatory findings. In summary, systematic biopsies are performed in the peripheral zone as 4 lateral and para-median biopsies on the left and right side, in the base and mid part of the gland. In the apical third of the gland one lateral left and right biopsy is performed.

Pathology

Pathology is centralized to Unilabs/Capio St Görans hospital. Dr Axel Glaessgen is responsible for the integrity of analyzes of pathological specimen. 2-3 uro-pathologists at dr Glaessgens department assesses all pathological specimen with intermittent cross-validation between them. Pathology preparation and reporting follow ISUP 2014 guidelines.

The pathology preparation is done by Unilabs as part of the normal clinical routine. Biopsy specimens are analyzed according to local practice.

Localisation of biopsies in the prostate are described using Swedish National Guideline nomenclature (A1-4; B1-4; C1-4; anterior/posterior). Gleason Score, mm cancer and % Gleason 4 is reported on each needle specimen.

Pathologist notes results in the usual way in the laboratory system. The result of the pathological analysis is submitted in accordance to existing clinical routines to the referring urologist. A copy of the result is delivered to the study administration.

13.5. Outcomes

Primary otcome:

1. Diagnosed ISUP grade ≥ 2 cancers

Key secondary outcomes:

2. Diagnosed ISUP grade 1 cancers
3. Performed biopsies
4. Performed MRI examinations

See statistical analysis plan (SAP) for details.

Additional endpoints

We are collecting data on a large number of endpoints in the study. See SAP for detailed information regarding the definition of these endpoints.

13.6. Follow-up

All participants were followed a minimum of 200 days after receiving blood test results. Main study outcomes are assessed after prostate biopsy procedures (plus 30 days of follow-up for post-biopsy infections). Additional participant data will be secured in the following circumstances:

No suspicious lesion on MRI:

Men in the experimental arm without suspicious lesions on MRI will be informed and recommended follow-up by the responsible, local urologist. After additional ethical application, the co-investigators might initiate retrospective follow-up of these participants.

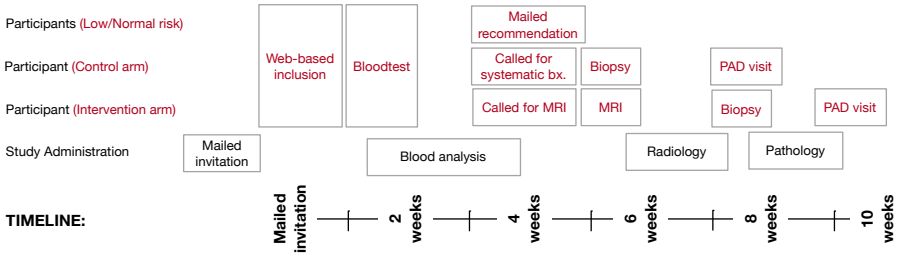
Men with diagnosed prostate cancer

Participants with prostate cancer diagnosed on biopsy within the study will be followed up after the biopsy to secure data on the following: Treatment modality (Active Surveillance, Surgery, Radiation); Treatment lead-time and site; Pathological report after surgery (positive margins, T-stage, etc). Data will be assessed through medical records intermittently.

13.7. Serious adverse events

Study nurse will monitor serious adverse events after the prostate biopsy procedures (up to 30 days post biopsy). To ensure this, the study nurse will check medical journals for hospitalization within 1 week after the biopsy procedure in the journal systems Take Care and Cosmic (covering the main part of hospitals in Stockholm region). This will be initiated as individual biopsy results are registered at the study administration. Results will be provided to the Data Safety and Monitoring Board.

13.8. Participant timeline



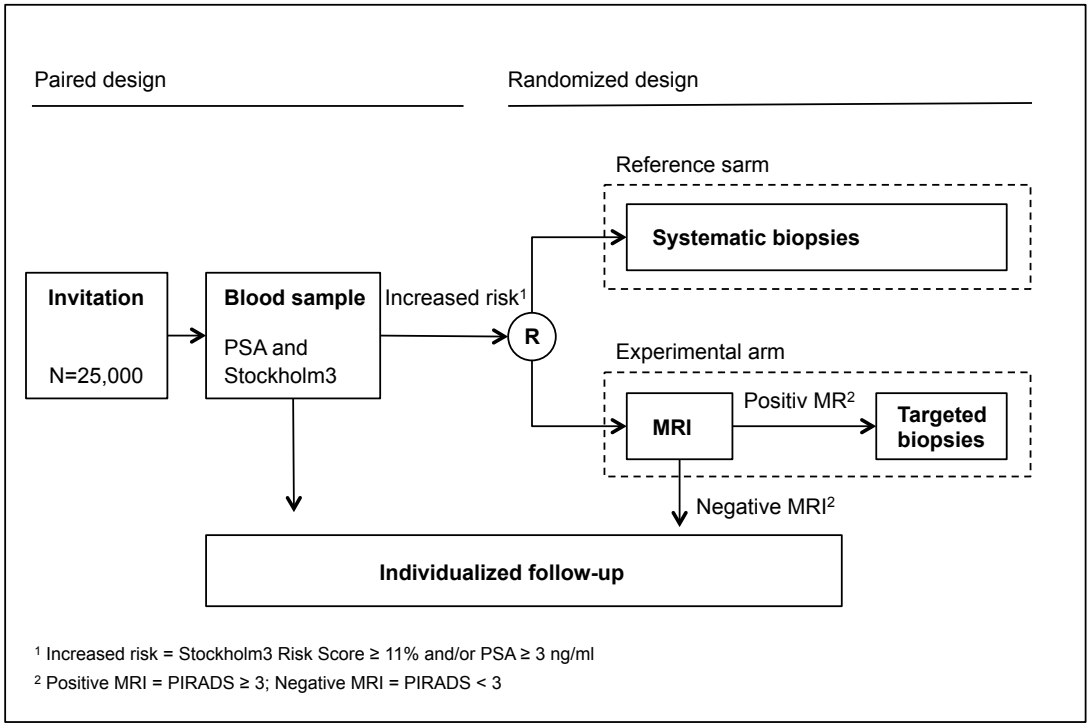
13.9. Sample size

Sample size calculations are described in the Statistical Analysis Plan (SAP).

13.10. Recruitment and Process Description

The STHLM3-MR Phase 2 will use existing solutions developed and optimized in the previous studies STHLM3 and STHLM3-MR Phase 1 where all major components of the process have been tested.

Figure 2: STHLM3 MR Phase II: Overall design and main process steps. First, participants will follow the *paired design study process* where inclusion, blood-test and delivery of recommendation letter is performed. Men with increased risk of high-grade prostate cancer then enter the *randomized study process*, where extended work-up including biopsies are performed.



Study part 1 (All participants): Paired study process

Step 1: Selection and Invitation

A potential participant list is created by collecting names and addresses for men between ages 50-74 with a permanent mailing address in Stockholm county. Name and address details of potential participants are bought from an external address source, such as SPAR or InfoTorg.

The address list of potential participants is loaded into STHLM3 Participant Handling Register (Microsoft Dynamics CRM configured for this purpose). For each potential participant a unique study ID is created.

An invitation letter is sent to each potential participant, asking if they are willing to participate in the STHLM3 MR Phase II study. The invitation includes:

1. Invitation letter with brief information about the study, including what the potential participant need to do in order to participate
2. Study information brochure with extensive information on the study
3. List of about 60 laboratories in Stockholm that participate in the study including addresses, opening hours, phone numbers, etc.
4. The proposed participant is directed to the website medicinskastudier.se for inclusion. At this website, a secure login using "Mobilt BankID" is used and the participant is included after answering studyrelated questions (family history, previous prostate biopsy and current use of selected medicine (finasterid, avodart, dutasterid, proscar and testosteron)). Informed consent is acquired and a unique Study ID is created.
5. An electronic referral with a unique referral ID (RemissID) is generated and activated for blood sampling at any of the blood-sampling stations.

Step 2: Blood Sampling

The research participants who choose to join the study visit one of the 60 laboratories that collaborate with STHLM3. For many participants, this means that sampling can be conducted close to home or work.

The lab personnel first check the research participant's identity. This is done in the normal way, i.e. by checking the man's identity card and checking that the photo matches the man. The lab personnel then scan the following information on the combined referral and consent form: RemissID, laboratory analysis code (=code that steers robots and transport routines at the laboratory, barcoded) and Kombika (=code for automatic payment through the health care system, barcoded).

The lab personnel then samples a Stockholm3 test from the participant. This is normal venous blood samples of 12 ml.

The blood tests are then passed through the regular health care logistics to A23 laboratory for analysis. A23 conducts the Stockholm3 analysis and sends the results together with the referral ID (RemissID) to the STHLM3 research group. KUL also sends the combined referral and consent form to the STHLM3 research group.

Step 3: Randomization

Research participants with a total PSA ≥ 3 ng/ml and/or a Stockholm3 risk score ≥ 11% (see above) will be randomized 2:3 to either the reference arm with traditional, systematic biopsies, or to the experimental arm with MRI followed by targeted biopsies.

4: Response letters to the participant

Each participant is placed in a group based on the test results:

Green: PSA < 1.5

Yellow: 1.5 ≤ PSA < 3 and Stockholm3 risk score < 11%

Red: PSA ≥ 3 and/or Stockholm risk score ≥ 11%

Each participant will receive any of four response letters (snail mail) within 5 weeks of sampling. The response letter is sent to the participants' registered permanent home address:

Green: Low risk for prostate cancer, follow-up test within 6-10 years is recommended. For participants > 60 years of age, no more testing is recommended

Yellow: Normal risk for prostate cancer, follow-up within 2-4 years is recommended

Men with increased risk of prostate cancer will enter the randomized part of the study. They receive letters according to study arm:

Red (reference arm): Increased risk of prostate cancer, urology consultation and biopsy is recommended. The biopsies will be performed using traditional technique.

Red (experimental arm): High risk of prostate cancer and extended work-up is recommended. The participant will be referred for MRI examination and subsequent visit to a participating urology office where targeted biopsies will be completed to suspicious lesions. If no lesions are detected, the urologist will recommend structured follow-up.

A list with participants with high risk (Red) are created weekly and delivered from the study administration to the participating urology department, including information on STHLM3 risk (%), PSA, responsible urology department (St Göran , Odenplan, Sophiahemmet) and study arm (experimental, control)

Participants are contacted from each department to book time for MRI (experimental arm) or systematic biopsy).

The participant that choses to continue with urology visit or MRI will become patients in the normal Swedish health care system, i.e. tax paid health care.

Step 5a: Reference arm (Traditional biopsies)

Men randomized to the reference arm will be referred for systematic biopsies at any of the participating urology centers according to Definition of systematic biopsies

After performed biopsy, men are followed in line with clinical practice.

STEP 5b: Experimental arm (MRI and targeted biopsies)

Men randomized to the experimental arm will be referred to MRI at S:t Göran Hospital or at Unilabs Globen. Radiology data is transferred in accordance to clinical practice to Karolinska Hospital or Capio St Görans radiology department and evalutated in accordance to PI-RADS v2 by study radiologists. Suspected lesions are marked and the report is transmitted to S:t Görans Hospital.

Men with PI-RADS≥3 lesions will undergo targeted biopsies followed by systematic biopsies. MRI data is loaded into the local Fusion software by the responsible urologist at the time of the biopsy procedure. All procedures include local anestethics and antibiotic

prophylaxis as recommended by National Guidelines. Separate referrals for systematic and targeted biopsies are used (see appendix).

Men with no visible PI-RADS≥3 lesions will be informed by participating urologist and given instructions for systematic follow-up. This follow-up is recommended to include a renewed MR and a STHLM3 test after 12 months.

14. Methods (Data Collection, management, analysis)

14.1. Data collection

Primary data sources are

i. clinical variables collected from laboratory referral
ii. biopsy referrals and reports
iii. pathology reports
iv. MRI reports
v. blood analysis reports

Collection of i. – iv. is performed by study nurses (C Cavalli-Björkman) on a weekly basis from participating urology sites, participating radiologists. For v., this is digitally transferred from A23 laboratory.

14.2. Data management

Data is collected, entered, coded and stored at Department of Medical Epidemiology and Biostatistics, Karolinska Institutet. Data is entered by Study Nurse using predefined database sheets. This is blinded from study co-investigators and data is stored at the department under supervision by the study database administrator (SDA, Astrid Björklund). Any extraction of study data is performed by the SDA after approval of PI Tobias Nordström.

14.3. Data analysis

Analysis of data is described in the Statistical Analysis Plan (SAP).

14.4. Auditing and Monitoring

A Data Safety and Monitoring Board (DSMB) is assembled and consist of dr Hans Garmo (Statistician), prof Ola Bratt (Urology) and prof Holmberg (Urology/Study Design). The DSMB audits protocol and process descriptions and interim data extraction performed by the study database administrator and the study statistician (AD). The co-investigators are blinded to

the interim data and analysis results. The work of the DSMB is regulated in the DSMB Charter.

15. Ethics and dissemination

15.1. Research ethics approval

The study has approval from the regional ethical board in Stockholm (2017-1280/31).

15.2. Protocol amendments

Minor changes to this protocol made after 2018-04-04 is noted in the protocol. Major changes including changes to eligibility criteria, outcomes, analyses are registered at clinicaltrials.gov and communicated to the DSMB for recommendations on further disseminations.

15.3. Consent

Participant consent is secured when the participant is included to the study at www.kliniskastudier.se. This includes secure identification using Mobilt BankID. Additional approval on use of biological specimen data is collected on the biopsy referral.

15.4. Confidentiality

Study data is collected and stored at Department of Medical Epidemiology and Biostatistics, Karolinska Insitutet using secure Oracle servers. All data extractions are made by database administrator and are anonymized (personal id number is removed) before dissemination to researchers.

15.5. Declarations of interest

Henrik Grönberg has five prostate cancer diagnostic related patents pending, has patent applications licensed to Thermo Fisher Scientific, and might receive royalties from sales related to these patents. Martin Eklund is named on four of these five patent applications. Karolinska Institutet collaborates with Thermo Fisher Scientific in developing the technology for the Stockholm3 test.

15.6. Access to data

Co-investigators will have access to the anynomized, final data-set. Publication of any post-hoc analyses are permitted after communication with Tobias Nordström or by him delegated person. The data-set might be accessible for external validation of trial results on communication with Tobias Nordström.

15.7. Dissemination

Analyses results on the posed aims will be submitted for peer-reviewed publication and submitted for presentation at scientific congress. Communication of the results will be made to patient organisations (Prostatacancerförbundet) and non-scientific channels. No use of professional writers are planned.

The study protocol is made publicly availiable through clinicaltrials.gov.

16. Specific considerations

Is there a risk of over diagnosis of low risk prostate cancer in men participating in STHLM3-MR?

There is a risk of over-diagnosis when performing systematic biopsies of the prostate, which is the current golden standard in clinical practice. While targeted biopsies are performed only towards visible lesions on MRI, and the problem of up-grading is substantially lower in targeted biopsy cohorts, the risk of causing over-diagnosis using targeted biopsies are low.

Is there an increased risk of missing aggressive cancers?

The sensitivity of finding high-grade cancers is very limited in current clinical practice, using PSA with a poorly defined cut-off and non-targeted, systematic biopsies for extended diagnostic work-up. Depending on the cut-off used for recommending biopsy (e.g. STHLM3-test cutoff and MR-cutoff using PIRADS-grade) this sensitivity can be adjusted. Previous studies show that the sensitivity/specificity balance of the STHLM3-test is better than PSA and that this balance is better for targeted biopsies than traditional biopsies. Using these individually superior components, we aim to keep the sensitivity to high-grade cancers stable, while improving specificity significantly. Thus, a similar number of aggressive cancers will be detected, but hopefully to a lower cost in terms of biopsies performed and un-harmful disease detected.

How do we protect the personal integrity?

Information obtained in the study will be collected in one database. The purpose of the database is to collect study data in a proper and safe way for a long time. All information about the participants will be treated with utmost confidentiality and with

strong safeguards to preserve their anonymity. Information that can be used to identify the participant (such as name, address and social security numbers) is always kept separate from other data (such as survey responses and blood tests). All questionnaire data and test results will be treated to prevent unauthorized access to them. The samples will get a unique code so that outsiders cannot identify them. The participants' samples are treated in accordance with the Swedish Biobank Act.

Everyone who works with STHLM3 are under confidentiality agreements. Results from the study are presented only as statistics in which individual answers cannot be traced.

Treatment of the personal data is in accordance with the Swedish Personal Data Act (1998:204). All participants may request in writing to find out what information about them self from which information has been collected and to whom the data has been disclosed. STHLM3's adherence to the Swedish Personal Data Act has been reviewed by the Swedish Data Inspection Board. A preliminary decision has been issues (DNR 1278-2012).

16.1. Rationale for performing a randomized design in addition to the paired STHLM3-MR and STHLM3 studies

	Strengths	Weaknesses
Randomized design **(Phase 2)**	• **Study test entire diagnostic pathway prospectively in one study** • Minimizes bias and contamination • Compares fusion bx to "existing gold standard". • Current practice biopsies performed at network urologist • Only 50% of participants undergoes MRI. • Studies screening population in contrast to population coming for biopsy in current clinical practice (selection).	• Increased participant nr. • Increased costs (STHLM3 tests, biopsies) • Screening population, i.e. lower mean PCa risk, smaller mean tumor size

17. Authors contribution

TN was the Principal Investigator. TN, HG, ME, SC and MA designed the study. ME and TN interpreted preliminary data. FJ designed MRI protocols and collected data.

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19. Protocol Addendum STHLM3MR Main Study

Introduction

This document describes clarifications and adjustments of the study protocol made after its finalization and publication (Protocol version Nordström et al. BMJ Open 2019). All listed adjustments have been approved in consensus by the trial steering committee.

Table of additions and clarifications

Date	Type	Description	Ref Protocol
2019-11-13	Sample size calculation	Decided to increase study size. Power calculation based on assumtions: participation rate (DSMB2; 22%), 13% men randomized; 20% men not following protocol; 55% MRI-negative; size increased to enable secondary contrast comparison s3M+MRI/TBx vs PSA+MRI/TBx (Additional Hypotheses 4). See SAP for details.	13.9
2020-11-27	Clarification	Clarification of wording in hypothesis descriptions.	9
20200108	Definition, inclusion	Men creating referral before 2020-01-09 are included in main analysis.	13.2
20200108	Definition, inclusion	Age is defined as age at referral creation. Men aged 50-74 are included in main analysis	13.2
20200108	Definition, intervention	Men with PSA>40 ng/ml are directly transferred for prioritized clinical workup at Capio St Görans Hospital. Intervention are at the discretion of the clinician, possibly including MRI.	13.4

		Approximately 5/10000 tested men have PSA ≥40 ng/ml	
20200108	Definition, analysis PP	PP (per protocol) analysis: Include systematic pathology report for men in standard arm. Include systematic+target report (PIRADS≥3) for men in experimental arm. Men without systematic bx report are protocol violators and not included in PP. Men in PP must have PSA and Stockholm3-result, otherwise excluded.	13.4
20200115	Definition, inclusion	Invited are defined as men that had letter sent subtracted by letters that were returned	13.10
20200115	Definition, intervention	Complete **pathological report** is defined as containing cancer/benign AND Global Gleason. For men in the experimental arm, this should hold for both pathological reports (TBx/SBx). For men with a "negative MRI" but STHLM3>25% this should hold for SBx pathological report.	13.4
20200115	Definition, intervention	**Global Gleason Score** is defined as the highest of Systematic and Targeted Bx global scores	13.4
20200115	Definition, intervention	Complete MRI is defined as existing MRI report including PIRADS score.	13.4

		Complete MRI is mandatory for inclusion of experimental arm men in the PP.	
20200115	Definition, Study population	Study population is defined as participating men. See further in Statistical Analysis Plan	13.4
2020-01-29	Definition, Study population	Participating men are defined as men with registered consent and blood sample.	13.4