

Characteristics of Screening Failures in Prostate Cancer Screening

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Front cover: *Should everyone be screened equally?* By Gunnar Aus

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Ineko

To my family

Characteristics of Screening Failures in Prostate Cancer Screening

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ABSTRACT

Although prostate-specific antigen (PSA)-based screening has been shown to reduce prostate cancer (PC)-specific mortality with large variations in mortality reduction with different screening algorithms, the optimal screening strategy has not yet been established. This thesis aims at exploring aspects of underdiagnosis in PC screening, focusing on the impact of screening failures on screening effectiveness. All of its papers are based on the Göteborg randomized PC screening trial except for Paper I, which also includes data from the Dutch center of the European Randomized Study of Screening for Prostate Cancer (ERSPC). Paper I analyzes the frequency of interval cancers (IC) between a 2- and a 4-year screening interval, as high IC rates are recognized as a limitation for screening effectiveness in screening for other cancers. Extremely few IC cases were detected and no difference was found in cumulative incidences of IC with a 2- and 4-year interval. In Paper II, the risk of PC death is compared between attendees and nonattendees in screening. A large proportion of PC deaths occurred in nonattendees, and the majority of attendees dying from PC were men aged ≥ 60 years when detected at their first (prevalence) screen. Paper III analyzes the PC incidence after screening cessation (due to upper age limit). Compared to the control arm, the incidence of potentially aggressive PC was reduced in the screening arm up to 9 years post-screening but thereafter approached the incidence of the control group. In Paper IV, multiparametric magnetic resonance imaging (mpMRI) was evaluated as a screening tool. A lowered PSA cut-off (1.8 ng/ml) + mpMRI followed by targeted biopsy yielded a higher detection rate of clinically significant PC compared with “conventional” screening (PSA, cut-off ≥ 3 ng/ml followed by systematic biopsy), requiring a decreased number of biopsies.

In conclusion, better screening strategies are needed to improve on screening failures. One option may be to lower the PSA cut-off and introduce sequential testing with mpMRI to decide which men to refer for biopsy. Age at screening start and cessation greatly impacts efficiency; starting at age 60 is probably too late, and stopping at age 70 for all men is probably too early.

Keywords: screening failures, age, prostate-specific antigen, interval cancer, non-attendees, multiparametric magnetic resonance imaging, prostate cancer screening

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SAMMANFATTNING PÅ SVENSKA

Randomiserade studier har visat att screening med prostata-specifikt antigen (PSA) minskar dödligheten i prostata cancer (PC), men effektens storlek varierar mellan olika algoritmer. Syftet med denna avhandling var att studera faktorer av betydelse för underdiagnostik i PSA-screening. Går det att optimera screening algoritmen för att minska risken för underdiagnostik av potentiellt dödlig PC?

Avhandlingen baseras på Göteborgs screening studie som startade 1995. Av alla 50-64 åriga män boende i Göteborg vid den tiden randomiserades 20,000 till studien, 10,000 till screening gruppen (inbjuden med 2 års intervall till screening med PSA) och 10,000 till kontroll gruppen (inte inbjuden). Göteborgs screening studie är en del av en stor multicenterstudie, The European Randomized Study of Screening for Prostate Cancer (ERSPC).

I första arbetet undersöktes intervallcancer (IC) förekomst vid olika screening intervall. Vi fann en låg förekomst av prostata IC och ingen signifikant skillnad i kumulativ incidens mellan ett 2- och ett 4-årigt screening-intervall. Däremot sågs en signifikant ökad risk för screening upptäckt PC med tätare screening-intervall. **I andra arbetet** studerades risk för PC bland de som deltar i screening och de som inte deltar. En stor andel (16/39) av män som dog av PC under 13 års tid hade aldrig deltagit i screening. Av de som deltog och ändå dog av PC diagnosticerade majoriteten med en avancerad cancer redan vid första screening tillfället, vid vilket alla var >60 år. **I tredje arbetet** analyserades PC-incidens och dödlighet efter att screeningen avslutats vid 69 års ålder. Screening gruppen hade en lägre incidens av hög-risk cancer i upp till 9 år efter screeningens avslut, varefter screening gruppens risk för denna typ av PC nådde samma nivå som kontroll gruppens. **I fjärde arbetet** analyserades magnetkamera (MR) undersökning av prostata som screening metod. Med tillägg av MR i screening algoritmen kunde ett stort antal biopsier undvikas (ökad specificitet) samtidigt som fler potentiellt aggressiva cancrar hittades.

Nuvarande PSA screening för PC är suboptimal och behöver förbättras. Screening programmets effektivitet avspeglas inte i frekvensen IC, som är extremt låg. Screening bör påbörjas innan 60 års ålder och tiden för screeningens avslut bör individualiseras. En intensivare screening algoritmen ger större effekt på PC-dödligheten, och MR verkar lovande för en ökad detektion av aggressiv cancer.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

- I. Robool, M, Grenabo, A, Schröder, FH, Hugosson, J. Interval Cancers in Prostate Cancer Screening: Comparing 2- and 4-Year Screening Intervals in the European Randomized Study of Screening for Prostate Cancer, Gothenburg and Rotterdam. *J Natl Cancer Inst* 2007; 99: 1296-303.
- II. Grenabo Bergdahl, A, Aus, G, Lilja, H, Hugosson, J. Risk of Dying From Prostate Cancer in Men Randomized to Screening: Differences between Attendees and Nonattendees. *Cancer* 2009; 115: 5672-9.
- III. Grenabo Bergdahl, A, Holmberg, E, Moss, S, Hugosson, J. Incidence of Prostate Cancer After Termination of Screening in a Population-Based Randomised Screening Trial. *Eur Urol.* 2013; 64: 703-9.
- IV. Grenabo Bergdahl A, Wilderäng, U, Aus, G, Carlsson, S, Damber, JE, Frånlund, M, Geterud, K, Khatami, A, Socratous, A, Stranne, J, Hellström, M, Hugosson J. Role of MRI in Prostate Cancer Screening: Results from a Pilot Study Nested Within the Göteborg Randomized Screening Trial (*in manuscript*).

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ABBREVIATIONS

AS	Active Surveillance
CT	Computer Tomography
DCE	Dynamic Contrast Enhanced (imaging)
DHT	Dihydrotestosterone
DRE	Digital Rectal Examination
DWI	Diffusion Weighted Imaging
EAU	European Association of Urology
ERSPC	European Randomized Study of Screening for Prostate Cancer
f/t PSA	Free/Total PSA Ratio
FDA	US Food and Drug Administration
GS	Gleason Score
IC	Interval Cancer
LUTS	Lower Urinary Tract Symptoms
mpMRI	Multiparametric Magnetic Resonance Imaging
MRI	Magnetic Resonance Imaging
MRSI	Magnetic Resonance Spectroscopic Imaging
NNB	Number Needed to Biopsy
NPCR	Nationella Prostata Cancer Registret
NPV	Negative Predictive Value

PC	Prostate Cancer
PCA3	Prostate Cancer Antigen 3
Phi	Prostate Health Index
PPV	Positive Predictive Value
PSA	Prostate-Specific Antigen
PSAD	PSA Density
PZ	Peripheral Zone (of the prostate)
QoL	Quality of Life
RARP	Robot Assisted Radical Prostatectomy
RCT	Randomized Controlled Trial
RP	Radical Prostatectomy
SB	Systematic Biopsy
SBU	Statens beredning för medicinsk utvärdering
T2WI	T2-Weighted Imaging
TB	Targeted Biopsy
TRUS	Transrectal Ultrasound

1 INTRODUCTION

The purpose of cancer screening is to detect cancers at an early stage when treatment is more effective to prevent cancer progression and death. Screening is a search for disease in the absence of symptoms, and one important distinction from clinical practice is that it targets apparently healthy people. This requires a systematic evaluation of all potential effects of screening before any recommendations for mass screening can be made, to ensure that the likely benefits outweigh any possible harm. Nationwide screening programs have already been implemented in parts of the developed world for cervical, breast and colorectal cancer. Studies are ongoing for prostate cancer (PC) screening and this thesis is based on one of those, the Göteborg randomized population-based PC screening trial that started in 1995.

Continual evaluation of a screening is vital to ensure that effectiveness is maintained and improved where possible. In population-based PC screening, any man dying from PC can be regarded as a failure of the screening strategy. This thesis analyzes limitations for PC screening effectiveness and explores areas of improvement.

1.1 The Prostate

The prostate is a gland in the male reproductive system located posterior to the pubic symphysis, inferior to the bladder, superior to the perineal membrane and anterior to the rectum. The prostate is in continuity with the bladder neck at the base, and then it surrounds the urethra and ends at the apex where it becomes the external urethral sphincter.

The prostate starts to develop from the urogenital sinus during the third month of fetal growth, and development is directed by dihydrotestosterone (DHT). DHT is synthesized by the conversion of fetal testosterone, through the action of the enzyme, 5α -reductase. DHT binds to the androgen receptor in the prostate and regulates growth, differentiation, and functions of the prostate. Two major cell types are present in the prostate, epithelial and stromal cells. In the normal prostate, the most common epithelial cells are secretory. These cells express PSA, acid phosphatase and androgen receptors and are rich in secretory granulae and enzymes. Secretory epithelial cells release their products into acini that are drained via ducts into the urethra(1). Together with sperm cells and fluids produced by the seminal vesicles and

bulbourethral glands, the prostate secretion makes up the semen. The prostatic secretion is thought to play a role in optimizing conditions for fertilization by increasing sperm motility and by enhancing transport in both the male and female reproductive tract.

The prostate can be divided into zones, a concept first proposed by McNeal in 1968. The peripheral zone (PZ) forms the outermost layer and constitutes the main part of the prostate tissue mass, and this is where most PC arises (about 80%)(2, 3). The central zone (CZ) is the second largest fraction of the gland and the least common site for cancer development. The transition zone (TZ) forms the innermost layer and surrounds the urethra. The TZ grows throughout life and is responsible for BPH development.

1.2 Prostate Cancer

1.2.1 Epidemiology

PC is a major public health concern. It is the most common cancer among men in Europe. Worldwide, it is the second most frequently diagnosed cancer and the sixth leading cause of cancer death in men(4). Globally, incidence rates vary largely, with the highest rates observed in the industrial world. In Europe, the highest incidence rates are observed in the northern and western Europe with an age-standardized incidence >200 per 100,000 men (using the European standard population)(5, 6). All European countries have experienced an increase in incidence during the last 20 years, and the main reason for this is the widespread PSA use for early detection of PC(7). Another reason is the steadily ageing population and the fact that PC commonly affects elderly men(8). However, the incidences have plateaued and even dropped in some Northern and Western countries, including Sweden, during recent years(6). According to the Swedish Cancer Registry¹, 9,663 and 9,678 new cases were reported in 2012 and 2013, respectively, accounting for a little over 200 per 100,000 men and corresponding to over a third of all male cancers(9). In the younger ages, the incidence rate is still increasing, and the highest incidence rate in the year 2012 was observed in the age group 65 to 69 years, compared with 75 to 79 years in the year 2000(10).

¹ A national registry was founded in 1958 where all cancers that are diagnosed in the population are registered. It is required for every health care provider to report all newly diagnosed cancers to the registry.

PC is the most common cause of cancer death among Swedish men. With a mean of 2,414 yearly deaths during the years 2008 to 2012, PC accounts for about 20% of all cancer deaths and about 5% of deaths from all causes(11, 12). A slow rise in mortality was seen from the 1970s until 2003, after which a slow decrease has been observed(13) (Figure 1). In Europe, PC is the third leading cause of cancer death after lung and colorectal cancer.

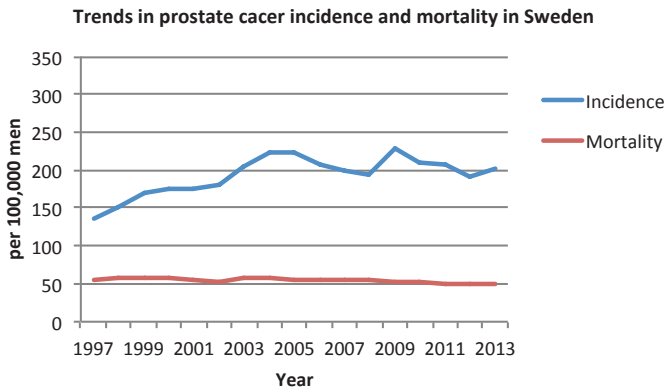


Figure 1. Incidence and mortality of prostate cancer in Sweden during the years 1997 to 2013. Graphics by A. Grenabo Bergdahl. Source: National Board of Health and Welfare, www.socialstyrelsen.se

Autopsy studies

Because incidence is influenced by the diagnostic intensity (i.e. PSA use and biopsy regimen), it does not necessarily reflect the true prevalence of PC. Autopsy studies may provide useful information on prevalence. Franks performed some of the classical work in this field back in the 1950s. These studies revealed a surprisingly large pool of indolent PC in adult men. Franks demonstrated a 31% incidence of histological PC in men >50 years of age who died of other causes(14). In a more recent study of 152 prostate glands from young men (98 African-American, 54 white) in the ages 10-49 years who died of other causes, Sakr found that PC was histologically evident in 27% and 34% among those in the ages 30-39 and 40-49 years, respectively.(15)

In a recent review, pooled data from 25 autopsy studies of men without a clinical diagnosis of PC during their lifetime or death due to PC revealed estimates of histological prevalence of 16%, 27%, and 37% in white men aged 50-59, 60-69 and 70-79 years, respectively. Higher prevalence was

reported for African-American men(16). A Hungarian autopsy study from 2005 reported prevalence estimates of 32%, 50%, and 65% in men in the ages 51-60, 61-70 and 71-80 years, respectively(3). Zlotta compared the prevalence of PC at autopsy in men who died of other causes than PC across two populations, Moscow (Caucasian) and Tokyo (Asian). The estimated prevalence was 29%, 46%, and 44% for Caucasian men in the ages 51-60, 61-70, and 71-80 years, respectively, and for Asian men it was 8%, 31%, and 44% for the same age groups(17).

The gap between observed clinical incidence and histological prevalence is of certain interest in screening because early detection narrows this gap, which raises concerns about overdiagnosis of harmless cancers that never will cause any symptoms during lifetime (“latent” cancers). Present tools for early detection of PC lack the ability to discriminate between the clinically significant cancers, that might lead to death if left untreated, and “latent” PC that are better left undetected.

Cystoprostatectomy studies

Another way to estimate prevalence is to examine specimens from men undergoing cystoprostatectomy for bladder cancer. It has been estimated that 25-40% of these men also have PC(18). In a landmark study from 1993, Stamey et al. found that PC was prevalent in 40% (55 of 139) of cystoprostatectomy specimen. The authors further explored the association between the lifetime risk of being diagnosed with clinically significant PC (8% at the time according to calculations from the Surveillance, Epidemiology, and End Results (SEER) Program of the National Cancer Institute) and the size of PC present in these cystoprostatectomy specimens. Based on the assumption that volume and tumor progression are correlated, they identified the 8% (11 of 55) of tumors with the largest volume (ranging from 0.5-6.1 ml). The investigators concluded the cancers with volumes of 0.5 ml or greater were clinically significant, a volume that included 20% of all PC in their series. Hence, “latent PC” was nothing more than a tumor smaller than 0.5 ml, according to this reasoning(19). More recently, associations between urothelial and prostate carcinoma have been suggested, making PC prevalence estimates drawn from cystoprostatectomy studies less reliable(20).

1.2.2 The natural course of prostate cancer

Knowledge of the natural course of PC is needed to understand the epidemiology but also to get an indication of prognosis once a tumor is diagnosed. Screening advances diagnosis and causes a stage-shift, which increases number of small organ-confined cancers. Localized cancers generally have a good prognosis even though they might become aggressive in the long term. Johansson et al. performed a population-based cohort study on men with early, initially untreated cancers (T0-T2, NX, M0, see Chapter 1.3) that had been clinically diagnosed between the years 1977 and 1984. After 15 years of follow-up, the reported PC-specific mortality for well-differentiated tumors was low, at 6%, whereas cancers with intermediate and poor differentiation had a worse prognosis (11% and 56% PC-specific mortality, respectively)(21). However, in a recent report with longer follow-up, it was shown that even the well-differentiated tumors (T0-T1 and WHO grade 1) continued to progress, and after 25 years, the PC-specific mortality was around 50% for these cancers(22). Another well-known study on the natural history of PC is Albertsen's retrospective cohort study, based on the Connecticut Tumor Registry of men diagnosed between the years 1971 and 1984. Among 767 men in the ages 55 to 74 years, PC-specific mortality was 42-70% for Gleason score (GS) 7 cancers and 60-87% for GS 8-10 after 15 years(23). For further description of grading in PC using Gleason score, see Chapter 1.3.

PSA-detected cancers

The Johansson and Albertsen studies recruited men before the PSA-era and thus report on the natural course for clinically detected PC. The knowledge of the natural course of PSA-detected cancers is still insufficient although studies on stored sera have showed that serum PSA levels are elevated years before the clinical diagnosis, adding to survival time for screen-detected cancers. Gann estimated a mean lead-time² for PC of 5.5 years using stored sera from the U.S. Physicians Health Study to determine PSA levels prior to diagnosis(24). Hugosson evaluated PSA in stored sera drawn from Swedish men 67 years of age in 1980 (epidemiological cohort of men born in 1930) and analyzed prognosis in those who subsequently developed clinical PC based on their PSA-level in 1980. The lead-time was calculated to around 10 years and PC-specific mortality to about 50% in 15 years for men with an initial PSA of <10 ng/ml(25).

² The amount of time by which the diagnosis has been advanced by screening is usually referred to as lead-time (see chapter 1.6.3). Lead-time may cause an artificial addition to the survival time of screen-detected cancers.

1.2.3 Risk stratification

Risk stratification is useful in trials and in the clinical practice to stratify patients based on prognostic factors. Depending on the risk group, different treatment options are available. Currently there are more than 100 risk-assessment tools for PC(26). One validated and commonly used system is the one developed by D’Amico and colleagues(27, 28). Originally developed to evaluate the risk of biochemical recurrence after radical prostatectomy (RP), cancers are stratified into low, intermediate and high-risk cancers based on PSA level, GS and T-stage (Table 1). One limitation of this classification system is that it does not account for multiple risk factors. For instance, a patient with PSA of 20 ng/ml, GS 4+3=7, and clinical stage T2b is classified as intermediate risk, but so is also a patient with serum PSA 2.0 ng/ml, GS 3+4=7 and clinical stage T1c. In addition, the D’Amico system does not encounter cancer extent or volume.

Table 1. D’Amico risk group. Obtained from D’Amico et al., Predicting prostate specific antigen outcome preoperatively in the prostate specific antigen era, J Urol 2001 Dec;166(6):2185-8.

Low risk	Intermediate risk	High risk
PSA ≤10 ng/ml, and GS 6, and T1c-T2a	PSA >10 - ≤20 ng/ml, or GS 7, or T2b	PSA >20 ng/ml, or GS ≥8, or T2c, or N1/M1

In 1994, Epstein formed a set of criteria to predict the presence of insignificant PC that never would metastasize or lead to death, which therefore made them suitable for active surveillance (AS) (see Chapter 1.7). Insignificant tumors fulfilling the Epstein criteria were tumors with the following characteristics: clinical stage T1c, PSA density (PSAD) <0.15, biopsy GS 6, presence of PC in fewer than 3 of the 6 cores obtained at biopsy, and ≤50% cancer involvement in any single core(29).

The concept was validated in a more recent series based on men undergoing RP (RP) during the years 2000-2003 at the Johns Hopkins Hospital in Baltimore. By using the Epstein criteria, as much as 91.6% of the 237 cases examined were correctly classified as organ-confined. However, the authors highlighted that age and health status were important factors to consider in addition to stage and grade, since insignificant cancers in healthy 50-year-olds very well may become significant some time later in life. Active treatment of insignificant cancers thus may be warranted in men with long

life expectancies(30).

More recently, the validity of the Epstein criteria has been questioned, especially since sampling of the prostate for histopathological evaluation has changed from sextant biopsy to more extensive biopsy protocols since the end of the 1990s, as will be explored in Chapter 1.3. Changes in sampling methods as well as in pathologists' reportings of Gleason grades (see paragraph 1.3.1) led to the development of the modified Epstein criteria, which is the same as the original except that bilateral cancer is substituted for >50% maximal involvement of a core(31).

Another approach to risk assessment is to incorporate multiple variables into mathematical models to predict the likelihood of recurrence, progression and similar outcome measures. These models are often referred to as nomograms, and several validated online options are available for clinical use(26). The utility of nomograms and risk calculators in population-based screening is limited because the models do not provide exact guidance as to what level of risk should prompt a biopsy. Hence, the results need to be interpreted in each individual case. A recent meta-analysis assessed the performance of existing risk calculators in predicting PC risk but concluded that many of them are still poorly validated and that further studies are needed to be able to make rigorous head-to-head comparisons of the most promising model for predicting risk of significant PC(32). Regardless, nomograms and risk calculators are considered useful in understanding risks and communicating it to patients.

1.3 Diagnosis of prostate cancer

Historically, the diagnosis of PC was based on palpable abnormalities at digital rectal examination (DRE). However, in recent decades, a marked shift has been observed towards earlier detection as a result of widespread PSA-use. Early PC rarely causes symptoms such as lower urinary tract symptoms (LUTS) because most cancers originate in the PZ, far from the urethra. Systematic symptoms including bone pain from skeletal metastases, urinary obstructive symptoms, renal failure and anemia, indicate an advanced tumor stage with distant metastases. Early detection, before symptoms arise, is therefor crucial for increasing the chance of cure.

1.3.1 Tools for aiding in the detection of prostate cancer

Digital rectal examination

The oldest and least invasive tool for early detection of PC is DRE. However, since PSA was introduced, its role has decreased. DRE can detect cancers in the posterior and lateral parts of the prostate, but it is subjective and may be normal, even in men with advanced disease. Studies specifically aimed at determining the value of DRE for the detection of PC are rare. A meta-analysis from 1999 on DRE performance in detecting PC estimated a sensitivity of 59% and a specificity of 94%. The positive predictive value (PPV) of an abnormal DRE was estimated to be 28%(33). DRE and PSA in conjunction have been evaluated, and their combined use can increase the overall cancer detection rate(34, 35). The 1994 study by Catalonia(35) involved 6,630 male volunteers aged 50 years or older, all undergoing PSA and DRE, and the reported cancer detection rate was 3.2% for DRE, 4.6% for PSA, and 5.8% for the two methods combined(35). Another study evaluating the combination of DRE and PSA was performed in the Dutch branch of the ERSPC. That study found that the PPV of a suspicious DRE in conjunction with PSA ≥ 3 ng/ml for the detection of PC was 49% compared to 22% for men with a normal DRE. In addition, an abnormal DRE was associated with an increased risk of GS>7 cancers(36). The Dutch ERSPC branch also reported a strong relationship between DRE and PSA, with enhanced sensitivity of DRE as PSA values increased(37, 38).

Although these data suggest a benefit of combining PSA and DRE, it has not been confirmed by randomized studies of PC outcomes. The ERSPC did not consistently require a DRE, and the Prostate, Lung, Colorectal, Ovarian Cancer Screening Trial (PLCO)³ found no beneficial outcome on PC mortality by using PSA and DRE(39, 40). Nevertheless, DRE is included in the urologic work-up following a suspicious PSA measurement and may be useful in differentiating between other non-cancerous conditions of the prostate such as inflammatory states.

Prostate-specific antigen

PSA is a serine protease and a member of the kallikrein family. It is expressed by the prostatic luminal epithelial cells and released into seminal fluid, where it plays a role in liquefying the semen following ejaculation.

³ A large, American, population-based, randomized trial initiated in 1993 to determine the effects of screening on cancer-related mortality and secondary endpoints in men and women aged 55 to 74 years.

Normally, only small proportions of PSA leak out into the serum, but elevated levels can be measured in conditions like PC, infection, inflammation and BPH. PSA that enters the circulation is immediately bound to protease inhibitors, mainly alpha-1 antichymotrypsin although a fraction is inactivated in serum by proteolysis and circulates as free PSA(41). Two main isoforms of PSA are normally measured in serum: free and bound PSA. Total PSA in serum is the sum of these two isoforms. The ratio of free PSA is measured as free PSA / total PSA (f/t PSA) and could be used for diagnostic purposes. The bound form is predominantly present in cancer patients, leading to a decreased ratio f/t PSA, while free PSA is higher in men with BPH, resulting in a higher f/t PSA.

The detection of PSA

PSA has revolutionized the diagnosis of PC and is considered the most effective test currently widely available for early PC detection. The discovery of PSA was a result of several researchers' work during the 1960s and 1970s, when antigens of the semen and prostate were explored. The original work was carried out to study the association between seminal proteins and infertility but also to find specific proteins that could be used for forensic purposes(42). In 1979, Wang was the first to purify PSA from prostatic tissue(43). In a landmark study from 1987, Stamey demonstrated that PSA levels increased with advancing tumor stages and that PSA was a better tumor marker than prostatic acid phosphatase, which had previously been used(44). In 1986, the Food and Drug Administration (FDA)⁴ approved PSA as a tool for monitoring disease status, and in 1994 PSA was accepted for aiding in the detection of PC in men aged 50 years and older(45). The widespread use in clinical oncology began during the 1990s.

Clinical use of PSA

As already mentioned, incidence rates have increased in large parts of the world since the advent of PSA. According to the National Prostate Cancer Register (NPCR), which captures 98% of PC cases in the Swedish Cancer Register(46), the proportion of men diagnosed as a result of screening increased from 29% in 2004 to 49% in 2013. However, figures from 2013 vary largely by region and hospital, reflected in part by different attitudes towards screening among different geographical regions in Sweden(47). The remaining 51% of PCs diagnosed during 2013 were detected due to LUTS

⁴ A federal agency within the U.S. Department of Health and Human Services that is responsible for protecting and promoting public health through regulation and supervision of drugs, vaccines, and other biological products and medical devices.

(30%), other symptoms (18%) and unknown reasons (2%). The median PSA value at diagnosis decreased from 23 ng/ml in 1998 to 8.4 ng/ml in 2013, also indicating a shift towards earlier stages at diagnosis during recent years(48).

Transrectal ultrasound and prostate biopsies

TRUS-guided SB under local anesthesia is the standard diagnostic modality in men with suspected PC(49). TRUS-guided sextant biopsy was introduced in 1989 and originally termed random systematic TRUS-guided biopsy(50). Before that, biopsy was performed through the perineum or transrectally using digital direction ad modum Franzén(51). In 1995, Stamey suggested that the TRUS-guided sextant biopsies would be moved more laterally to better cover the anterior horns of the PZ(52). Later it was shown that a more extensive sampling using 10 to 12 cores increased the cancer yield further (with about 30%), adding laterally directed cores to the standard 6 cores. Today, most urologists have abandoned the sextant biopsy in favor of these more extensive sampling methods(53). Other approaches, including transperineal prostate biopsy, are used under special circumstances with ultrasound, computer tomography (CT) or magnetic resonance imaging (MRI) guidance.

1.3.2 Staging

The stage of PC is determined by the size and extent of local growth and whether it has spread to lymph nodes or to distant organs. The stage is classified according to the Tumor, Node, and Metastasis (TNM) system(54). The T-stage is established by DRE. Non-palpable tumors are referred to as T1, palpable tumors considered confined to the prostate as T2, tumors penetrating through the capsule as T3 and tumors penetrating into adjacent organs as T4 (Table 2). After surgical removal of the prostate, the pathological stage is evaluated based on the histological findings (pT1-4). To determine N-stage, CT and/or MRI can be used. N-stage can also be determined after surgical excision of regional lymph nodes. M-stage has traditionally been assessed by bone-scan but is more and more often being replaced by MRI of the vertebral column and pelvis.

Table 2. Tumor, Node, Metastasis (TNM) stage definitions for prostate cancer (7th edition, 2009). Adapted from Sobin LG, Gospodarowicz MK, Wittekind, C. TNM Classification of Malignant Tumors, 7th Edition. Oxford, UK: Wiley-Blackwell; 2009.

T - Primary tumor	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
T1	Clinically unapparent tumor not palpable or visible by imaging
T1a	Tumor incidental histological finding in 5% or less of tissue resected
T1b	Tumor incidental histological finding in more than 5% of tissue resected
T1c	Tumor identified by needle biopsy (e.g. because of elevated PSA)
T2	Tumor confined within the prostate ¹
T2a	Tumor involves one half of one lobe or less
T2b	Tumor involves more than half of one lobe, but not both lobes
T2c	Tumor involves both lobes
T3	Tumor extends through the prostatic capsule ²
T3a	Extracapsular extension (unilateral or bilateral) including microscopic bladder neck involvement
T3b	Tumor invades seminal vesicle(s)
T4	Tumor is fixed or invades adjacent structures other than seminal vesicles: external sphincter, rectum, levator muscles, and/or pelvic wall
N - Regional lymph nodes ³	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Regional lymph node metastasis
M - Distant metastasis ⁴	
MX	Distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis
M1a	Non-regional lymph node(s)
M1b	Bone(s)
M1c	Other site(s)

¹ Tumor found in one or both lobes by needle biopsy but not palpable or visible by imaging is classified as T1c.

² Invasion into the prostatic apex or into (but not beyond) the prostate capsule is not classified as pT3, but as pT2.

³ Metastasis no larger than 0.2 cm can be designated as pN1 mi.

⁴ When more than one site of metastasis is present, the most advanced category should be used.

1.3.3 Grading

The histopathological differentiation defines the grade of the disease and is evaluated based on resected tissue. Grading of PC is performed by using the Gleason grading system, initially presented in 1966 by Donald F. Gleason (1920-2008). The system is solely based on the architectural pattern of the tumor. These patterns are divided into 5 different grades ranging from 1 to 5, where the highest grade is the most dedifferentiated. The grade of the cancer used to be defined as the sum of the two most common grade patterns and reported as the Gleason score (GS) or Gleason sum. If there is only one histological grade pattern, the primary (predominant) and the secondary (second most prevalent) are given the same number.

In 2005 however, the International Society of Urological Pathology (ISUP) organized a consensus conference to update the Gleason grading system and to standardize both the perception of histological patterns and the reporting of grades. For core needle biopsies, it was agreed that the GS should be the sum of 1) the most common pattern and 2) the highest-grade pattern even if the amount is minute. It was also decided that Gleason grades 1 to 2 rarely, if ever, should be used on needle biopsy tissue. In practice this means that all PC diagnosed today are GS 6 to 10, based on biopsy material(55). In prostatectomy specimens, grades are evaluated according to the same scaling system, but the score is the sum of the primary (predominant) pattern and the secondary (second most prevalent) pattern. In addition, any presence of smaller foci of higher grades is mentioned as tertiary grades but is not included in the GS(56).

1.4 Shortcomings of today's diagnostics

1.4.1 Limitations of PSA

High PSA values are predictive of PC, and an elevated PSA can precede clinical PC by 5-10 years(24, 57). However, PSA is not cancer-specific. It is strongly influenced by androgens and age. At puberty, when testosterone peaks, PSA becomes detectable in serum, and thereafter it increases with age(58). PSA also varies with volume, race, and can be elevated by non-cancerous conditions of the prostate such as benign prostate hyperplasia (BPH), inflammation and infection. These are all limitations of PSA as a screening test for early detection of PC.

Cut-off for biopsy and test performance

According to the Prostate Cancer Prevention (PCPT) Trial⁵, in which all men at all PSA levels underwent a biopsy, PSA should be considered as a continuum of PC risk at all levels(59). This means that PSA ideally should be evaluated together with age, co-morbidity, presence of symptoms, and patient preferences before recommendations for further urologic work-up should be made. In mass screening, however, an individual approach to each PSA measurement usually is difficult to maintain. Therefore, PSA cut-offs are useful, but establishing a general threshold for biopsy is controversial. A value of 4.0 ng/ml has been commonly used, but the sensitivity and specificity at this cut-off is fairly low, 20.5%, and 93.8% respectively, according the PCPT trial(60). Lowering the cut-off for biopsy increases sensitivity, but at a cost of reduced specificity.

Several factors influence sensitivity estimates, including PSA cut-off, population characteristics, background prevalence, biopsy strategy, and the reference test used to confirm the results of biopsy. The American Cancer Society (ACS) reviewed the literature on test performance of PSA and included prospective studies of PC screening that used either 3.0 or 4.0 ng/ml as cut-offs. Pooled estimates of sensitivity and specificity of 32% and 85% with a cut-off of 3.0 ng/ml were reported, plus 21% and 91% with a cut-off of 4.0 ng/ml. The sensitivity for GS \geq 8 PC was 68% with cut-off 3.0 ng/ml, compared to 51% with 4.0 ng/ml. Eighteen percent had a positive screening test (“elevated PSA”) with a cut-off of 3.0 ng/ml, compared to 12% with a cut-off of 4.0 ng/ml. Clearly, there are pros and cons with each of these two thresholds, with more men requiring a biopsy when the cut-off is lowered, but with improved detection of high-grade PC(61).

In the Göteborg randomized screening trial, a PSA cut-off of 3.0 ng/ml was used for biopsy (corresponding to a PSA of 2.54 ng/ml if calibrated to WHO standardization). Out of all men with PSA \geq 3.0 ng/ml, about 25% had cancer at TRUS-guided systematic biopsy (SB), corresponding to the positive predictive value (PPV) of PSA at this cut-off(62). According to the ACS review referred to previously, PPV decreased from 30% with a cut-off of 4.0 ng/ml, to 28% with a cut-off of 3.0 ng/ml, which is similar to the Göteborg results. The consequence of a low PPV at 25% is that a large proportion of men are biopsied unnecessarily whenever PSA is used as a sole biopsy indicator.

⁵ A randomized, placebo-controlled study designed to determine whether Finasterid (a 5- α reductase inhibitor) could prevent PC in men 55 years of age or older.

1.4.2 Limitations of systematic biopsy

Risks with the procedure

Even the diagnostic test used for verifying or ruling out disease has shortcomings. Although generally well tolerated, TRUS-guided biopsy is an invasive procedure and should be restricted only to a defined subset of men. Complications of biopsy include bleeding (hematuria, hematospermia, hematochezia or rectal bleeding) and infectious complications (sometimes serious enough to require hospitalization). To reduce the risk of infectious complications, all men receive antibiotic prophylaxis at biopsy, and fluoroquinolones are most often used. According to a recent review, the risk of sepsis after biopsy ranges from 0-6.3% (depending on varying definitions between studies included in the review)(63). In a Swedish population-based study of men undergoing prostate biopsy from 2006 to 2011, 6% were prescribed a urinary tract antibiotic within 30 days after biopsy, and 1% were hospitalized with infection. An increased risk of hospitalization was reported during the 5-year study period (OR 2.14, 95% CI=1.55-2.94) (64). Increased rates of infectious complications have also been observed in other parts of the world during recent years (65, 66), probably due to the increasing problem with fluoroquinolone-resistant E-coli strains(67).

Over- and undersampling

PC is the only solid-organ tumor diagnosed by a non-targeted systematic sampling. In the standard biopsy it is mainly the PZ that is sampled with the risk of missing anterior and apex cancers that the needles cannot reach. Villers et al. have shown that 20% of all PC are located anteriorly and will in almost half of the cases be missed by standard TRUS-guided biopsies(68). At the same time, overdiagnosis occurs because the needles frequently hit the small, indolent tumors present in about 30-60% of men aged 50-70 years (see paragraph 1.2.1).

A prostate biopsy may be performed in several different ways. It is difficult to estimate the diagnostic accuracy of specific biopsy regimens because men with negative biopsies do not undergo RP. Nevertheless, several studies have compared biopsy results with histopathological prostatectomy results, to evaluate the accuracy of different biopsy regimens. One study reported that 28% of significant PC (≥ 0.5 ml or GS ≥ 7) were not detected by the traditional sextant method according to the RP specimen(69). Haas et al. performed needle biopsies on autopsy prostates from men with no history of PC and compared cancer yield between different biopsy strategies. In 164

men, 47 cancers were found, of which 20 were clinically significant (≥ 0.5 cm³, or GS ≥ 7 , or >1 tumor focus). The sensitivity of sextant biopsy (of the mid-PZ) for detecting clinically significant cancer was 55% (95% CI=32-77). When another 6 cores towards the lateral PZ were added, sensitivity increased to 80% (95% CI=56-94) (70).

Another approach to measuring false negatives at TRUS-guided SB is to evaluate PC incidence on repeat biopsies in men with an initial negative biopsy. Such studies report that the original sextant method missed about 30% of cancers(71, 72). It may be wrong to interpret all cancers found at repeat biopsy as missed at the initial biopsy because size progression may have occurred (depending on the time elapsed between two biopsy sessions). One study addressed this problem by studying the incidence of false-negative sextant biopsies in men undergoing RP, who participated in a randomized trial in which all subjects were biopsied twice before enrollment in the trial. Although all subjects had a biopsy-proven PC at the initial biopsy (inclusion criteria for enrollment), 23% of cancers were missed at the repeat biopsy(73). Hence, undersampling is substantial with today's sampling techniques and a major limitation for diagnostic accuracy.

Poor concordance between biopsy and prostatectomy Gleason grades

Since all clinical decisions are based on the diagnostic biopsy, a representative sample and correct classification is essential. Several studies have compared the concordance of biopsy GS and the final GS of prostatectomy specimens. Noguchi reported in 2001 that of 222 men diagnosed with T1c PC using a mean of 6.4 biopsy cores, only 36% of those with a Gleason grade of 4 or 5 were correctly classified on biopsy when compared to prostatectomy Gleason grades; 46% were underestimated and 18% were overestimated(74). More recent studies have shown better concordance with extended biopsy protocols, though the underestimation of grades still occurs in about 35-38% of biopsies(75, 76).

1.4.3 Addressing the shortcomings

Improving the screening test

Several strategies have been proposed to increase the diagnostic performance of PSA. New biomarkers for early detection have emerged, showing promising potential to detect and differentiate between potentially aggressive and insignificant PC. Advances in biomarkers as well as imaging will most likely play a role in the future screening and early diagnosis of PC.

PSA derivatives

Serum PSA levels overlap in men with BPH and those with cancer, especially in the PSA range of 3-10 ng/ml(77). *PSA density (PSAD)* is a concept that relates PSA to prostate volume (measured at TRUS or other imaging) and has been suggested as a tool to differentiate BPH from cancer. A higher density (>0.15 ng/ml/cm³) is suggestive of PC, but the method is limited by the inaccuracy of volume measurements and logistical problems that make it impractical for screening purposes.

Another measure proposed as a tool to differentiate cancer from BPH is *PSA velocity*, i.e. the rate of change in PSA over time. PSA velocity is an absolute measure, defined as the annual increase in PSA (ng/ml/year). Carter originally described the concept in 1993 using stored sera from the Baltimore Longitudinal Study of Ageing to measure PSA accelerations. A significant association was observed between a high PSA velocity and later development of PC(78). A minimum of three consecutive PSA measurements was required according to the original definition, drawn during at least 18 months. Later studies have reported contradictory results on the usefulness of PSA velocity, partly because different definitions have been used (for instance, only two measurements during one year), which has diluted the concept. In summary, PSA velocity adds little predictive information to PSA alone in screening for PC(79).

A third concept, the *f/t PSA* ratio, may be used to distinguish BPH from cancer, especially in the “grey zone” of PSA levels, between 2 to 10 ng/ml(80, 81). Lower values of f/t PSA are associated with a higher likelihood of cancer, but the optimal cut-off is uncertain. The f/t PSA may be valuable in risk stratification(82), but its role in screening is unclear.

Prostate cancer antigen 3, PCA3

PCA3, also called *differential display clone 3 (DD3)*, was originally described by Marion Bussemakers in 1999 and was shown to be highly overexpressed in PC(65). PCA3 is a “house-keeping gene” that expresses non-coding mRNA that can be detected in urine following prostate massage, and the levels have been shown to increase up to 100 times in cancer tissue compared with normal prostatic tissue. PCA3 is approved by the FDA for aiding in the decision regarding repeat biopsy in men with one or more previous negative biopsies(83). There is also evidence of an association between PCA3 and clinical and pathological features such as Gleason grade and positive surgical margins(84). Although several studies have

demonstrated the usefulness of PCA3 (high specificity, not volume-dependent), the published estimates of sensitivity and specificity display large variations, and there is no consensus regarding the optimal cut-off (84-86). Further studies are needed to establish the role of PCA3.

[-2]Pro PSA and Prostate health index, Phi

Pro PSA is an inactive precursor of PSA that is cleaved by several proteases, for instance, kallikrein-related peptidase 2 (hK2), to form the mature PSA. One form of pro PSA is the [-2]pro PSA, which has been shown to be more cancer-specific than PSA. Phi is another new test that actually is a mathematical formula of 3 biomarkers ($[-2]pro\ PSA/fPSA \times \sqrt{total\ PSA}$). These biomarkers are still not fully evaluated but have shown promising potential both for detecting PC and differentiating between aggressive and indolent PC. A recent meta-analysis reported pooled estimates of sensitivity and specificity for [-2]pro PSA of 0.86 (95% CI=0.84-0.87) and 0.40 (95% CI=0.39-0.42), and for Phi, 0.85 (95% CI=0.83-0.86) and 0.45 (95% CI=0.44-0.47)(87). Increasing evidence indicates that Phi is a significantly stronger predictor of PC than PSA(88) and superior to f/tPSA in predicting PC in the “grey zone” of PSA levels(89, 90).

4 kallikrein panel, 4K

Another new biomarker is the 4K panel, or 4K score, which is a panel of 4 kallikreins (total PSA, fPSA, intact PSA, and kallikrein-related peptide 2, hK2) combined to generate a score. 4K is currently undergoing validation, but recent studies indicate that the 4K score can be useful in differentiating between insignificant and aggressive PC and in reducing the number of unnecessary biopsies(91, 92). A recent study based on the STHLM2⁶ cohort found that 4K, with a cut-off for biopsy at 10%, predicted risk of high-grade cancer and reduced the number of men undergoing biopsy by 29% at a cost of missing 10% of high-grade cancers, and it was reported to perform similarly to Phi, at a cut-off of 39(72).

Genetic markers

Although a positive family history is one of the strongest risk factors for developing PC, no specific genes underlying the disease have been identified. However, several alleles have been associated with an increased susceptibility to PC. There are now almost 100 single nucleotide

⁶A Swedish population-based study where serum samples were collected from almost 25,000 men in the Stockholm region who had a PSA-test during the years 2010 to 2012.

polymorphisms (SNPs) that are associated with risk for PC(93) and it has been demonstrated that a combined genetic risk score based on SNPs can be used to identify men at an increased risk for harboring PC even in the lower PSA ranges (1-3 ng/ml)(94). Presence of BRCA1 and BRCA2 mutations has also been shown to increase the risk of developing PC(95). Ongoing studies will hopefully shed light on the potential role of genetic markers for improving sensitivity and specificity in screening for PC.

Age- and race-specific reference ranges for PSA

Since PSA varies with age(96) and race(97), reference ranges adjusted for these factors have been proposed. According to the most recent National Health Care Program for Prostate Cancer (Nationellt vårdprogram för prostate cancer), age-specific reference ranges were proposed for use in clinical practice (<50 years: 2.0-2.9 ng/ml, 50-70 years: ≥ 3 ng/ml, 70-80 years: ≥ 7 ng/ml)(98). The National Health Care Program for Prostate Cancer follows the recommendations from the National Board of Health and Welfare in Sweden regarding PC management.

A couple of studies have evaluated the impact of age-specific reference ranges in PSA-based screening. Bangma performed a simulation study in 1995 and showed that 37% fewer (sextant) biopsies would be needed with age-specific reference ranges, but at a cost of 12% loss in sensitivity. Another study by El-Galley et al. reported similar findings, though less pronounced, in men ≥ 60 years referred for urological work-up due to a suspicious PSA or DRE. In that study, age-specific reference ranges would have decreased the number of biopsy referrals by 12% compared to using the normal reference range of <4 ng/ml, but at a cost of missing 2.5% cancers(99). Consequently, age-adjusted PSA reference ranges mainly aims at reducing overdiagnosis and unnecessary biopsies. Underdiagnosis, however, may not be resolved by age adjustments. In addition, about 1% of cancers are not PSA-producing at all, which is worth noticing(100).

Improving sampling

To resolve the issue of sampling errors with today's SB, some have advocated the use of template-guided mapping biopsy, where cores are obtained at 5 mm intervals throughout the prostate(101). A template-guided mapping biopsy is performed transperineally under the guidance of a brachytherapy template. Although this method increases the cancer yield, it requires anesthesia and increases the risk of post-biopsy complications and is therefore not widely available(102, 103). "Saturation biopsy" is a more investigational method, where >20 cores are sampled transrectally with an

improved anterior coverage. Apart from increasing the number of cores, a repeat biopsy at a second visit is another way to improve coverage. Ideal numbers of cores and timing of repeat biopsy is debatable(104). The European Association of Urology (EAU) guidelines recommend that a repeat biopsy should be performed in men who have negative first biopsies but persistent suspicions of PC(49). The cancer yield increases with age and time since the first biopsy(105).

Even with increased sampling there is a risk that the sampling is not representative. Therefore, instead of targeting the whole organ over and over again, a more strategic approach aims at targeting the lesion once and for all. This seems logical and efficient, saving biopsies while reducing the risk of infectious complications (which in turn reduces the consumption of antibiotics). The most promising methods currently available to visualize and target lesions will be further reviewed in the next chapter.

1.5 Imaging in prostate cancer diagnosis

1.5.1 TRUS

TRUS has become every urologist's tool in evaluating patients with prostate problems, and its importance cannot be underestimated. However, this method has some shortcomings. PC is typically characterized as foci of low echogenicity (hypoechoic), located in the PZ. However, all malignant foci are not seen on TRUS, as some are isoechoic or only slightly hypoechoic as compared to the normal PZ(106). Therefore, standard greyscale TRUS has a PPV of a biopsy targeted at a hypoechoic lesion in the PZ of only 25-30%(107).

Contrast-enhanced ultrasound, CE-TRUS

CE-TRUS is a novel method that can enhance the visualization of perfusion changes related to cancer. The contrast agents administered intravenously are made up of microbubbles with specific ligand molecules that bind to receptor targets that are upregulated in angiogenesis (present in cancerous tissue). However, these receptors can also be upregulated in prostatitis, yielding false positive signals. A recent meta-analysis reported on test performance of CE-TRUS in detecting PC and the pooled sensitivity and specificity estimates were 70% and 74% (in more than 2,500 patients pooled). The authors concluded that CE-TRUS is a promising tool but that it should not be used as sole biopsy guidance and cannot completely replace SB at this point(108).

Elastography

Pathological changes such as cancer generally affect the stiffness of the tissue. Elastography is a recently developed ultrasound method that evaluates the elasticity, the “stiffness,” of the tissue being examined. There are several techniques under development. The principle of strain elastography is to apply slight pressure on the examined organ with the ultrasound probe. The elasticity and deformation of the tissue following this pressure is processed and computer analyzed, and the result is reported in real time as a color map called elastogram. Different elasticity scores are coded with different colors. Color-scaled elastograms can be lapped over the grey-scale ultrasound images and allow for analysis of visible lesions and to guide biopsy needles(109).

There is also another technique that does not require compression of the rectal wall (reducing inter-observer variability), called shear-wave elastography. Elastography has been demonstrated to increase sensitivity and NPV compared with standard TRUS and SB and has been suggested as a tool to avoid unnecessary biopsies(110, 111). Much research has been done in recent years on different ultrasound modalities, which has given the technology a multiparametric character. These emerging technologies are very promising but need to be further validated and standardized(112). The high diagnostic accuracy with MRI will be discussed in the next chapter, but one clear advantage with ultrasound technology is the accessibility for office-based urology and the easy-to-interpret images for a TRUS-experienced urologist. It is also possible that lesion targeting at biopsy is facilitated with the real-time TRUS-approach (at least compared with cognitive targeting, using the MRI image as map, without fusion technology). Combinations of techniques are also possible, as one modality does not exclude another.

1.5.2 MRI

MRI technology has become increasingly valuable in the imaging of PC and is an emerging technique for detecting and classifying PC. Several sequences are available with MRI but what seems to be the best approach for PC is a combination of T2-weighted imaging (T2W), mainly evaluating anatomy, and at least two additional functional techniques, including dynamic contrast enhanced (DCE), diffusion weighted (DWI) or MR spectroscopic imaging (MRSI). This combination is usually referred to as multiparametric MRI (mpMRI)(113).

T2-weighted imaging, T2W

T2W gives a picture of the anatomy of the prostate but is not sensitive enough to detect PC alone because benign conditions of the prostate (BPH, prostatitis, hemorrhage, scarring, atrophy) and changes following hormonal and radiation therapy can mimic tumor on T2W. Instead, T2W should be interpreted together with functional techniques for optimal detection of PC.

Diffusion-weighted imaging, DWI

DWI examines the diffusivity of water molecules, which is inversely related to the density of the cellular microenvironment. Owing to a high cellular density, cancers typically exhibit restricted diffusion and appear hyperintense on DWI corresponding to a low Apparent Diffusion Coefficient (ADC). ADC is a biomarker for diffusion and represents the net displacement among water molecules (mm^2/s). The ADC value is lower in PC lesions than in the normal central gland, PZ, prostate cysts and BPH(114). In addition, the ADC of a suspicious lesion has been shown to be inversely related to the Gleason grade and can help in differentiating between low-risk, intermediate-risk, and high-risk tumors in the PZ(115-118). DWI along with T2W are therefore particularly useful in differentiating cancer from benign abnormalities (i.e. postbiopsy hemorrhage, BPH, prostatitis), and detecting extra prostatic tumor growth (119).

Dynamic contrast enhanced imaging, DCE

DCE examines the dynamic distribution of the intravenously administered contrast agent between the tissue and blood pool. Due to tumor angiogenesis, the dynamics of cancer tissue differ from that of normal gland tissue. Typical signs of cancer are more intense tumor enhancement and earlier contrast washout compared with the normal prostate tissue(119).

Magnetic resonance spectroscopic imaging, MRSI

MRSI examines the metabolic and biochemical environment of the tissue. The spatial distributions of the metabolites choline, creatine, polyamines and citrate are assessed. Specifically, the ratio of choline + creatine over citrate is used as a tumor marker, with higher ratios seen in PC. MRSI offers the potential for determining tumor aggressiveness, and its performance is comparable to that of ADC values attained through DWI even though the two methods have somewhat different performance results in different

regions of the prostate. ADC values perform better in the PZ, whereas MRSI (choline + creatine /citrate ratio) does better in the TZ(116).

The role of MRI in the diagnosis of PC

Increasing evidence suggest that MRI has an important role in detecting PC and classifying PC. Somford studied men with Gleason 3+3 PC who underwent MRI before RP and compared the accuracy of ADC in predicting high-grade PC. According to the prostatectomy evaluation, 48% of cancers classified as Gleason 3+3 had a Gleason 4 or 5 component that was missed by the diagnostic TRUS-guided biopsy. The diagnostic accuracy of ADC as a marker for discriminating the undergraded from those with “true” Gleason 3+3 cancers was strong, with an AUC of 0.88 (95% CI=0.64-1.00), compared with 0.58 (95% CI=0.32-0.83) for PSA in discriminating patients into these 2 groups(120). In a more recent study by the same researchers from Nijmegen, MRI was used on 54 men with low-risk PC managed with AS within the Prostate Cancer Research International Active Surveillance (PRIAS) study⁷, and the ability of ADC in identifying high-grade Gleason components not suitable for AS was evaluated. The diagnostic accuracy of ADC for predicting PC in cancer-suspicious regions was calculated with an AUC of 0.73 (95% CI=0.61-0.84). The ADC was also correlated to grade, and the conclusion was that ADC could predict presence and grade of PC in cancer-suspicious regions on MRI(121).

Bittencourt demonstrated similar findings in a study where 35 consecutive patients with biopsy-proven PC underwent a preoperative MRI. Compared to the ability of TRUS-guided SB to predict Gleason grades in patients undergoing RP, the ADC value attained at MRI correlated significantly better (Pearman’s correlation coefficient) with the final prostatectomy Gleason grade, with a 13-fold difference(117). It should be noted that this study was small, used 1.5T MRI and that a sextant biopsy was used for diagnosis. Nevertheless, the concept of ADC as a non-invasive biomarker for tumor aggressiveness has been raised by others(122, 123), and analyzed using prostatectomy specimens as reference(124). DWI may potentially be utilized not only at detection, but also for staging purposes and for assessing therapy response and tumor relapse in various cancer types. Currently, the clinical oncological areas where DWI is utilized the most are neurooncology, prostate, breast and liver cancer (125).

⁷ A Dutch trial on AS where the following criteria was used for inclusion: asymptomatic T1c/T2 PC, PSA \leq 10.0 ng/ml, PSA density $<$ 0.2, TRUS-guided SB Gleason score \leq 3+3=6, and \leq 2 positive TRUS-guided biopsy cores. Initial TRUS-guided biopsies were performed according to local protocols, with 9 to 13 cores taken.

Comparison of MRI-targeted biopsy and TRUS-guided systematic biopsy

As pointed out earlier there is an urgent need for improving biopsy of the prostate to reduce the risk of overdiagnosis as well as underdiagnosis. Could MRI be one way forward to change from today's random SB to TB? MRI-TB has been suggested to have several advantages compared with TRUS-guided SB. For instance, MRI can aid in guiding biopsies in the repeat biopsy setting in men with persistently suspicious PSA but previous negative SBs(126, 127). According to a recent study by Sonn, MRI-TB increased the diagnostic yield 3-fold (21% vs. 7%) compared with standard SB in men undergoing a pre-biopsy MRI due to either an AS yearly biopsy protocol, or suspicious PSA but prior negative SB(128). In a review by Moore et al., it was concluded that MRI-TB and standard SB detect clinically significant PC in an equivalent number of men but that MRI followed by TB does this more efficiently, requiring fewer biopsies (mean 3,8 cores) in a third of men, and about 10% fewer insignificant PC are detected(129).

Techniques for targeting biopsies

TB can be obtained by different manners. One way is through “cognitive” targeting, where the TRUS-performing urologist reviews the MRI results before the procedure and guides the needles towards the most appropriate region on TRUS, believed to correspond with the MRI location. Another way is by using fusion technology, where specific software incorporates the location of an MRI-suspicious lesion into the TRUS image. A third way of targeting is to do in-bore targeting within the magnet. The most frequently used method so far is the “cognitive” TRUS-guided method, but fusion and in-bore techniques are upcoming and are under continuing evaluation.

The optimal method for targeting is controversial. In a study by Rastinehad et al., 105 patients with suspicious findings on MRI underwent MRI/TRUS fusion-guided biopsies before standard 12-core SB. The investigators reported a 27.7% relative increase in the detection of clinically significant PC (Epstein criteria), using the fusion biopsy approach compared to the SB approach. Also, when comparing positive core length, fusion biopsies yielded significantly longer cancer lengths compared with SB, and the overall cancer detection rate per core was higher. They also concluded that if only TB would be used, 12.4% of PC would be missed, of which 3.8% would have been clinically significant(130).

However, whether fusion biopsy is the most appropriate targeting technique remains unclear. Recently, biopsy performance of cognitive TB, fusion TB,

and SB was compared in a prospective study from three specialized centers: Lille, Lyon and Paris. According to the results published in *Radiology*, both targeted methods yielded higher detection rates of clinically significant PC compared with SB, and the cognitive and fusion techniques were equally accurate(131). There is great heterogeneity among imaging studies using MRI in the diagnosis of PC. Factors like patient characteristics, MRI criteria for biopsy, gold standards used as reference, and whether men are biopsy-naïve or not differ. Villers et al. concluded in a 2015 review comprised of 12 articles (many others excluded due to heterogeneity), that MRI-TB has a high NPV for detecting clinically significant PC (63-98%) and that the overall performance of MRI-TB is about 2-3 times better than that of SB(132).

1.6 Screening

1.6.1 Principles for screening

In the 1960s, the WHO published a paper by Wilson and Jungner on the “Principles and Practice of Mass Screening for Disease”(133). The authors stated 10 fundamental criteria for evaluating screening tests and deciding on whether a particular screening strategy was effective or not. If the criteria could not be fulfilled, there would be no implication for screening since it is expensive, time consuming, and lays an excessive burden on the screened population. The 10 criteria were the following:

1. The condition sought should be an important health problem.
2. There should be an accepted treatment for patients with recognized disease, and treatment should be better at an earlier stage.
3. Facilities for diagnosis and treatment should be available.
4. There should be a recognizable latent or early symptomatic stage.
5. There should be a suitable test or examination.
6. The test should be acceptable to the population.
7. The natural history of the condition, including development from latent to declared disease, should be adequately understood.
8. There should be an agreed-upon policy on whom to treat as patients.
9. The cost of case-finding (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole.
10. Case-finding should be a continuing process and not a “once and for all” project.

These principles are still valid today. If the principles are met for a certain disease, further research should be performed. Effects on mortality should be systematically evaluated and the benefit vs. harm balance should be assessed. If early diagnosis can be demonstrated to be cost-effective and lead to a measurable reduction in disease-burden, implementation of mass screening might be justified.

1.6.2 Evaluating screening

When evaluating screening, two key factors are important – the quality of the evidence and the impact of screening upon clinically relevant outcomes. The Oxford Centre for Evidence-Based Medicine (OCEBM) Levels of Evidence is one of several evidence-ranking systems, grading evidence for diagnostic tests, prognostic markers and screening. According to the OCEBM, the best study design to answer the question of whether a specific screening program is worthwhile is a systematic review of randomized trials(134). Hence, randomized controlled trials (RCT) provide the highest potential to determine the actual effects of screening (Figure 2).

Question	Level 1	Level 2	Level 3	Level 4	Level 5
Is this (early detection) test worthwhile (screening)?	Systematic review of randomized trials	Randomized controlled trial	Non-randomized controlled cohort/ follow-up studies*	Case-series, case-control or historically controlled studies*	Mechanism-based reasoning

Figure 2. Oxford Centre for Evidence-Based Medicine 2011, Levels of Evidence.

*As always, a systematic review is generally better than individual studies. Adapted from OCEBM Levels of Evidence Working Group. "The Oxford 2011 Levels of Evidence". Oxford Centre for Evidence-Based Medicine.

<http://www.cebm.net/index.aspx?o=5653>

The main purpose of screening for cancer is to reduce mortality. However, it is important to report on all possible outcomes of screening(135, 136). A wide range of outcomes measuring benefit (+) and harm (-) may therefore be of value, including:

- + **Overall survival** (most robust outcome but requires very large samples and may fail to pick up clinically important, cancer-specific mortality reductions)
- + **Cancer-specific survival** (requires long follow-up, sensitive to lead- and length-time biases)
- + **Cancer-specific mortality** (requires long follow-up, avoids lead- and length-time biases)
- + **Proportion of low-grade tumors** (might indicate screening effectiveness but may also indicate overdiagnosis)
- + **Proportion of high-grade tumors and interval cancers** (indicates sensitivity of the screening program, might be related to cancer-specific mortality)
- **Anxiety and other psychological consequences** (includes weighing benefits vs. harms but often difficult to measure)
- **Procedural risks and discomfort related to screening activities** (depends on invasiveness, frequency, potential gain)
- **Burden of false positives and false negatives** (psychological consequences, depending on sensitivity and specificity of the screening test)
- **“Labeling”** (i.e. going from apparently healthy to diseased)
- **Number of clinically insignificant lesions** (contributing to overdiagnosis)
- **Economic considerations** (societal costs but also possible savings owing to potentially decreased morbidity and mortality)

1.6.3 Bias in prostate cancer screening

Several biases were addressed in the assessment of screening outcome in the early PSA-era. One was *lead-time bias* since screening caused a stage shift towards more organ-confined PC, causing what seemed to be extended survival after diagnosis when, in fact, no prolongation of lives were achieved(137). Lead-time is defined as the amount of time by which diagnosis is advanced due to screening(138, 139). Calculations of lead-time for PC vary in the literature, with mean lead-times ranging from 3 to 12 years(137). As stated above, this bias is overcome when mortality rates are reported instead of survival rates.

Length-time bias is another possible confounder in the interpretation of PC-specific survival. Screening programs, especially those with regular intervals, are more likely to pick up slow-growing tumors. On the other

hand, rapidly progressing tumors with aggressive features are more likely to surface clinically during a screening interval (Figure 3). Thus, screen-detected cancers will appear to have improved survival, incorrectly ascribed to screening(136). This is especially important in the initiation of a screening program rather than during subsequent screening rounds. It is important that all cases are counted, regardless of detection mode (i.e. both screen-detected and interval detected), to overcome this bias.

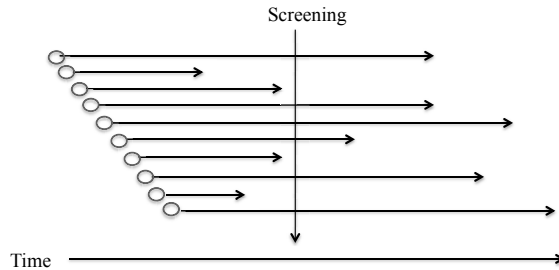


Figure 3. Schematic illustration of length-time bias. “0” indicates when tumors arise, end of arrow indicates when the patients die from the cancer. Consequently, screen-detected cancers will, by selection, appear to have a better prognosis than cancers diagnosed outside the screening program. Adapted from Alibhai S, “Cancer screening: The importance of outcome measures.” *Critical Reviews in Oncology/Hematology* 57 (2006) 215–224.

Closely related to length- and lead-time is the quantity of overdiagnosis. Overdiagnosis is often defined as the rate of screen-detected cancers that would not have been detected in absence of screening. Calculations of overdiagnosis in PC screening also vary in the literature, ranging from about 30% to 50% (57, 140).

Another potential bias was the upgrade in Gleason scoring that occurred during the 1990s, causing stage-specific survival improvements. Although the Gleason grading system itself was unchanged, its application changed so that more cancers were classified as moderate- and high-risk that previously had been classified as low-risk PC. Several factors contributed to this upgrade, including improved TRUS-technologies and modernized biopsy-guns as well as increased numbers of RP, yielding larger pathology specimens(141). The phenomenon is sometimes called the Will Rogers phenomenon and occurs whenever patients are reclassified, often after the introduction of more sensitive staging tools(142). The ISUP revision from 2005 contributed further to this development. A Swedish study recently confirmed that Gleason upgrading has occurred gradually from 1998 and

onward, but that it became more evident after 2005 (according to data from the NPCR). For instance, the proportion of T1c tumors, PSA 4-10 ng/ml graded as GS 7-10 increased from 16% in 1998 to 40% in 2011, with the largest increase observed after 2005(143).

In studies where the primary outcome measure is cause-specific mortality, an accurate *cause of death (COD) determination* is crucial. To minimize bias, it is common to appoint an independent COD committee consisting of experts in the field. This committee then reviews available medical records blinded to trial arm allocation, and determines the underlying cause of death. Although it has been shown that the Swedish COD certificates for men with PC are highly accurate(144), specific COD committees are valuable in multicenter studies such as the ERSPC for a systematic approach to COD determination(145). However, blinding is sometimes difficult to obtain due to the fact that screen-detected cancers differ from those detected due to symptoms and there is a potential that errors occur whenever human judgment of the COD is involved. An alternative approach to evaluate the effect of screening on mortality is to compare the excess mortality in PC patients in both trial arms(146).

1.7 Treatment and prognosis of localized prostate cancer

One prerequisite for introducing screening is that efficient treatment alternatives are available, as screening itself does not affect mortality. Most screen-detected PC are estimated to have a slow-growing natural history, posing little threat to the patient's life, as discussed previously. The following paragraphs on treatment will only cover localized cancers, as these are the ones most frequently detected by screening.

Screening increases the detection rate of small, localized tumors(147, 148). Treatment options for men with localized PC include RP, radiation therapy (RT), or AS. An overall assessment of prognostic factors and patient characteristics guides clinicians in choosing the right treatment for the right patient. Factors like biological age, comorbidity and life expectancy are especially important to consider because they are closely linked to the risk of dying from competing risks(23). That is not to say that treatment (or screening) should be suspended completely in older men. Most Swedish men who die from PC are ≥ 80 years of age(12), and a high age at diagnosis is associated with more aggressive features at diagnosis(149).

Radical prostatectomy

Surgically, the prostate is removed either through retro pubic RP or through robot-assisted RP (RARP). The latter approach has recently gained increasing popularity in Sweden, with shorter hospital stays and less blood loss(150). However, evidence of its superiority in cancer control compared to RP is lacking(49, 151).

The evidence of a beneficial effect on mortality with RP includes an often-cited study by the Scandinavian Prostate Cancer Group, study number 4 (SPCG-4). This study randomized 695 patients with clinical stage T1-T2 PC in 1989-1999 to either RP or watchful waiting (observation). The recently published 23-year results confirmed a substantial reduction in overall mortality with RP compared to watchful waiting, RR 0.71 (95% CI=0.59-0.86; $p<0.001$) as well as cancer-specific mortality, RR 0.56 (95% CI=0.41-0.77; $p=0.001$). However, the benefit decreased with age and was largest in men <65 years of age and minimal in men >70 years of age(152-154). It should be noted that this study was initiated at a time when PSA testing was uncommon in Scandinavia, reflected in the fact that only a small proportion of cancers were low-risk cancers at diagnosis (12% T1c).

Another large study, the Prostate Cancer Intervention Versus Observation Trial (PIVOT) showed results conflicting with those of the SPCG-4. In the PIVOT trial, 731 men were randomized to RP or observation between 1994 and 2002(155). After a follow-up of 10 years, there was no statistically significant difference in overall (47.0% vs. 49.9%; $p=.22$) or PC-specific mortality (5.8% vs. 8.4%; $p=.09$) between the RP and the observation groups. A possible survival benefit for those in the RP group was suggested only in men with intermediate- and high-risk PC(156). Different study populations in terms of risk group distribution and indications for RP reflect the major differences between the SPCG-4 and the PIVOT results. With the inclusion of a large number of low-risk cancers and a very high non-cancer mortality (50% at 10 years), the PIVOT trial is probably underpowered. Both the SPCG-4 and the PIVOT support the efficacy of surgery in men with intermediate- and high-risk prostate cancer.

Radiation therapy

Another treatment option in localized PC is radiation therapy (RT), which may be delivered either by an external beam source (EBR) or by brachytherapy. Transperineal brachytherapy is an effective choice in low-risk PC(157). Combined techniques are also available.

Active surveillance

According to EAU guidelines, AS is suitable in men with localized, low-risk PC with life expectancies of >10 years who may be offered curative treatment at a later stage(158). The idea with AS is to monitor patients closely and to reduce overtreatment by postponing treatment until there is evidence that the patient is at increased risk of progression. However, there is no consensus on eligibility criteria for selecting men for AS(159). In addition, the optimal way of monitoring men on AS has not yet been defined, although it is currently being investigated in the Study of Active Monitoring in Sweden (SAMS)(160).

Information on long-term outcomes after AS as well as from RCTs is so far lacking. According to a 2012 review by Dell'Era, PC-specific mortality appears to be very low with AS (0-1%), at least in the short to intermediate term (median follow-up ranging from 1.8 to 6.8 years between reviewed studies). An early confirmatory biopsy is essential, and about one-third receive secondary therapy after a median of 2.5 years on AS, according to Dell'Era(159). A study by Arnsrud Godtman from the Göteborg randomized screening trial showed that almost half of all screen-detected PC (442 of 968; 46%) were managed with AS initially, with only one PC death and one man developing metastases during a follow-up of 6 years. However, a total of 37% received deferred active treatment (RP, RT or hormonal therapy), a risk that increased cumulatively with time and age(161). It may be unwise to choose AS in young patients as it has been shown that there is a small risk of missing the opportunity for curative treatment(162).

AS should be differentiated from watchful waiting (WW), which is a non-curative option, initiated when symptoms arise. WW is characterized by initial conservative management and delayed hormonal therapy and is a reasonable choice in men with localized PC and a life expectancy of 10 years or less(163, 164).

2 AIM

The overall aim of this thesis is to explore aspects of underdiagnosis in PSA-based screening for PC, with special reference to the impact of screening failures on screening effectiveness. Objectives of each paper were as follows:

Paper I

- To study the number and characteristics of IC in two centers of the ERSPC, one using a 2-year screening interval (Göteborg) and one using a 4-year screening interval (Rotterdam).

Paper II

- To analyze PC mortality among men randomized to biennial screening, comparing attendees with nonattendees.

Paper III

- To analyze the incidence of PC after screening cessation in men who had reached the upper age limit for further invitations to screening.

Paper IV

- To evaluate whether the addition of mpMRI as a screening tool may improve on the benefits and harms of PSA-based screening.

3 PATIENTS AND METHODS

3.1 Study population

The Göteborg randomized screening trial

This thesis, in all its papers, is based on the Göteborg randomized screening trial, a population-based study approved by the ethical review committee at the University of Gothenburg in 1994. According to the Population Register, a total of 32,298 men in the age group 50-64 years (born between January 1, 1930 and December 31, 1944) lived in Göteborg as of December 31, 1994. Of those, 20,000 were randomly selected via computer-randomization to form a screening and a control group. Randomization was performed before informed consent (upfront), meaning that the study population forms a representative sample of about two-thirds of all men in this age group living in Göteborg at the time. Men with prevalent PC as well as those who emigrated or died before randomization (not yet recorded in the Population Register at time of randomization) were excluded from the study.

Procedures

Men in the screening group were invited biennially to PSA testing. A PSA value exceeding the cut-off level was regarded as a positive screening test and led to further workup, including DRE and TRUS-guided biopsy (laterally directed sextant biopsies until 2009, 10-core biopsy thereafter). The PSA cut-off was originally set at 3.0 ng/ml but because of calibration issues regarding the PSA assay (Prostatus Total/Free PSA-Assay from Perkin-Elmer [Turku, Finland]), the actual cutoff values used differed slightly from the target value. On the basis of the current WHO PSA calibration standard 96/670, the actual PSA cutoff was 3.4 ng/ml during 1995 to 1998 (screening rounds 1-2), 2.9 ng/ml during 1999 to 2004 (rounds 3-5), and 2.5 ng/ml from 2005 onward (rounds 6-10). A PSA value below the cut-off did not lead to any further examination, but a re-invitation was extended 2 years later. Re-invitations were no longer extended when the upper age limit had been reached (median 69 years, range 67-71). The oldest age group received 3 invitations before reaching the upper age limit, whereas the youngest age group received as many as 10 invitations before reaching the upper age limit. The 10th and last screening round took place during 2013-2014. The control group was not invited. The study design is depicted in Figure 4.

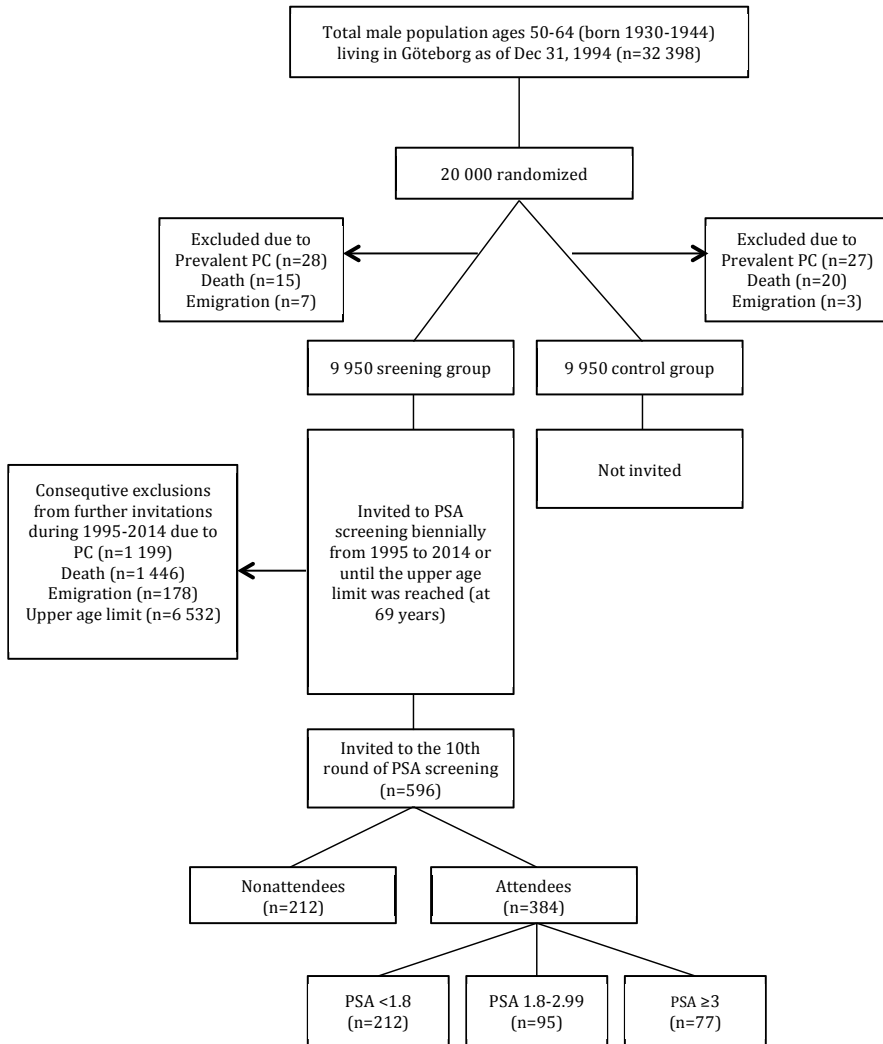


Figure 4. CONSORT diagram showing the design of the Göteborg randomized prostate cancer screening trial including the outcome of the last (10th) screening round. PSA=prostate specific antigen, PC=prostate cancer.

Approximately 4 times per year, the study cohort was linked with the Regional Cancer Register and the Swedish Population Register to identify all PC diagnosed in both study groups and to identify all men who emigrated or died. Information on stage, grade and treatment was gathered from medical records and charts. An independent cause of death (COD) committee was assigned to determine COD in men with PC. In a blinded and independent fashion, each of three members of the COD committee reviewed available information on deaths among men with PC to determine COD. Ambivalent cases were resolved by discussion.

The Rotterdam branch of the ERSPC

In Paper I, data from the Rotterdam center of the ERSPC was included in addition to Göteborg data to answer specific research questions. The Rotterdam branch of the ERSPC started to randomize men on November 17, 1993. A total of 42,376 men in the ages 55-74 years were assigned using computer randomization to a screening arm (n=21,210) and a control arm (n=21,166) through December 31, 1999. Men in the screening arm received invitations to PSA screening every 4th year until 75 years of age. The control arm was not invited. Generally, a PSA cut-off of 3.0 ng/ml was used for biopsy referral. However, in the beginning of the Rotterdam study, a PSA level of 4.0 ng/ml or higher and/or an abnormal DRE and/or TRUS was required to indicate a sextant biopsy, but from May 1997 and onward the PSA cut-off was lowered to 3.0 ng/ml and DRE/TRUS was omitted from the study protocol. A COD committee reviewed all causes of deaths occurring in men with PC and ambivalent cases were resolved by discussion.

3.2 Methods

An overview of study populations, follow-up times, aims, methods and statistics used in each paper is depicted in Table 3. In the following chapter, material and methods used in Papers I-IV of this thesis are presented, followed by a paragraph called “methodological considerations” for each paper, in which the strength and limitations of the chosen methods are discussed.

Table 3. Overview of studies in this thesis.

Paper	Aim	Population and follow-up	Method	Statistics
I	To compare IC rates with 2- and 4-year screening intervals.	Göteborg and Rotterdam: Men ages 55-65 years who attended initial and subsequent screenings followed up to December 31, 2005 (or maximum 10 years).	Detection rates of IC were compared between Göteborg (2-y interval) and Rotterdam (4-y interval). IC= all PC detected during the screening interval.	Rates of IC were analyzed and cumulative incidence calculated using K-M estimates, differences tested with log rank test.
II	To describe the intermediate-term mortality in men randomized to screening.	Men randomized to screening, divided into attendees and nonattendees. Followed from randomization date up to December 31, 2007.	Cumulative overall survival and cumulative PC-specific mortality was compared between attendees and nonattendees. PC deaths were analyzed with regard to previous screening pattern.	K-M estimates, log rank test. Cox regression was used to calculate HR.
III	To analyze PC incidence and mortality in the screening and control groups after screening cessation.	Men who had reached the upper age limit as of June 30, 2012 without a PC diagnosis. Follow-up defined as date of last invitation (or equivalent) until date of an event (PC, death, emigration), or maximum 12 years.	IR of PC (overall) and high-risk/advanced PC were reported, divided into 3-year, post-screening periods.	K-M estimates, life table models, and competing-risk analysis.
IV	To evaluate sequential testing with PSA followed by MRI in screening for PC.	Men attending the 10 th and last screening round, which took place during 2013-2014 (men born 1944).	Detection rates with 3 screening strategies were compared: 1) PSA \geq 1.8 ng/ml + MRI + TB only 2) PSA \geq 3.0 ng/ml + MRI + TB only 3) PSA \geq 3.0 ng/ml + SB	Accuracy of strategies was analyzed by comparing sensitivity and specificity.

PC = prostate cancer, PSA = prostate-specific antigen, IC = interval cancer, K-M= Kaplan-Meier, MRI = magnetic resonance imaging, HR = hazard ratio, IR = incidence rate, TB = (MRI-) targeted biopsy, SB = systematic biopsy

3.2.1 Paper I

The aim of Paper I was to study incidence of IC with a 2- and a 4-year screening interval. For this reason, Göteborg data was compared with Rotterdam data. To achieve comparable age distributions, only men in the ages 55-65 were included in the analysis. In addition, only men responding to the first invitation to screening were included. The cumulative incidence of PC, IC and aggressive IC was compared between the centers. An IC was defined as any cancer diagnosed outside the screening protocol during a screening interval, following a negative screen. Aggressive IC was defined as IC with at least one of the following characteristics: M1 or N1, PSA ≥ 20.0 ng/ml, or GS >7 . The follow-up time was measured from date of randomization until 1) date of PC diagnosis, or 2) date of death, or 3) date of last follow-up (December 31, 2005), which was when the last cross-matching with the cancer registry was performed (and no PC registered).

Statistics

Differences in cumulative incidence of PC and IC between the two centers were analyzed by using Kaplan-Meier estimates and tested for significant differences by the log rank test. The rate of IC was also compared with the rate of PC detected in the control arm of each center (“rate” calculated as number of IC diagnosed during the follow-up period divided by number of men at risk at start of follow-up). All statistical tests were two-sided. P values less than .05 were considered statistically significant.

Methodological considerations – Paper I

Outcome measure

In breast cancer screening, IC has been monitored as an interim outcome measure of screening efficacy. The sensitivity of a screening program is often evaluated by comparing the IC rate to the expected cancer incidence in absence of screening (i.e. in the control group), a measure referred to as the proportional IC rate (PICR). This measure is preferred when comparing different screening programs(165).

In Paper I, the primary measure of comparison was absolute IC rate. Ideally, when two groups are compared to look at the impact of one factor (screening interval) on an outcome measure (IC detection rate), all other factors influencing the outcome of interest should be equal. To improve comparability between Rotterdam and Göteborg, we adjusted for age and

follow-up time. However, the populations differed in other ways, for instance with respect to indicators for biopsy and randomization procedure, which should be kept in mind when interpreting the results. Rotterdam used DRE and TRUS in addition to PSA in the beginning of the study (until 1997). This strategy might have been unequally effective compared to the PSA-only strategy used in Göteborg. If so, the comparison between the centers regarding PC and IC frequency would be biased. However, similar detection rates of PC and IC were observed even after DRE/TRUS had been omitted in Rotterdam, indicating that these tools added little to screening efficacy at the time(37).

Another difference between the groups worth considering was the method of randomization. In Rotterdam, randomization was performed after informed consent whereas men in Göteborg were randomized upfront. As discussed by Zhu et al. in a paper from 2012, randomization after informed consent in Rotterdam led to a lower than expected overall and PC-specific mortality in both trial groups, corresponding to a healthy volunteer bias(166). This type of selection bias occurs whenever people who volunteer to participate in a trial differ from the general population in important ways(136). However, these “healthy volunteers” in the Dutch center were randomized and therefore equally distributed in the screening and control groups, which allows for valid comparisons between the trial groups (such as using PICR). Another argument for comparability between the two populations despite different randomization procedures was that only attendees (i.e. men who volunteered to participate) in the first round were analyzed, which makes the cohorts more alike. In the Swedish cohort, 64% participated compared to about 55% in the Dutch cohort.

The concept of interval cancers

Breast IC tend to have more aggressive characteristics than cancers detected at mammography screening(167, 168). IC rates give an estimate of the proportion of cases picked up at previous screening, which would otherwise have become clinically manifested during the screening interval, at least in other cancer screening(169). At the time of writing Paper I, almost nothing was known about the characteristics, frequency, and importance of prostate IC.

3.2.2 Paper II

This paper aimed at describing the intermediate-term mortality (overall and PC-specific) in men randomized to screening, comparing attendees with nonattendees. According to the ethical review from 1994, a first mortality analysis of the Göteborg randomized screening trial was not planned until 15 years after study start (i.e. in 2010). Hence, no comparisons between the trial groups were performed in Paper II, but mortality between attendees and nonattendees among those invited to screening was compared. Cancers that eventually led to PC death were described according to risk group, as presented by D’Amico et al.(170), and according to participation and compliance with the screening protocol (Table 4).

Table 4. Subgrouping of cancers detected in men randomized to screening based on adherence and compliance with the screening protocol.

	Subgroup	Definition
Attendees	Completely on-protocol	Participated according to the protocol (PSA test at 2-year intervals and biopsy if PSA \geq 3 ng/ml).
	Interval	Participated in the screening program but diagnosed during the 2-year screening interval.
	Irregular intervals	Participated at irregular intervals, >2 years.
	Biopsy refusal	Refused biopsy despite a PSA level \geq 3 ng/ml but were later diagnosed with prostate cancer.
	After-study (previous attendee)	Diagnosed >2 years after last screening visit (after screening had stopped) but had previously attended.
Non-attendees	Nonattendee	Did not participate at all.
	After-study (previous nonattendee)	Diagnosed >2 years after last screening invitation due to the upper age limit (previous non-attendee).

Methodological considerations

Non-participation in the screening group and contamination (occult screening) in the control group are well-recognized problems in the evaluation of RCTs. Intention-to-screen analyses are recommended to overcome these issues, in which subjects are analyzed according to randomized groups. Intention-to-screen analyses ignore non-compliance, protocol deviations, withdrawal, and anything that happens after randomization(171). However, to fully understand the effects of population-

based screening programs, it is important to understand how mortality from PC is affected by compliance with the screening protocol. Therefore, analyses comparing attendees with nonattendees may be justified and highly informative. However, nonattendees should not be regarded as controls because they might differ from the general population in important ways(172).

The optimal algorithm for PC screening has not been established. Factors thought to influence screening efficacy are age, screening interval and compliance with the protocol. Paper II assessed the distribution of these factors in men who died from PC in the screening group to answer the question: Who dies from PC despite being enrolled in a PSA screening program?

Statistics

Cumulative survival plots were calculated with Kaplan-Meier estimates, and differences were tested for significance by using the log rank test. The Cox proportional hazards model was also used, which is a regression method for survival data that provides an estimate of the hazard ratio (HR) and its 95% CI. Cox regression analyzes the impact of a risk factor (attendance/non-attendance) on the outcome (mortality). A hazard is the instantaneous event rate, i.e. the probability that an individual at time t has an event at that time (assuming event-free survival up to time t).

Kaplan-Meier survival curves rely on three assumptions: that censoring is unrelated to prognosis, that survival probabilities are the same for subjects recruited early and late in the study, and that the events happened at the specified time(173). In the PC-specific mortality calculations of Paper II, non-informative censoring can be questioned as men who died from other causes probably were older and had a poorer general health. It has become increasingly recognized that Kaplan-Meier analyses overestimate cause-specific survival in the presence of competing risks(174). A standard Cox proportional hazards model is also inadequate in the presence of competing risks because competing risks are treated as censored observations(175). Hence, a competing risk analysis would have been preferred. We performed such an analysis (Fine and Gray) for the purpose of this thesis to complement the already published results.

3.2.3 Paper III

This study investigated the incidence rate and characteristics of PC diagnosed in men above screening age. The median age at last invitation was 68.7 years (67.0-70.8). No re-invitations were thereafter sent; instead, men in

the screening group received a letter declaring that no re-invitations were scheduled due to the uncertainty of any screening-benefit in men >70 years. Men in the control group received a letter of information in 1995 stating that they belonged to a control group for a cancer study but no further contacts were made after that.

Defining follow-up time

The screening protocol of the Göteborg randomized screening trial has already been described. In Paper III, men were followed from the time they reached the upper age limit for further invitations. The starting point of this “post-screening” period was the date of the last invitation to screening for men in the screening group (ranging from year 1999 to 2012, depending on age at first invitation). Due to the fact that men in the control group had not been invited to screening, dates defining the start of follow-up (corresponding to dates of the last invitation) were imputed after age matching with men in the screening group. Men were censored at 1) PC diagnosis, 2) death, 3) emigration/lost to follow-up, 4) June 30, 2012, or 5) after a maximum of 12 years, whichever came first. All men were closely followed through regular cross-matching with the Regional Cancer Register and the Swedish Cancer Register as described previously. Men with cancers diagnosed at the last screening round were excluded.

Grouping of cancers

All tumors were classified into risk groups: low, intermediate, high and advanced risk (Table 5). Men with high risk and advanced PC were grouped together. Men in the screening group were classified as attendees (attending at least once) and nonattendees (never attending). Information regarding mode of detection (opportunistic screening, incidental, LUTS, other symptoms, or unknown) was consecutively gathered from medical journals.

Table 5. Definition of risk groups used in Paper III.

Low	T1, not N1/M1; Gleason score ≤ 6 ; and/or PSA ≤ 10 ng/ml
Intermediate	T1–2, not N1/M1; Gleason score ≤ 7 ; and/or PSA < 20 ng/ml and not meeting the criteria of low risk
High	T1–4, not N1/M1; Gleason score ≤ 8 ; and/or PSA < 100 ng/ml and not meeting the criteria of low or intermediate risk
Advanced	N1/M1 or PSA 100 ng/ml

Statistics

Incidence rate of PC was calculated for the screening group as well as the control group as number of cancers diagnosed per 1,000 person-years (py), both during the whole follow-up period and divided into 3-year intervals. Possible differences in incidence rate ratios were analyzed by using a two-sided test, based on the binominal probability. Survival was analyzed through Kaplan-Meier estimates and life table models. A competing risk method (Fine and Gray) was also used.

Methodological considerations

In this study, we wanted to compare the incidence rate of PC in the screening group with that of the control group after screening had been discontinued. As explained above, we used a sort of imputation method to establish the dates at which men entered the “post-screening” period. Due to the exclusion of men with cancers detected at the last screening, the follow-up for the screening group was slightly shorter than that of the controls (4.8 vs. 4.9 years). Although all men were randomized in 1995, the groups were no longer equal with regards to cancer risk at the start of the follow-up period of this study (a difference caused by invitations to regular screening vs. no organized screening).

Data on reasons for diagnosis was reported as explained above. A research nurse recorded this consecutively based on information from medical documents. It was not always perfectly clear why men sought medical attention and some may have argued that they had symptoms when in fact they wanted a check-up (screening). Hence, figures on modes of detection should be interpreted with caution.

Paper III also reports on PC mortality post-screening, which can be questioned due to the low number of events. Our original aim was just to describe the incidence, but in the review process, referees requested that we added mortality data as well, especially since incidence depends on diagnostic intensity and because of the uncertain association between incidence and mortality/survival. However, one may argue that incidence data reported by risk groups add valuable information even without mortality results because of the strong association between high-risk/advanced PC and PC death(176). A competing risk analysis was also performed, but since the mortality in both trial groups was equal, it did not change the results.

3.2.4 Paper IV

This pilot study, investigating the role of MRI in screening for PC, was nested within the 10th and last screening round of the Göteborg randomized screening trial that took place during 2013-2014. In this screening round, changes were made in the algorithm in order to test the potential role of MRI in PSA screening for PC. The PSA cut-off for further work-up was lowered from 3.0 ng/ml to 1.8 ng/ml and a pre-biopsy MRI was offered to all men with a PSA above this cut-off. The algorithm of the 10th screening round is shown in Figure 4. Men with a positive MRI and/or PSA ≥ 3.0 ng/ml were referred for biopsy, whereas men with a negative MRI and PSA < 3.0 ng/ml were released without further work-up.

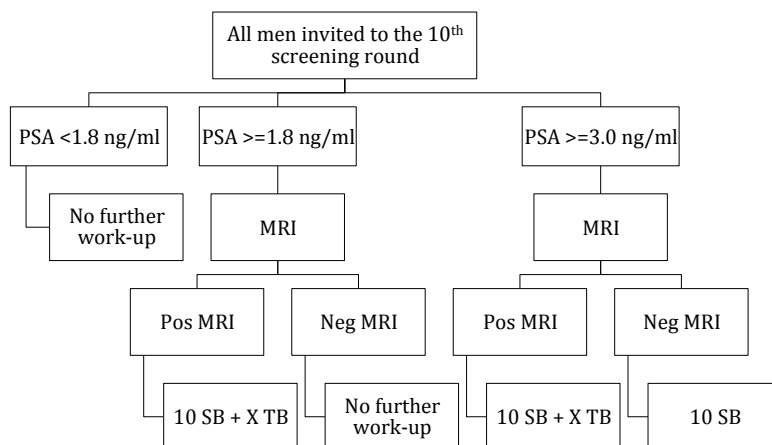


Figure 5. Flowchart showing the algorithm of the 10th screening round of the Göteborg randomized screening trial. PSA = prostate specific antigen, SB = systematic biopsy, TB = (MRI)-targeted biopsy

MRI

All examinations were performed using a 3Tesla system (Philips Achieva 3.0, Philips Healthcare, Best, the Netherlands). During the first part of the study, a SENSE Cardiovascular Array Coil with 32 overlapping elements was used. During the study period the system was upgraded and a digital coil system (dStream Torso with integrated anterior and posterior coils) was used (no endorectal coil). The following sequences were used: T2W, DCE and DWI. For DWI, b-values 0-1000 were used. Apparent Diffusion Coefficient (ADC) maps were calculated and qualitatively assessed. Suspicious lesions

were described by region in the transversal and sagittal plane and scored according to the validated Prostate Imaging Reporting and Data System (PIRADS) for each sequence, ranging from 1 (unlikely) to 5 (highly likely) according to the likelihood of significant PC being present(113, 177). If the PIRADS score in any of the 3 sequences was ≥ 3 (equivocal), the MRI was regarded as positive. All images were read in consensus by 3 experienced radiologists.

Biopsy procedures and cancer classification

A biopsy was performed at a second visit by one single urologist. In order to be able to make comparisons between different diagnostic pathways, all men with a positive MRI and an indication for TB also underwent SB. The 10-core SB was sampled first, blinded to the MRI results. The MRI results were then revealed and the TB was performed in all men with a positive MRI (PIRADS 3, 4, or 5). Targeted sampling of suspicious sector(s) was performed “cognitively,” meaning that the biopsy-performing urologists reviewed the MRI image first and then targeted the TRUS biopsy needles towards the area in the grey-scale TRUS picture thought to correspond to the sector where the lesion was located on MRI. Hence, men without suspicious findings on MRI only underwent SB, whereas men with tumor-suspicious findings on MRI underwent both SB and MRI-TB. Three cores were sampled from each suspicious region on MRI. Cancers were classified as significant / insignificant based on the modified Epstein criteria for insignificant cancer(31): clinical stage (DRE only) T1c, PSAD < 0.15 , Gleason score ≤ 6 , ≤ 2 positive cores, and unilateral cancer.

Statistics

Three different screening strategies were analyzed. The strategies overlapped partially, which meant that that it was possible for a man to be detected with more than one strategy. Strategy “1” was regarded as reference in comparisons and referred to as the “reference strategy.” The three strategies were

1. PSA ≥ 3.0 ng/ml followed by SB
2. PSA ≥ 1.8 ng/ml + MRI followed by TB, if positive MRI
3. PSA ≥ 3.0 ng/ml + MRI followed by TB, if positive MRI

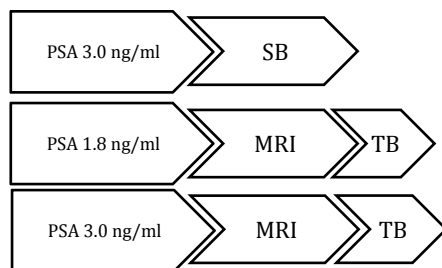


Figure 6. Schematic presentation of screening strategies compared in Paper IV.

Detection rates of cancers were calculated as number of cancers detected among those with a positive screening test according to each screening strategy. We observed a higher MRI attendance among men with PSA ≥ 3 ng/ml than among men with PSA 1.8-2.99 ng/ml. Therefore, in comparisons between the screening strategies, we corrected for this imbalance by calculating the cancer yield with screening strategy number 2 and 3 as if all men with an indication for MRI consequently underwent MRI, followed by TB if the MRI was positive. Similarly, we calculated the outcome with the reference strategy as if all men with an indication for SB actually underwent SB.

Point estimates for the statistics sensitivity, specificity, and positive and negative predictive values were calculated. The binominal option provided exact confidence intervals. Analyses were made using the free statistical software R, utilizing the package DTComPair(178, 179). P values for comparing sensitivities and specificities were calculated using McNemar's test, and P values for comparing PPV and NPV were calculated using the method described by Moskowitz et al. (180).

Methodological considerations

Men with PSA < 3 ng/ml and a negative MRI were not biopsied but assumed cancer-free and released without further work-up. This might have introduced a verification bias since cancer exists at all PSA levels. Although MRI has been shown to have a high NPV at 80-90% (132), we performed a “sensitivity analysis” to test the effect of a hypothetical situation where MRI was assumed to have “missed” cancers in the PSA interval 1.8-2.99 ng/ml. We hypothesized that 5 undetected cancers were present among those men, an estimation of prevalence based on the results from the PCPT trial on PC detection at SB at this PSA interval(60). We then performed calculations on sensitivity and specificity estimates in the presence and absence of those 5 hypothetical cancers and compared the results.

Another methodological concern was that men were attendees in the 10th round of a large PSA screening trial where the vast majority had been screened previously. The screening test used in the Göteborg randomized screening trial was PSA, using a cut-off of 3.0 ng/ml, i.e. equal to what was regarded as the reference strategy in the pilot analysis. This must be kept in mind when interpreting the results because the reference strategy might have appeared less useful in detecting PC than it actually is only because it was applied on a selected group of men.

4 RESULTS

4.1 Paper I

This study compared the IC rate of a 2-year and a 4-year screening interval because IC might give an indication of screening effectiveness and appropriateness of the screening interval. As shown in Figure 7, 21,210 men were randomized to screening in Rotterdam and of those, 13,301 (62.7%) were in the ages 55-65 years and eligible for analysis. During a mean follow-up of 7.16 years, these men had been screened a maximum of 3 times. During this time, 1,061 PC were detected in the screening group, yielding a cancer detection rate of 7.98% (i.e. without the IC). A total of 57 IC (15 aggressive IC) were detected, yielding an IC detection rate of 0.43%.

In Göteborg, 9,973 men were randomized to screening and of those, 4,202 (42.1%) were in the ages 55-65, eligible for analysis. These men had been screened a maximum of 6 times during a mean follow-up of 7.38 years. In total, 521 PC were screen-detected, yielding a cancer detection rate of 12.4% (i.e. without the IC). A total of 31 IC (5 aggressive IC) were detected, corresponding to a detection rate of 0.74%.

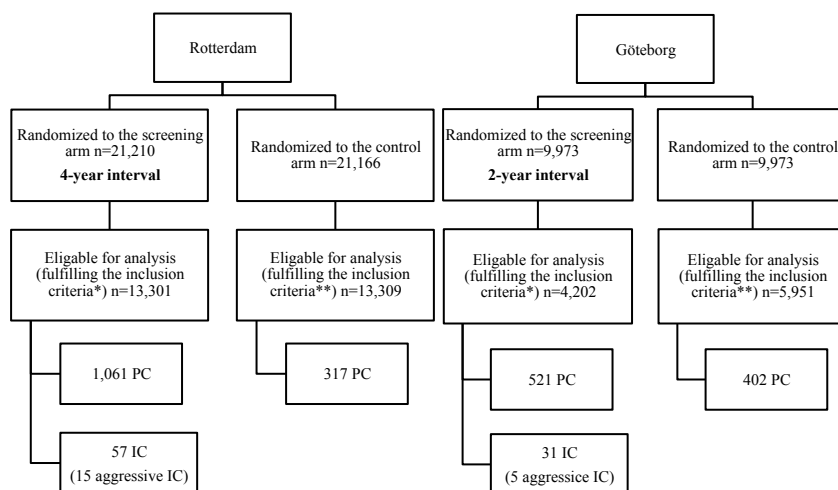


Figure 7. CONSORT diagram of the Rotterdam and Göteborg centers up to December 31, 2005. Aggressive interval cancer was defined as stage M1 or N1, prostate-specific antigen ≥ 20 ng/ml, or a Gleason score ≥ 7 at diagnosis. *This analysis was restricted to 55-65 year olds who responded to their first screening invitation. ** In the control group, only men aged 55-65 years at randomization were included. PC = prostate cancer, IC = interval cancer

The clinical stage, grade and serum PSA concentrations of IC were assessed in both centers, and the majority of IC had low-risk features at diagnosis (T1c, GS 2-6, and PSA levels of 3.0-10.0 ng/ml) with very low proportions as well as absolute numbers of aggressive IC (Table 6).

Table 6. Clinical characteristics of interval cancers and screen-detected cancers at the time of diagnosis in the Rotterdam and Göteborg centers of the European Randomized Study of Screening for Prostate Cancer.

Characteristics	Interval cancers, n (%)		Screen-detected cancers, n (%)	
	Rotterdam (n = 57)	Göteborg (n = 31)	Rotterdam (n = 1,061)	Göteborg (n = 521)
T stage				
T1a or b	8 (14.0)	5 (16.1)	0	0
T1c	30 (52.6)	14 (45.2)	536 (50.5)	388 (74.5)
T2	14 (24.6)	12 (38.7)	432 (40.7)	117 (22.5)
T3-4	5 (8.8)	0	93 (8.8)	14 (2.7)
Unknown	0	0	0	1 (0.2)
M stage				
M0	45 (79.0)	20 (64.5)	949 (89.4)	366 (70.2)
M1	2 (3.5)	4 (12.9)	2 (0.2)	0
Mx	10 (17.5)	7 (22.6)	110 (10.4)	155 (29.8)
Gleason score				
2-6	34 (59.6)	22 (71.0)	798 (75.2)	420 (80.6)
7	12 (21.1)	6 (19.4)	209 (19.7)	89 (17.1)
8-10	3 (5.3)	2 (6.5)	48 (4.5)	12 (2.3)
Unclassified	8 (14.0)	1 (3.2)	6 (0.6)	0
PSA, ng/ml				
<3	6 (10.5)	0	136 (12.8)	0
3-10	28 (49.1)	24 (77.4)	793 (74.7)	455 (87.3)
>10-20	6 (10.5)	4 (12.9)	88 (8.3)	44 (8.4)
>20-100	10 (17.5)	3 (4.6)	43 (4.1)	19 (3.6)
>100	2 (3.5)	0	1 (0.09)	3 (0.6)
Missing	5 (8.8)	0	0	0

Numbers in parenthesis are percentages of total number of interval cancers of each center (left column) and percentages of total number of screen-detected cancers in each center (right column). T = tumor, M = metastases, PSA = Prostate-specific antigen

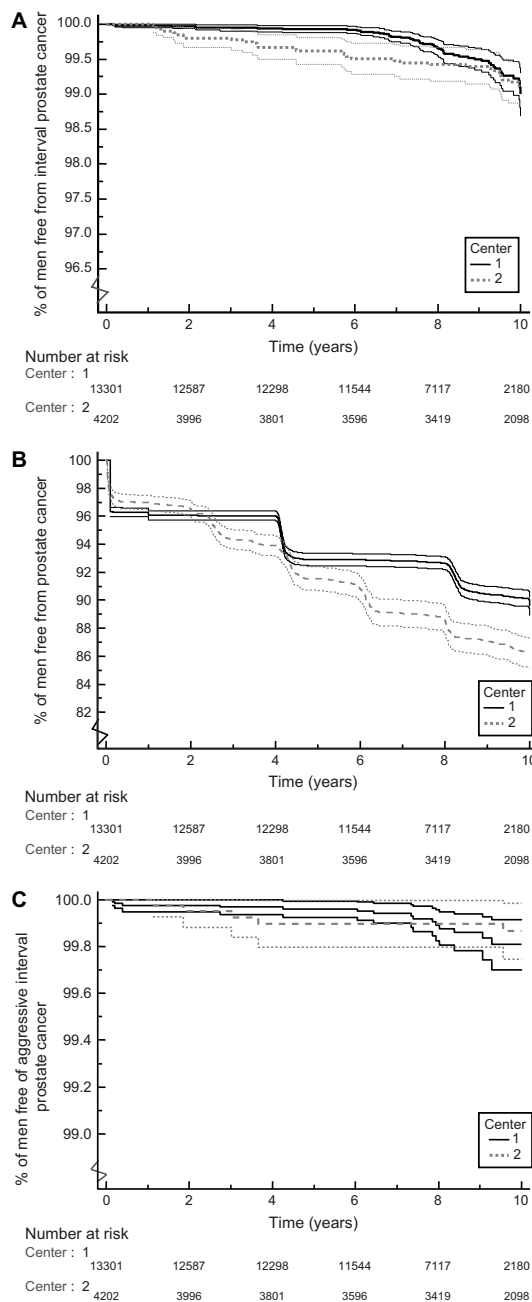


Figure 8. Kaplan-Meier estimates. A) Percentage of men free from interval prostate cancer as a function of time. $P = .51$. B) Percentage of men free from prostate cancer as a function of time. $P < 0.001$. C) Percentage of men free of aggressive interval cancer as a function of time. $P = .72$. Outer lines represent 95% confidence intervals. Center 1=Rotterdam, Center 2=Göteborg

The estimated percentages of men free from PC, IC and aggressive IC were analyzed using Kaplan-Meier estimates. A significant difference between the centers was observed only when comparing the overall PC detection (13.5% vs. 8.40%, $p < 0.001$) (Figure 8b). The cumulative incidence of IC and aggressive IC was low in Göteborg and Rotterdam; 0.74% vs. 0.43%, $p = .51$ (Figure 8a), and 0.11% vs. 0.12%, $p = .72$ (Figure 8c). When the rate of IC was compared to the rate of PC diagnosed in the control group, the PICR was 11% in Göteborg and 18% in Rotterdam.

4.2 Paper II

This study aimed at describing the intermediate-term mortality from PC in men invited to screening in the Göteborg randomized screening trial and to compare attendees with nonattendees. Patterns of attendance were explored among those who had been invited to screening but subsequently died from PC. During a mean follow-up of 12 years (until December 31, 2007), a total of 1,076 PC were detected in the screening group. Of those, 92% (990/1,076) was detected among attendees (having attended at least once). The remaining 8.0% (86/1,076) were detected in nonattendees (never attending) (Figure 9).

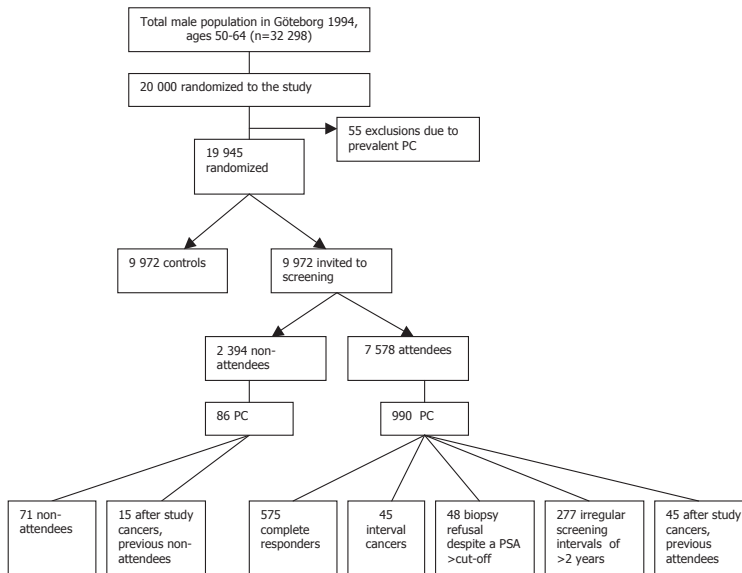
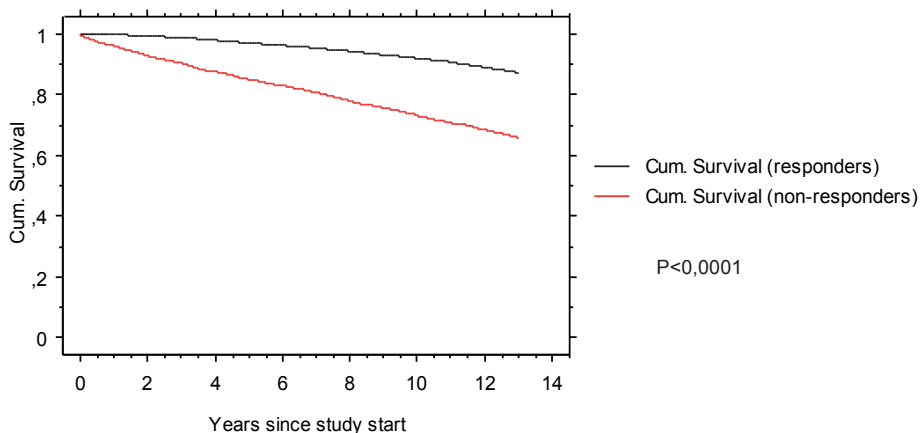


Figure 9. Flowchart depicting cancers diagnosed in the Göteborg randomized screening trial up to 13 years after randomization, including subgrouping of cancers diagnosed in men invited to screening.

As shown in Figure 10, Kaplan-Meier estimates of cumulative mortality revealed that nonattendees had an almost 3-fold higher risk of dying from any cause (34%; 816 of 2,394 men) during follow-up compared with that among attendees (13%; 962 of 7,578 men), $p < .0001$. PC-specific cumulative mortality was also higher among nonattendees (0.8%) compared to that of attendees (0.3%), resulting in a HR of 0.375 (95% CI=0.198 – 0.722; $P < .005$).

A)



B)

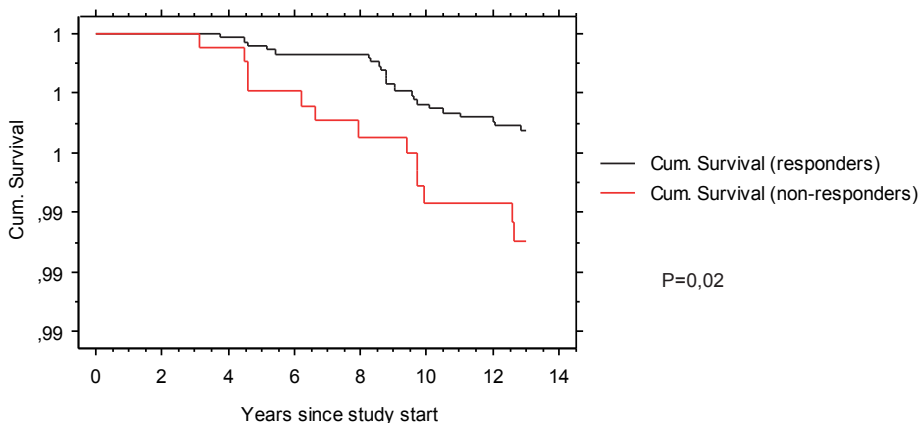
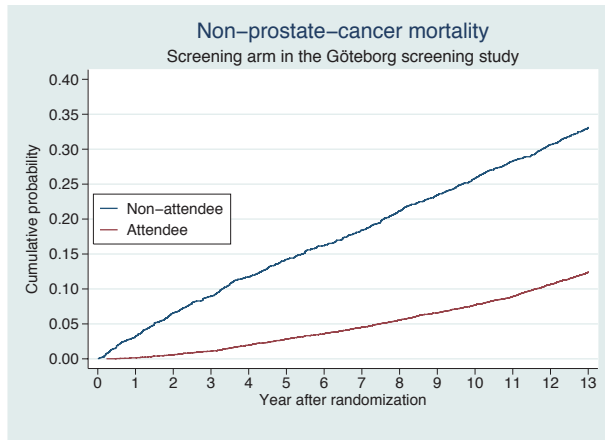


Figure 10. Kaplan-Meier estimates of overall (A) and prostate-cancer specific (B) survival in the screening group, comparing attendees (responders) with nonattendees (non-responders). All P values were calculated using the log-rank test

As mentioned previously, Kaplan-Meier estimates and Cox regression analyses overestimate mortality in the presence of competing risks. Therefore, a complementing competing-risk analysis was performed (Figure 11). As shown, the PC-specific mortality among nonattendees was overestimated with the Cox regression method (0.8%) compared with the competing risk analysis (0.6%). HR for non-PC-mortality according to the competing risk method was 3.1 (95% CI=2.8-3.4), $p < 0.001$ and for PC-specific mortality, 2.1 (95% CI=1.1-4.0), $p = 0.027$.

A)



B)

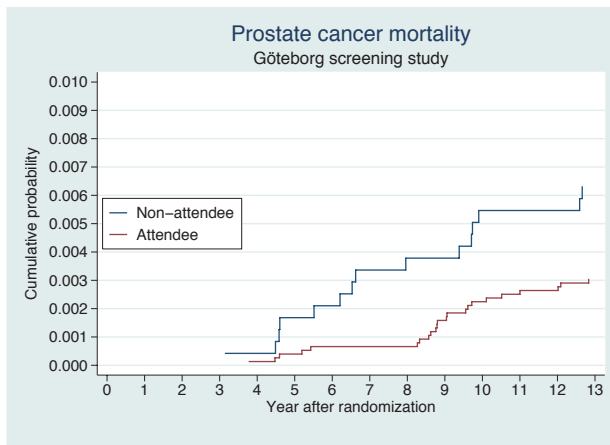


Figure 11. Competing risk analyses of A) non-prostate cancer mortality and b) prostate cancer-specific mortality among men invited to screening, comparing attendees with nonattendees.

Subgroup analysis of the deceased

Of 1,778 deaths due to all causes, 39 (2.2%) were due to PC. Clinical characteristics and screening attendance in men dying from PC are depicted in Table 7. The majority (80%; 31 of 39 men) had high-risk PC at time of diagnosis. In total, 23 of 39 PC deaths occurred among attendees. Fifteen had been complete attendees, but 12 of those were detected at their initial screen (prevalent cases). All of those were ≥ 60 years of age at diagnosis. Three deaths were related to IC. 16 PC deaths occurred among nonattendees and 15 of those were classified as high-risk cancers at diagnosis.

Table 7. Characteristics of invited men who died from prostate cancer within 13 years, stratified into groups based on attendance.

		Median age at diagnosis, years (range)	Median PSA at diagnosis, ng/ml (range)	Risk group		
				Low	Inter-mediate	High
Attendees	Completely on-protocol, n=15*	63 (61-69)	10.7 (3.6-210)	2	3	10
	Interval, n=3	68 (67-69)	7.6 (5.1-23.6)			3
	>2 yr interval, n=1	63	92.4			1
	Biopsy refusal, n=2	61 (56-66)	1404 (8.6-2800)	1		1
	After study, n=2	72.5 (72-73)	15.6 (10-21)		1	1
	All attendees, n=23	64	10.7	3	4	16
Nonattendees	Nonattendees, n=14	62 (54-68)	45 (3.3-2100)	1		13
	After study, n=2	70.5 (69-72)	720 (340-1100)			2
	All nonattendees, n=16	63	66.5	1		15
All, n=39		63	24	4 (10%)	4 (10%)	31 (80%)

* Of those, 12 were detected at the first (prevalence) screening.

Update of the results

An update was performed with data on mortality up until the last matching with the Regional Cancer Registry and Population Register as of January 14, 2015. Up to this date, a total of 82 men had died of PC in the screening group (and 123 in the control group). Of 27 complete attendees (Table 8), 16 were detected at the first screening round. The majority of those were above age 60 at detection, but one was 54 years, one was 56 years, and three were 59 years at first invitation.

Table 8. Characteristics of invited men who died from prostate cancer within 20 years, stratified into groups based on attendance.

		Median age at diagnosis, years (range)	Median PSA at diagnosis, ng/ml (range)	Risk group			
				Low	Inter- mediate	High	Mis- sing
Attendees	Completely on- protocol, n=27*	63 (59-66)	8.8 (4.4-20.8)	2	9	16	
	Interval, n=4	68 (65-69)	15.6 (6.4-49.3)			4	
	>2 yr interval, n=3	66 (64-67)	139 (38.3-1505)		1	2	
	Biopsy refusal, n=4	65 (60-69)	11.0 (5.1-20.0)	1	0	3	
	After study, n=13	74 (73-76)	39 (14.0-298)		2	10	1
	All attendees, n=51	66 (60-71)	139 (38.3-1505)	3	12	35	1
Nonattendees	Nonattendees, n=23	64 (59-67)	39.5 (21-134)	1	1	18	3
	After study, n=8	72 (71-74)	590 (280-1100)		1	7	
	All non- attendees, n=31	66 (61-72)	88 (24-750)		2	25	3
All n=82		66 (62-69)	4.9 (3.7-8.6)	4 (5%)	14 (17%)	60 (73%)	4 (5%)

*16 of 27 cancers detected at the initial screening.

Hence, 11 deaths occurred in men who had attended repeated screenings and 4 deaths were related to IC, but the remaining 82% of PC deaths (67 of 82) occurred in nonattendees or in men who were incomplete screeners or were detected after reaching the upper age limit for screening. Consequently, even after this update, the results from Paper II are still valid.

4.3 Paper III

This study compared the PC incidence and PC-specific mortality in the screening group with that of the control group “post-screening,” i.e. after re-invitations to further screenings had been dismissed due to the upper age limit.

At the last follow-up, a total of 13,423 men (6,449 men in the screening group and 6,974 men in the control group) had reached the upper age limit without a PC diagnosis. Twenty percent (1,287 of 6,449 men) in the screening group had been nonattendees during the active screening period. The follow-up was 4.8 years (IQR: 2.3-8.7) for the screening group and 4.9 years (IQR: 2.4-8.8) for the control group.

Incidence of PC after screening termination

During follow-up, 173 cancers were diagnosed in the screening group and 371 in the control group. Overall, 42 of the 173 cases of PC detected in the screening group were among previous nonattendees. Another 10 had presented with elevated PSA (≥ 3.0 ng/ml) within the study but had been noncompliant to further diagnostics and were therefore analyzed together with nonattendees. A total of 121 men (70%) had been attendees and compliant with the protocol, and 75 of those attended their last screening round (Table 9).

Table 9. Number of prostate cancers and characteristics of patients diagnosed after screening cessation in the screening and control groups.

	Screening group		Control group			
	<i>Attendees</i>	<i>Nonattendees</i>	<i>Total screening group</i>		<i>Control</i>	
	Median (range)	n	Median (range)	n	Median (range)	n
No. of men		5,162		1,287	6,449	6,974
Age at "upper age limit"	68.7 (67.0-70.4)		68.7 (67.1-70.8)		68.7 (67.1-70.8)	
Age at diagnosis, yr	73.9 (68.1-81.4)		73.3 (68.8-79.9)		73.8 (68.1-81.4)	72.1 (67.5-81.5)
Time to PC from last invitation, yr	5.4 (0-11.9)		5.1 (0.1-11.6)		5.23 (0-11.9)	3.3 (0-11.9)
FU, yr	4.8 (0-12.0)		4.7 (0-12.0)		4.8 (0-12.0)	4.9 (0-12.0)
No. of PC		121		52	173	371

PC = prostate cancer, SA = screening arm, FU = follow-up time, yr = years

IRs of PC according to risk-groups and time since last screening are shown in Table 10. All tumor risk groups were more commonly diagnosed in the control group after screening termination but after 9 years, the screening group caught up (except for the low-risk groups). Subgroup analyses showed that attendees had a 50% reduced risk of being diagnosed with PC up to 9 years compared with controls; RR 0.26, 0.46, and 0.50 during years 0-3, 3-6, and 6-9. (Table 10). Although opportunistic screening seems to have commonly occurred (Table 10; figures within brackets), the majority of cancers were classified as intermediate- or high-risk at diagnosis (80%, 139 of 173 in the screening group and 77%, 286 of 371 men in the control group). Figure 12 and 13 show Kaplan-Meier estimates on cumulative incidence of PC and high-risk and advanced PC, respectively, by age.

Table 10. Absolute numbers and incidence rates of prostate cancers diagnosed per attendance and risk group after termination of screening, by 3-year intervals.

Time	Screening						Control									
	Attendees			Non attendees			Total screening arm			Control						
	Low	Inter med	High/Adv	Low	Inter med	High/Adv	Low	Inter med	High/Adv	Low	Inter med	High/Adv	Total			
0-3	14(5) 1,09	13(6) 1,01	6(5) 0,47	33(16) 2,58	3(1) 0,96	7(2) 2,25	6 1,93	16(3) 5,14	17(6) 1,07	20(8) 1,26	12(5) 0,75	49(19) 3,08	48(27) 2,80	69(37) 4,02	52(11) 3,21	169(75) 9,86
3-6	9(5) 1,08	20(9) 2,39	12(3) 1,43	41(17) 4,90	0	5(2) 2,50	13(1) 6,49	18(3) 6,98	9(5) 0,87	25(11) 2,41	25(4) 2,41	59(20) 5,69	22(8) 1,98	46(20) 4,14	48(7) 4,32	118*(35) 10,63
6-9	4(1) 0,82	10(5) 2,06	10(4) 2,06	24(10) 4,94	1 0,89	1 0,89	8 7,13	11† 9,80	5(1) 0,84	11(5) 1,84	18(4) 3,01	35†(10) 5,85	9(1) 1,40	25(13) 3,88	29(5) 4,50	63(19) 9,78
9-12	1 0,44	8(3) 3,55	14(5) 6,22	23(8) 10,21	1(1) 2,00	4 7,99	2 4,00	7(1) 13,99	2(1) 0,73	12(3) 4,36	16(5) 5,81	30(9) 10,90	4 1,35	9(5) 3,05	8(2) 2,71	21(7) 7,11
Total	28 0,99	51 1,80	42 1,48	121 4,28	5 0,74	17 2,51	29 4,29	52 7,71	33 0,94	68 1,94	71 2,03	173 4,94	83 2,21	149 3,96	137 3,64	371 9,86

Prostate cancers classified in risk groups (low, intermediate, high/advanced). Bold figures represent absolute numbers, figures within brackets represent number of cases detected at opportunistic screening and small figures below represent incidence per 1000 person-years. Attendees = attended screening at least once, Nonattendees = never attended screening. The p values reflect the difference in incidence rate between total screening group and the control group.

† One case with unknown risk factors excluded.

‡ Two cases with unknown risk factors excluded.

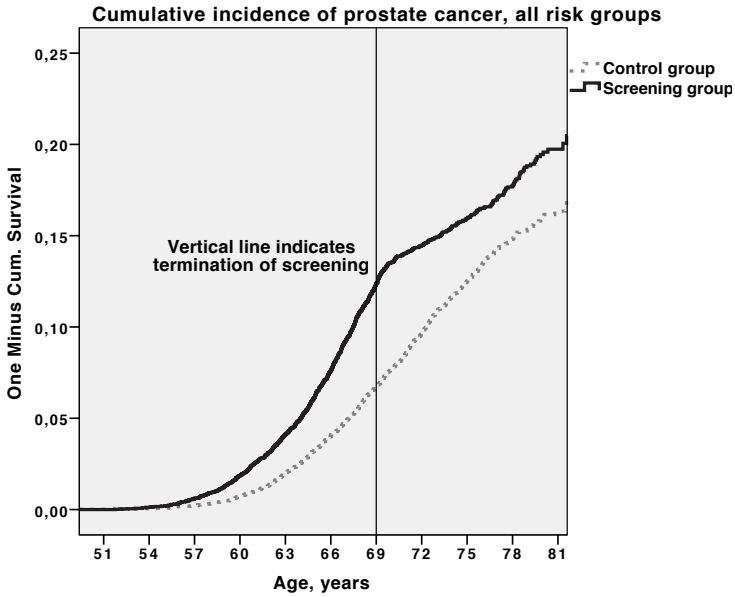


Figure 12. Kaplan-Meier estimates of cumulative incidence of prostate cancer (all), by age, in the screening and control groups.

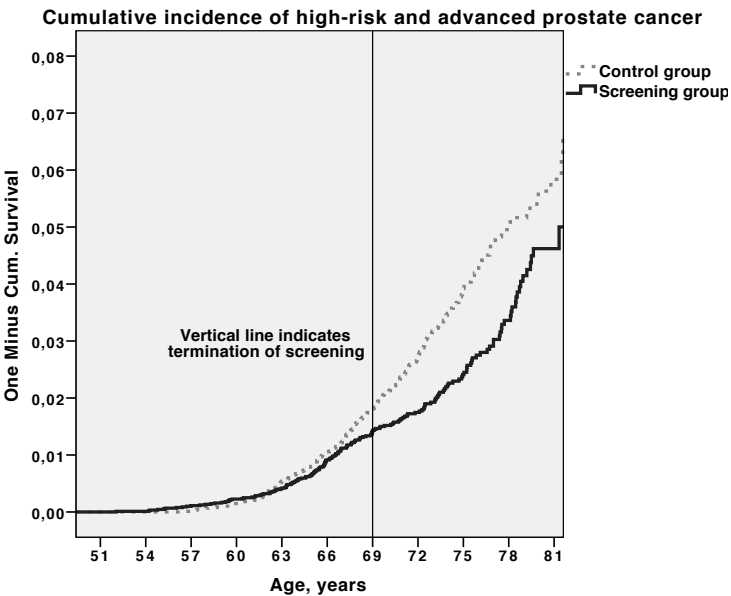


Figure 13. Kaplan-Meier estimates of cumulative incidence of high-risk and advanced prostate cancer, by age, in the screening and control groups.

Mortality

During follow-up, the number of PC deaths was 18 in the screening group and 37 among controls, and a tendency was observed toward increased PC mortality in the screening group with time, reaching a similar rate as controls after about 9 years (Table 11).

Table 11. Number of prostate cancer deaths and death rates in each study groups after termination of screening, by 3-year intervals.

Time interval	Screening group		Control group		RR
	Number of PC deaths	DR per 1,000 py	Number of PC deaths	DR per 1,000 py	RR
0-3	0	0	5	0.86	0
3-6	6	1.70	14	3.62	0.43
6-9	6	2.86	13	5.59	0.46
9-12	6	6.23	5	4.63	1.2
Total	18	0.50	37	0.94	0.49

PC=prostate cancer, DR=death rate, py=person-years, RR=relative risk (relative death rate)

4.4 Paper IV

This pilot study investigated the role of imaging of the prostate with MRI as an additional tool in screening for PC. Of 596 men invited to the 10th and last screening round of the Göteborg randomized screening trial, 384 (64%) attended (median age 69.3 years). Of those, 172 had a PSA above the cut-off (1.8 ng/ml) and were offered an MRI, which 127 (74%) attended. One third of the MRIs were positive (PIRADS 3, 4, or 5) and almost half (19/40) of TBs were positive for cancer. Totally 28 PC were detected, of which 7 were detected in the PSA range 1.8-3.0 ng/ml (Figure 14).

The outcome of each screening strategy (PSA \geq 1.8 ng/ml + MRI, PSA \geq 3 ng/ml + MRI, and PSA \geq 3 ng/ml) in men attending the 10th screening round is shown in Table 12. Depending on PSA cut-off, 19.8% men had an MRI indication when 3.0 ng/ml was used, compared with 44.8% when 1.8 ng/ml was used as cut-off for MRI. The proportion of men with a biopsy indication differed between the strategies, ranging from 6.48% with PSA \geq 3.0 ng/ml + MRI to 20.05% with the reference strategy. The strategy PSA \geq 1.8 ng/ml + MRI yielded a 36% higher overall cancer detection rate compared with the reference strategy and detected 48% more significant cancers (modified Epstein criteria).

Table 12. Cancer detection rates in the 10th screening round of the Göteborg randomized screening trial, using three different screening strategies (PSA ≥ 3.0 ng/ml followed by systemic biopsy, PSA ≥ 3.0 ng/ml + MRI followed by targeted biopsy, and PSA ≥ 1.8 ng/ml + MRI followed by targeted biopsy).

Attendees in the 10th screening round (n=384)	PSA ≥ 3 (SB)	PSA ≥ 3 + MRI (TB)	PSA ≥ 1.8 + MRI (TB)
No. of men with elevated PSA	77	77	172
No. of men with MRI indication (proportion)	0	77/384 (19.8%)	172/384 (44.8%)
No. of men undergoing MRI (proportion)	0	65/77 (84.4%)	127/172 (73.8%)
No. of men with positive MRI (proportion)	0	21/65 (32.3%)	42/127 (33.1%)
No. of men with bx indication (proportion†)	77 (20.05%)	21 (6.48%)	42 (14.81%)
No. of men biopsied (proportion)	70/77 (90.9%)	20/21 (95.2%)	40/42 (95.2%)
No. of PC detected (rate††)	18 (5.16%)	12 (3.89%)	19 (7.04%)
No. of significant PC (rate)	14 (4.01%)	11 (3.56%)	16 (5.93%)
No. of insignificant cancer (rate)	4 (1.15%)	1 (0.32%)	3 (1.11%)
No. of GS ≥ 7 PC (rate)	9 (2.58%)	7 (2.27%)	10 (3.70%)
No. of GS 6 PC (rate)	9 (2.58%)	5 (1.62%)	9 (3.33%)
NNB per PC	4	2	2
NNB per significant PC	5	2	3
NNB per GS ≥ 7 PC	8	3	4
NNMRI + TB per PC	0	5	7
NNMRI + TB per sign PC	0	6	8
NNMRI + TB per GS ≥ 7 PC	0	9	13

†Proportions calculated as number of men with a positive MRI/number of men attending MRI multiplied by proportion of men with elevated PSA. †† Rates calculated as number of cancers detected/number of men biopsied multiplied by proportion of men with biopsy indication. Cancers classified as significant/insignificant according to the modified Epstein criteria (insignificant cancer = Clinical stage T1c, Gleason score ≤ 6 , PSA density ≤ 0.15 , ≤ 2 sectors with cancer, unilateral cancer). TB = targeted biopsy, SB = systematic biopsy, GS = Gleason score, MRI = magnetic resonance imaging, bx = biopsy, NNB = number needed to biopsy, NNMRI = number needed to refer for MRI, PSA = prostate-specific antigen, PC = prostate cancer

Significant cancers were missed with all three screening strategies (Table 13). Compared with the reference strategy, sequential testing with PSA, cut-off 3.0 ng/ml + MRI significantly improved specificity but decreased sensitivity. Sequential testing with PSA, cut-off 1.8 ng/ml + MRI on the other hand, significantly improved specificity *without* decreasing sensitivity compared with the reference strategy. Sensitivity was significantly improved with PSA, cut-off 1.8 ng/ml + MRI compared with PSA, cut-off 3.0 ng/ml + MRI (Figure 15, Table 14).

The results of the sensitivity analysis with 5 simulated “missed” cancers in the non-biopsied men with PSA 1.8-2.99 ng/ml showed that these “missed” cancers affected all 3 screening strategies negatively but proportionally, meaning that the significant differences observed between the strategies remained even in such a scenario (data not shown).

Table 13. (A) Characteristics of 7 prostate cancers detected at systematic biopsy in men with a PSA ≥ 3.0 ng/ml and no abnormality on MRI and (B) characteristics of 7 prostate cancers detected with MRI followed by TB in men with PSA 1.8-2.99 ng/ml (and missed by the reference strategy as well as PSA ≥ 3.0 ng/ml + MRI).

A)

	PSA	T-stage	Gleason	Biopsy mode	No. of sectors with cancer	Modified Epstein criteria
1	3.47	T1c	3+3=6	SB	1/10	NS
2	4.05	T1c	3+3=6	SB	3/10	S
3	3.53	T1c	3+3=6	SB	1/10	NS
4	3.32	T1c	3+4=7	SB	1/10	S
5	6.83	T1c	3+4=7	SB	4/10	S
6	4.04	T1c	3+3=6	SB	1/10	S
7	3.03	T1c	3+3=6	SB	1/10	NS

B)

	PSA	T-stage	Gleason	Biopsy mode	No. of sectors with cancer	Modified Epstein criteria
1	2.32	T2a	3+4=7	SB + TB	5/ 10	S
2	2.57	T2a	3+4=7	SB + TB	6/10	S
3	1.82	T1c	3+4=7	SB + TB	5/10	S
4	1.94	T2a	3+3=6	SB + TB	2/10	S
5	2.04	T1c	3+3=6	SB + TB	2/10	NS
6	2.94	T1c	3+3=6	TB	1/10	S
7	2.89	T1c	3+3=6	TB	2/10	NS

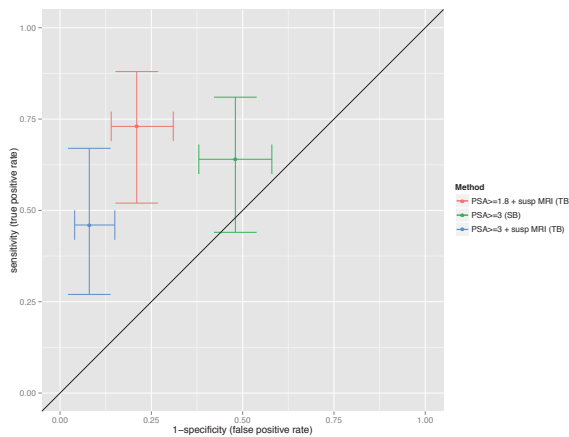


Figure 15. Plot showing sensitivity and 1- specificity and confidence intervals of three screening strategies (PSA cut-off 1.8 ng/ml followed by MRI, PSA ≥ 3.0 ng/ml followed by MRI, and the reference strategy PSA ≥ 3.0 ng/ml).

Table 14. Test performance (A) with the three screening strategies and comparison between strategies for significant differences (B).

	1. PSA \geq 3 (SB)		2. PSA \geq 3 + MRI (TB)		3. PSA \geq 1.8 + MR (TB)	
	%	95% CI	%	95% CI	%	95% CI
Sensitivity	0.64	0.47 - 0.82	0.46	0.27 - 0.65	0.73	0.56 - 0.90
Specificity	0.52	0.43 - 0.62	0.92	0.86 - 0.97	0.79	0.70 - 0.87
PPV	0.27	0.16 - 0.37	0.60	0.39 - 0.81	0.48	0.32 - 0.63
NPV	0.84	0.75 - 0.93	0.87	0.80 - 0.93	0.92	0.86 - 0.98
LR+	1.35	0.96 - 1.90	5.65	2.58 - 12.37	3.41	2.19 - 5.32
LR-	0.68	0.40 - 1.16	0.59	0.41 - 0.84	0.34	0.18 - 0.65
Accuracy	0.55	0.46 - 0.63	0.82	0.76 - 0.89	0.77	0.70 - 0.85

	Strategy 1 vs. 2	Strategy 1 vs. 3	Strategy 2 vs. 3
Sensitivity	0.21	0.47	0.008
Specificity	<0.001	0.001	<0.001
PPV	<0.001	0.006	0.09
NPV	0.55	0.17	0.03

PPV=positive predictive value, NPV = negative predictive value, LR+ = positive likelihood ratio, LR- = negative likelihood ratio, PSA = prostate-specific antigen, MRI = magnetic resonance imaging, TB = targeted biopsy, SB = systematic biopsy, CI = confidence interval.

Biopsy

Attendees in the 10th screening round were previously screened to a large extent. Only 2% were first-time screenees in the 10th round, while the remaining 98% had been screened with PSA 1-9 times before. As many as 37% (33/90) of men referred for biopsy were previously biopsied and 63% were never biopsied (57/90). Of those attending MRI, 53 were biopsy-naïve and 29 previously biopsied. The biopsy-naïve men were three times more likely to have a positive MRI (64%; 34/53 vs. 21%; 6/29). However, the risk that cancer was found at TB was about the same, irrespective of whether the man had been biopsied before or not (16 PC found in 34 (47%) biopsy-naïve men undergoing TB and 3 PC found in 6 (50%) previously biopsied men undergoing TB). Of all biopsies performed in this study (irrespective of screening strategy), TB was significantly more effective than SB on a per-patient basis, with a cancer-positivity rate of 48% (19/40) vs. 26% (23/90), p=0.014 and a cancer positivity-rate of significant PC of 40% (16/40) vs. 20% (18/90), p=0.017.

5 DISCUSSION

5.1 Paper I: Interval cancers

Paper I compared IC rates between two centers of the ERSPC that used different screening intervals (Göteborg and Rotterdam). The 10-year cumulative incidence of PC was significantly higher (13.14%) with the Göteborg 2-year interval compared with the Rotterdam 4-year interval (8.41%), $p < 0.001$. The 10-year cumulative incidence of IC was very low in both centers, at 0.74% in Göteborg vs. 0.43% in Rotterdam, $p = .51$.

It seems surprising that the shorter interval yielded an increased detection rate of IC but caution should be taken when comparing absolute IC rates between two different populations (see chapter 3.3). A more appropriate measure of comparison, the PICR (relating IC rates to the rate of PC in the control groups), revealed more intuitive figures, 11% in Göteborg and 18% in Rotterdam. Corresponding figures reported in mammographic screening is considerably higher, around 30-50%(181). One interpretation of a PICR of 30% in breast cancer screening is that $100 - 30 = 70\%$ of all cancers that would otherwise have become clinical during the screening interval actually were detected at the previous screening. This reasoning cannot be directly applied in PC screening according to Paper I, because the majority of IC were low-risk T1c tumors without symptoms, suggesting that most of these men were diagnosed due to opportunistic screening rather than due to symptoms from rapidly growing tumors. Nevertheless, the PICR is a measure of a program's ability to detect cancers, and a low PICR is preferable since it means that cancers are detected at screening rather than in between screenings.

The demonstrated low PICR and the significantly increased detection rate of PC overall in Göteborg compared with Rotterdam indicates a more effective screening strategy. A lower proportion of advanced cancers at diagnosis in Göteborg according to reports from Hugosson(182) and van der Cruijsen-Koeter(183) were other indicators of this already at the time of writing Paper I. Later on, in 2012, Van Leeuwen compared the Göteborg 2-year strategy with Rotterdam's 4-year strategy by using the ratio of observed number of advanced cancers ($\geq T3a$, or N1/M1, or PSA > 20 ng/ml, or GS ≥ 8) to the expected number of advanced cancer based on the control group. That study demonstrated a 43% reduced incidence of advanced cancers with a 2-year interval compared with a 4-year: relative risk (RR) of advanced cancer 0.40 (95% CI=0.22-0.71) vs. 0.69 (95% CI=0.50-0.96), yielding a RR of 0.57 (95% CI=0.33-0.99; $p = 0.048$)(184). Thus, it appears that more advanced

cases are detected with a more intensive screening strategy. Whether this outweighs the harms associated with screening in terms of overdiagnosis, unnecessary biopsies, and costs remains to be established, but fresh data from Göteborg indicate that overdiagnosis reaches a steady state after about 4 screenings, so there does not seem to be a large concern regarding number of screenings(185).

In conclusion, the frequency of IC is a poor indicator for PC screening effectiveness. Most IC are not aggressive or symptomatic which makes them different from IC described in mammographic screening. When it comes to comparing the efficiency of different PC screening strategies, proportional incidence rates of advanced cancer is the method of choice.

5.2 Paper II: Screening failures

This paper showed that attendees in repeated screening contributed little to the intermediate-term mortality, whereas nonattendees constituted a high-risk group both for death from any cause as well as for PC death. It is well-known that people who volunteer to participate in screening are more health conscious and generally healthier, a phenomenon referred to as “healthy screenee effect” or “healthy volunteer bias(186). This makes them different from those who choose not to participate. The observed improvement in PC-specific mortality in attendees in Paper II can therefore not be ascribed to screening only. However, a part of it was interpreted as an early indication of a screening benefit at the time of writing Paper II. Indeed, in the 2010 publication on mortality after 14 years in the Göteborg randomized screening trial it was demonstrated that screening was effective and that attendees had a 56% lower PC-specific mortality compared with the control group(62).

Others have described the considerably lower life expectancy in PC-screening nonattendees. In 2009, Kjellman et al. reported results from a screening study initiated in 1988 in the Stockholm region, where 2,400 men were randomized to a single screening with a combination of DRE, TRUS and PSA. Although the screening algorithm and the management of screen-detected cases can be questioned in this study, overall mortality among nonattendees was almost doubled that of attendees, with an incidence rate ratio of 1.89 (95% CI=1.65-2.16) after 13 years, a difference attributable to death from causes other than PC(187). It has also been demonstrated that participants in the PLCO trial have a lower-than-expected overall mortality compared with that of the general population (calculated based on SEER data)(188).

Nonattendance – a barrier for screening effectiveness

Researchers from the Finnish ERSPC center recently evaluated the impact of nonattendance on the effects of screening on mortality (189). Since the relatively conservative Finnish screening strategy yielded only a modest, statistically not significant mortality reduction (HR 0.85 (95% CI=0.69-1.04) after 11.9 years), the researchers studied possible factors for screening failure and compared them by using a counterfactual exclusion method(190). Of 9,251 men dying in the screening arm, 241 died from PC (cumulative PC-mortality 0.76%). When they corrected for non-participation in the screening arm, the cumulative mortality from PC was 0.64% (and HR compared to the uncorrected control arm was 0.71, 95% CI=0.59-0.86). Similar corrections were made for other potential explanations (PSA cut-off for biopsy of 4.0 ng/ml instead of 3.0 ng/ml and IC occurrence due to a 4-year screening interval instead of a 2-year interval) to evaluate which aspect contributed the most to screening failure. It was demonstrated that non-participation in the screening group had the greatest impact on the magnitude of mortality reduction, greater than the other factors assessed (189).

Thus, nonattendees limit screening effectiveness. It might be warranted to improve on participation in PC screening, especially since 15 of 16 nonattendees were classified as high-risk at diagnosis in Paper II. Efforts have been made to improve attendance in screening for other cancers. High attendance is especially important when nonattendees are at an increased risk of harboring advanced disease (as is typically the case in cervical cancer screening). It has been reported that sending reminder letters and self-sampling kits to nonattendees in routine cervical cancer screening in Finland increased the participation by 10% while increasing the detection rate of CIN3+⁸ cancers by 24%(191). Others have demonstrated similar results(192, 193).

When interpreting the results of Paper II, it is important to remember that there probably was a selection of more advanced cases already at randomization among those who later turned out to be nonattendees (due to a potential resistance towards seeking medical attention). This potential selection bias is overcome though randomization in intention-to-screen analyses.

⁸ CIN=Cervical Intraepithelial Neoplasia. CIN is graded from 1 to 3 (3 being the most severe dysplasia or cancer in situ).

Who dies from PC despite invitations to PSA screening?

Very few deaths were related to cancers detected at repeated screening. Apart from 3 IC, only 3 of the 39 PC deaths in the screening group occurred in men that had been screened at least twice and per-protocol. The majority of those who died were either nonattendees or incomplete attendees. Another important finding in this study was that almost one third of PC deaths (12 of 39) were related to prevalent cases detected at the initial screening, at which all of those men were ≥ 60 years of age. This indicates that starting screening at age 60 probably is too late. Had these men been invited at younger ages, it is likely that at least some of them would have had a better prognosis. The updated results confirm the findings that the majority of complete attendees dying from PC were detected at the first screening. Although five of those men with prevalent cancers were < 60 years of age, the vast majority were above age 60 when they were first invited. However, longer follow-up is necessary because lead- and length-time biases may still affect the results.

5.3 Paper III: Prostate cancer incidence above screening age

Screening should be restricted to those who might benefit from it, but how do we define those? One of the main concerns with screening is overdiagnosis, which involves unnecessary biopsies, psychological consequences and overtreatment. Age has been suggested to be a major driver for overdiagnosis because older men are more likely to die from other causes than PC (194). Nevertheless, most men dying from PC are 80 years or older, and age is a risk factor for being diagnosed with aggressive disease(195). Increasing life expectancies in the Western world has drawn growing attention to the incidence and characteristics of PC in elderly men.

In this study, where men had their last invitation to screening at a median age of 69 years, screening reduced the risk of being diagnosed with potentially aggressive PC for up to 9 years after screening cessation. Thus, screening had a “protective” effect that lasted for 9 years, but thereafter the screening arm caught up and had about the same risk as the controls.

Characteristics of cancers detected post-screening

Cancers detected in these elderly (69+ years) men were more advanced at diagnosis compared with screen-detected cancers. Only a small proportion (19%; 33 of 173) were low-risk cancers, compared with cancers detected

during the active screening phase (over 50% low-risk)(62). This finding is consistent with previous studies (195, 196). It has been shown that locally advanced PC has a considerably worse prognosis with higher PC-specific mortality compared with low- and intermediate-risk localized tumors, already at 4 years of follow-up and even in older men(197). In addition, Albertsen demonstrated that men ≥ 75 years of age at diagnosis have a higher PC-specific mortality than men aged 66-74 at diagnosis at a specific stage and grade of PC(198). Underuse of treatment with curative intent among older men with high-risk disease may partly explain these differences(199). However, there is also evidence that lead-time is shorter in older men(200). Taken together, these factors raise concerns over potential underdiagnosis and undertreatment of aggressive PC in older but otherwise healthy men.

The fact that a large proportion of PC was advanced at diagnosis post-screening may appear paradoxical, considering that opportunistic PSA testing seems to have been fairly commonly occurring. However, it appears that men in the screening arm were less prone to undergo opportunistic PSA testing post-screening as indicated by the marked drop in incidence immediately after screening cessation at age 69 (Figure 12 and 13). This might have been an effect of the letter that men in the screening group received in conjunction with their last invitation to screening, saying that the risk of death from PC was small and that they therefore did not need to be tested any further. The controls, on the other hand, were subjects for widespread PSA use seen in Sweden today. From NPCR data, it is estimated that about one third of all Swedish men in the ages 50-75 had a PSA test between year 2000 and 2007(201). An unequal “screening exposure” between the groups may bias the comparison. On the other hand, we do not know how this opportunistic testing was outlined or whether it was effective at all. Recent evidence indicates that unorganized and less intensive PSA testing does not reduce PC mortality(202, 203). One may question the ethics behind the potentially misleading letter regarding the lack of need for further testing that was given to men in the screening group at the time when they reached the upper age limit for further screenings, especially with the results of Paper III in hand. Even now, there is no consensus regarding when it is “safe” to stop screening (at least not based on chronological age).

To conclude, the beneficial effects from PSA screening in terms of stage at diagnosis seems to last for up to 9 years after screening cessation at age 69. Considering the high PC mortality rate in men >80 years, discontinuing screening at this age might be too early, at least in a subset of men. Instead, a flexible stop-age is suggested, based on risk stratification and life expectancy.

5.4 Paper IV: A novel tool in prostate cancer screening

Screening for PC has been shown to reduce PC-specific mortality, but the effect is moderate and risks are involved. There is an urgent need for a more selective screening strategy that can circumvent the shortcomings of PSA in terms of low specificity. In addition, we need to find strategies to increase the detection of potentially aggressive cancers and minimize the detection of indolent cancers. We conducted a pilot study to investigate whether imaging with MRI can be used to increase the benefits of PSA screening for PC. Three screening strategies were compared, including the “reference strategy” (PSA ≥ 3.0 ng/ml followed by SB) and two sequential screening strategies using PSA with different PSA cut-offs (1.8 and 3.0 ng/ml) followed by MRI to select men for TB.

According to this pilot study, sequential testing with PSA followed by MRI significantly improved specificity for PC detection compared with using PSA as the stand-alone screening test. However, this came at a cost of lowered sensitivity when the PSA cut-off for MRI was set at 3.0 ng/ml. By lowering the PSA cut-off to 1.8 ng/ml, specificity remained significantly improved compared with the reference strategy while sensitivity was maintained. Accuracy is a measure where both sensitivity and specificity are taken into account and according to this measure, PSA ≥ 3.0 ng/ml + MRI followed by TB was the most accurate of the three strategies compared. Consequently, the results of this pilot study indicate that sequential testing with MRI may be used to increase specificity, but the optimal PSA cut-off to indicate an MRI (and keep satisfactory sensitivity) remains to be established. The size of this pilot study was too small to allow for comparisons between, for instance, a cut-off of 2.5 ng/ml with 1.8 ng/ml or 3.0 ng/ml. However, such calculations will be possible in the newly launched large-scaled trial (the Göteborg-2 study), where 20,000 men will be randomized to sequential screening.

Sequential testing has been evaluated both in colorectal and cervical cancer screening as an attempt to reduce the number of false-positives and to make efficient use of available screening resources (204, 205). It is used to increase specificity, but it usually also decreases sensitivity as screenees are exempted from further testing(206). In this pilot study, all 3 screening strategies missed significant cancers. However, PSA ≥ 1.8 ng/ml + MRI detected slightly more significant cancers compared with the reference strategy (16 vs. 14) and fewer insignificant cancers (3 vs. 4). Strategy number 2 (PSA ≥ 3.0 ng/ml + MRI) detected only one insignificant cancer,

but had a significantly lowered sensitivity for PC detection overall compared with PSA ≥ 1.8 ng/ml + MRI, and had the lowest detection rate of significant cancers. Although these are encouraging results, validation in larger trials is necessary.

Our results are in line with several studies on pre-biopsy MRI in the diagnostic pathway in men with suspected PC. It has been demonstrated that MRI has a high NPV (over 90%) and can be used to select men for biopsy (207-209). TB has also been shown to be more effective than SB in detecting significant PC, according to a recent meta-analysis of 16 studies comparing TRUS-guided SB with MRI-TB(210). Siddiqui et al. recently assessed TB vs. SB and the 2 approaches combined, for their ability to diagnose high-risk PC. The results were published in JAMA and showed that TB detected 30% more high-risk cancers compared with SB (173 vs. 122 cases, $p < .001$), and that TB detected 17% fewer low-risk cancers compared with SB (213 vs. 258 cases, $p < .001$)(211).

MRI as a screening tool?

One may question MRI as a screening tool due to its costs and limited accessibility. In our pilot study, as much as 44.8% of attendees in the 10th screening round needed an MRI if the PSA cut-off was set at 1.8 ng/ml, or 19.8% if the cut-off was set at 3.0 ng/ml. However, these men were all born 1944 and the median age was 69.3. Had they been younger, the median PSA value would have been lower (and fewer would have an indication for MRI). Recent years have shown a rapid development in imaging techniques for PC detection, but covering the increasing demands is a challenge because it requires properly trained radiologists and access to the equipment. When considering increased MRI-use, one must weigh costs with today's screening that results in a huge problem with overdiagnosis, overtreatment, and loss in quality of life (QoL) due to side effects from treatment etc. Recently, de Rooij investigated the cost-effectiveness of integrating MRI and TB into the diagnostic pathway of PC detection and compared that to the standard diagnostic pathway. The investigators used a Markov model and analyzed Quality-Adjusted Life Years (QALYs) and health-care costs with both strategies, and reported that the cost of both strategies was equal but that the MRI strategy led to improvements in QoL by reducing overdiagnosis and overtreatment(212).

Another way to approach the cost issue is to search for a primary screening test with a higher specificity than PSA (reducing the number of men with indication for MRI). Much research is currently underway regarding novel

biomarkers, and emerging evidence suggests that Phi is especially useful in predicting significant PC(213). It is less expensive than PCA3 and does not require prostate massage before measurement, which makes it more suitable for screening purposes compared with PCA3(214). However, further validation of these novel tests as well as clinical studies comparing different screening strategies are needed before determining the optimal method of screening for PC.

To summarize, this pilot study indicates that MRI can be used to increase specificity in screening for PC and reduce unnecessary biopsies. Fewer men need a biopsy and fewer cores are sampled from each man. The results also suggest that MRI may aid in the detection of significant cancers. The Göteborg-2 study will provide more information on whether and how prostate MRI should be used in a screening setting. The following questions need to be assessed in future analyses:

1. Should MRI be integrated in the diagnostic pathway of early detection of PC?
2. Will MRI reduce the problem of overdiagnosis?
3. Will MRI reduce the problem of underdiagnosis?
4. What is the optimal screening interval after a negative MRI given the reported high NPV?
5. Can SB safely be abandoned in favor of TB?
6. What is the optimal approach for targeting biopsies?
7. Would screening with MRI be feasible? What about acceptance, logistics, costs-effectiveness, and accessibility?

6 GENERAL DISCUSSION AND FUTURE PERSPECTIVES

Screening with PSA for early detection of PC remains a controversial issue. Although large randomized trials have demonstrated a risk reduction of PC-specific mortality with screening, risks are involved (62, 215). The uncertain net benefit has called for research for more selective screening that reduces unnecessary harm while preserving or improving the benefit. One essential step on the way is to analyze the shortcomings of today's screening.

6.1 Overview of the results and implications for further research

Paper I studied screening intervals, as the optimal interval has not been established. By comparing a 2-year interval with a 4-year interval, we concluded that a 2-year interval detects significantly more PC and more potentially aggressive PC. Although absolute numbers of IC were low with both intervals, the proportional rate of observed vs. expected IC was lower with a 2-year interval. Hence it appears, quite intuitively, that a shorter interval is more effective in detecting cancers than a longer one, but this benefit must be balanced against the increasing risk of overdiagnosis. Although screening interval seems to have a smaller effect on overdiagnosis as compared to age and PSA cut-off, overdiagnosis is responsible for a major part of the predicted difference between life-years and QALYs gained with screening, according to modeling studies(140, 194).

Paper II described nonattendees in screening as a group with a significantly higher all-cause mortality and PC-specific mortality. Even after adjusting for competing risks, nonattendees had a doubled risk of PC death compared with attendees, and their cancers were more aggressive at diagnosis. If we want to increase the benefit of screening further, this group might need specific attention. To date, almost 40% of all PC deaths occurred in the approximately 20% that were nonattendees in the Göteborg study.

One field of further research includes looking into reasons for nonattendance. Reflected by the increase in all-cause mortality, nonattendees are probably generally unhealthier. However, there is a wide range of other potential reasons for nonattendance that might be important to identify.

Cultural barriers, language difficulties, costs⁹, and lack of proper information may result in involuntary non-participation. One American study investigated reasons for nonattendance in a free PC screening program in Southeastern United States. Through telephone surveys, the researchers found that “time problems” was the major self-reported reason for nonattendance, and that appointment reminders were critical(216). Considering today’s debate about the uncertain benefit of screening, nonattendees may also have made a well-informed decision not to participate in screening. Proper information seem to be important, as knowledge and beliefs about PC screening has been shown to predict attendance for PSA testing and biopsy(217). If screening were to be implemented in the general population, these issues are important in order to provide an equal care for everyone.

Paper III analyzed what happened after screening cessation at an upper age of 69 years. We found that the “protective” effect in terms of reduction in the risk of being diagnosed with advanced PC decreased with time and reached that of the screening group after 9 years. The median age for PC death is 80 years today in Sweden(11), and if we want to reduce PC-specific mortality further, we need to consider what to do with these elderly men affected with aggressive PC. Would it be possible to advance the diagnosis of those cancers by prolonging screening beyond age 69, at least in healthy 69-year-olds? If so, would these men benefit from radical treatment? An aging population will most likely increase the demand for answers to these questions in the near future. One way forward may be to consider a risk stratified and individualized screening in healthy older men.

Lastly, **Paper IV** looked further ahead towards what might become the future of early detection of PC. The results indicated that imaging with MRI increases specificity in screening for PC. MRI might also pave the way towards a more precise sampling of lesions instead of the blind random sampling of whole organs that has characterized early detection of PC for decades. Although encouraging, these results need to be confirmed in larger trials before any recommendations can be made regarding screening with MRI.

⁹ Cost is probably less of an issue in Sweden because the Swedish health care system is financed by a social insurance that provides all citizens with subsidized health care through the government. However, in countries where people need to have personal insurance or pay out of pocket to cover health care, costs might be a barrier for screening adherence.

6.2 How can we improve the performance of screening?

As discussed in this thesis, current screening strategies suffer from validity issues. It is our responsibility as researchers and physicians to continually look over and revise efforts towards improved public health. When it comes to the significant concern posed by PC, screening has the potential to reduce mortality. However, the optimal combination of methods with the largest gain and the least harm is yet to be defined. By describing the problem with current screening, potential areas of improvement are identified. The shortcomings of screening can very generally be divided into test-related (test performance, cut-off, gold standard/diagnostic test) and protocol-related issues (screening interval, age, nonattendance).

6.3 Test issues

The test used to screen for PC is suboptimal. PSA produces too many negative biopsies and PSA alone has a limited ability to predict significant cancer. But what are alternatives?

Several novel biomarkers have been presented including refinements and combinations of already available ones. One vital objective is to determine which available, acceptable, minimally-invasive and accurate test to use to screen for significant cancer. A French research group recently addressed this question by prospectively comparing the diagnostic accuracy of Phi and PCA3 in screening for overall and significant PC. PCA3 was demonstrated to be the most accurate predictor for PC overall, but for accurately predicting clinically significant PC, Phi outperformed PCA3 (AUC 0.80 vs. 0.55, $p=0.03$). In multivariate analysis, a PHI >40 was shown to be the only independent predictor after adjusting for PSAD >0.15 and PCA3 >35 (213). Loeb and colleagues compared Phi with f/t PSA and [-2]pro PSA in men aged >50 years with PSA between 4 and 10 ng/ml in a prospective multicenter trial investigating the role of Phi. This study found that Phi had a greater predictive accuracy for clinically significant PC than its individual components(218).

The diagnostic test (TRUS-guided SB) is also imperfect. It is prone to sampling error, and there is an increasing awareness of its limitations(219). Especially in times when conservative management is advocated to reduce overtreatment(220), it is essential that cancers be accurately classified. PC is now the only solid organ tumor diagnosed without tumor imaging and a

directed sampling method(221). Screening and early detection can be improved by using pre-biopsy imaging with MRI according to **Paper IV** of this thesis. In our study, MRI functioned as a secondary screening test to select men for further work-up and to exempt those with non-suspicious findings, which increased specificity substantially. In addition, it functioned as a method of improving the diagnostic biopsy.

Other studies support our findings. Recently, investigators from the National Institute of Health (NIH) led by Peter Pinto reported results from a study where 143 biopsy-naive patients underwent bi-parametric MRI (bpMRI), including T2WI and DWI, as a complement to PSA testing prior to any biopsy¹⁰. If lesions on bpMRI were suspicious for cancer, patients underwent fusion-guided TB in addition to a 12-core SB during the same session. Different diagnostic modalities were combined and compared and test performance was evaluated. The combined method of bpMRI + PSA yielded a high sensitivity (90%) with moderate specificity (63%). Specificity increased when PSAD + bpMRI was used together (86%), but with reduced sensitivity (74%). They also concluded that PSAD + bpMRI reduced the number of “test-positives” by 3.8 fold, compared to PSA alone. This meant that the efficiency of identifying men with PC increased from 67% of “PSA test-positive” men actually harboring cancer to 89% of “PSAD + B-MRI test-positives” harboring PC(222).

The Swedish Council on Health Technology Assessment (SBU) recently published two reports regarding imaging techniques in PC diagnosis and staging. MRI was evaluated together with positron emission tomography (PET) scan, PET with computer tomography (PET/CT), ultrasound with Doppler and other ultrasound-based techniques including elastography and histoscanning. Both reports concluded that the evidence on MRI in the detection and staging of PC was insufficient to draw any conclusions on the usefulness of MRI compared with today’s methods(223). This report has been criticized for its strict methodology in selecting evidence. As always when new techniques are emerging, there is great heterogeneity across studies, making systematic reviews particularly difficult. However, the body of evidence of MRI as an aid in the detection of PC is growing. It remains to be proven which imaging technique and biopsy targeting system is the most advantageous, but much evidence points towards a future where *lesions* are detected and targeted, not *organs*.

¹⁰ Bi-parametric MRI with DWI and T2W has the advantage that it does not require contrast injection and requires less than half in-bore magnet time compared with a complete mpMRI, making it a less invasive and more accessible method.

6.4 Protocol issues

Different screening strategies are unequally effective

Few clinical studies have compared different screening algorithms, although ERSPC may be regarded as such a study(184, 224). The reduction in PC-specific mortality differs substantially between individual ERSPC centers. Of the major centers, the relative risk reduction of PC-specific mortality ranges from 9% in Finland to 38% in Sweden at 13 years(215). Although screening intensity is not the only factor that differs between these populations, it appears as if the largest reductions are obtained with the more intensive programs (PSA cut-off 2.0 ng/ml instead of 4.0 ng/ml, 2-year instead of 4-year intervals, lower starting age for screening). However, it is important to remember that background risk and frequency of opportunistic screening in the control groups also influence the relative risk reduction as well as follow-up time.

Does this mean that the optimal screening strategy is intensive in order not to miss any lethal cancers? **Paper I** showed that more advanced cases were detected with a shorter interval. It is probably true that the more we look, the more we find, but as De Carvalho pointed out in a recent study where the MISCAN model was used to project the outcome of 83 different screening strategies, it is usually impossible to reduce PC-specific mortality without also increasing overdiagnosis(194). A modeling study by Gulati et al. demonstrated that the most intensive screening strategy yielded the highest cancer detection rate but also the highest overdiagnosis rate. According to their modeling estimates, one way to reduce overdiagnosis was to prolong the screening interval to 5 years in men with a PSA below the median, thereby lowering the average number of tests by one-third and overdiagnosis by one-quarter relative to a biennial strategy while only reducing the amount of lives saved by a relative 17%(225).

There is growing evidence that mid-life PSA testing can be used to predict future risk. One study from the Malmö Preventive Project demonstrated that men with PSA levels below median at age 45-49 years and 51-55 years have a very low risk, at 0.09% and 0.28%, of developing metastatic disease in 15 years(226). The same investigators also reported that it is very unlikely that men with PSA levels at or below median (≤ 1 ng/ml) at age 60 years have clinically relevant PC at 85 years of age (0.5% risk of M+ disease and 0.2% risk of PC death)(227). Therefore, it has been suggested that screening intervals (and time for screening cessation) ought to be individualized based on these mid-life PSA measurements(228). One recent report from the Swiss

branch of the ERSPC concluded that a “baseline PSA” measured at age 60.7 years was a powerful predictor for future PC. The authors proposed rescreening every 8 years for men with <1 ng/ml, every 4 years for men with PSA 1-2 ng/ml, and yearly screening in men with PSA 2-3 ng/ml at this age(229). There is also evidence that individualized screening intervals improve on QoL and have an advantage in cost-effectiveness over conventional uniform screening with annual or biannual PSA testing for every individual, which sounds very logical(230).

Age

There is no consensus regarding ages for screening initiation and cessation. According to **Paper II** and the updated results, screening starting at 60 years of age risks detecting a substantial proportion of cancers beyond cure. Regarding time for cessation, several investigators have proposed individualized approaches. Carter demonstrated in 1999 that no 60-year-old man with a serum PSA of ≤ 0.5 ng/ml developed PC in 15 years (in the Baltimore Longitudinal Study of Aging)(231). Carlsson et al. recommended against screening in men with PSA levels of ≤ 0.5 ng/ml at age 60 in a study mentioned previously, due to the very low risk of advanced disease within 15 years, according to data from the Malmö Preventive Project(228).

Few advocate screening in older men, but according to **Paper III**, stopping at 69 years might be too early. It has been shown that older men (≥ 70 years) have comparable clinical outcomes and cancer control after treatment for localized disease as younger men(232). In addition, older men do not seem to fare worse regarding QoL declines after treatment for localized disease, according to a recent study by Cooperberg et al. Rather, the authors reported, declines varied across all ages, and at 2 years, more men <60 years than those >70 years experienced declines in urinary function (14% vs. 9%), and sexual bother (39% vs. 17%)(233). To conclude, it seems that older men should not be declined treatment out of fear for loss in QoL. Active treatment is a viable option in healthy older men with high-risk disease and little comorbidity(234), and based on this, screening may be considered in a subset of elderly men.

Targeting risk groups

Another way of screening smarter might be to target specific risk groups. However, such risk groups with an increased predisposition for developing aggressive PC are not easily defined although men of African origin in the U.S. have a greater PC incidence and PC mortality compared with men of

European ancestry, and men in Sweden have an increased incidence and mortality compared with men in southern parts of Europe. There is a hereditary component, and men with a first-degree relative with PC have a higher risk of developing PC compared with those without a first-degree relative with PC (RR 2.48, 95% CI=2.25-2.74) (235). One multicenter study (the Identification of Men with a genetic predisposition of ProstAte Cancer, IMPACT study) has been initiated to evaluate targeted PSA screening in BRCA1 and BRCA2 mutation carriers, as these mutations are associated with an increased risk of PC and aggressive PC (236-238). Reports from the initial screening round were recently published, revealing a PPV for biopsy of 48% in BRCA2 carriers, using a PSA cut-off of 3.0 ng/ml, and 41% for BRCA1 carriers (to be compared with the PPV of cancer at biopsy in ERSPC of 24%). About two-thirds of cancers were classified as intermediate- or high-risk, according to D'Amico risk groups, also verifying that this is a group with an increased risk. More studies are needed to identify risk factors for PC.

6.5 Strengths and limitations with patient material and methods used

The Göteborg randomized screening trial forms a unique population. It is a large study comprised of about two thirds of all men, ages 50-64, living in Göteborg at the time of randomization. The study started in 1995 during a time when PSA testing was practically non-existent in the general population(47). However, during the study period PSA testing became increasingly used in the general population, which also affected the control group of the study. From NPCR data, it is estimated that about one third of all Swedish men, ages 50-75, had a PSA test between year 2000 and 2007(201). However, a recent study has shown very little, if any, effect on mortality with unorganized screening(202). Longer follow-up of the Göteborg screening trial will provide additional information on effects of screening on overdiagnosis, underdiagnosis and outcome of MRI-detected cancers.

6.6 Future perspectives

We need to find strategies to reduce unnecessary repeated testing in men with a low risk of developing advanced disease without missing out on the possibility of curing patients harboring significant cancer. This balance will probably always be fine, but modern diagnostic tools such as new biomarkers and imaging techniques may be of help in ambiguous cases.

According to studies on individualized screening, it seems appropriate to offer men a “baseline test” at a certain age and design rescreening based on the test outcome, possibly also considering comorbidity and hereditary factors. Much like today’s nomograms, each individual would fall into a certain risk category and be screened thereafter. This way, men at high risk of developing aggressive PC would be monitored and screened more intensively than men at low risk within a certain time frame. Whether such individualized screening would be efficacious remains unknown. Clearly, tailored strategies in mass screening require a large and robust organization, which would be resource-consuming. Another question is how well people would tolerate individually designed programs because one can argue that it violates principles of equal rights and lead to discrimination against certain groups in the society.

7 CONCLUSION

Before the implementation of a mass screening program, all aspects of the screening outcomes need to be thoroughly studied. Specifically, we need to search for more effective screening methods and the long-term effects must be well understood. Screening under present terms will not be recommended or even accepted among the general population or among clinicians before evidence shows that the benefit outweighs the harm.

This thesis explored factors associated with screening failures. It is essential to reduce screening failures and increase the detection of potentially aggressive cancers so that treatment can be offered and mortality can be further reduced. Much research has focused on lowering the harm associated with PSA screening, but we must not forget that screening first and foremost aims at saving men from PC death. Quite intuitively, the balance between benefit and harm is improved not only by diminishing harm but also by maximizing benefits. The magnitude of benefits depends on screening strategy, and it seems that screening should have a certain intensity to be worthwhile. Screening should probably be initiated before age 60 to reduce the risk of advanced disease at the point of diagnosis. Reasons for nonattendance need to be further explored, but this is a group with a large impact on crude as well as PC-specific mortality, which should be kept in mind when designing future screening programs. Age is a risk factor for being diagnosed with high-risk PC, and stopping screening at a chronological age of 70 years might be too early in healthy older men. MRI can aid in the detection and classification of significant PC and due to its high specificity and NPV, it appears to be a safe method to exempt men from unnecessary biopsy while possibly also increasing the detection of significant cancers. However, further studies are needed to determine the true value of MRI and how to best utilize this technology. The Göteborg-2 study will hopefully shed valuable light onto this relatively new field in screening for PC.

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