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PROSTATE CANCER: POPULATION BASED SCREENING AND MARKERS FOR LONG-TERM CLINICAL OUTCOME

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Prostate Cancer: Population Based Screening and Markers for Long-Term Clinical Outcome
THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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De ännu oföddas, de levandes

och de dödas stad. Med fotavtryck av

det förgångna och varsel om det kommande

inflätade intill varandra i nuets collage.

Ur *Stad i världen* Per Anders Fogelström 1968

POPULAR SCIENCE SUMMARY OF THE THESIS

Prostate cancer is the most common malignant disease in Swedish men. It is also the most common cause of cancer related death in Sweden. Roughly 10 000 men are diagnosed each year and 2 500 men die each year from prostate cancer. What makes matter complicated is that most men who are diagnosed with prostate cancer never, during their natural life span, develop symptoms from it. Eventually they die "*with* prostate cancer not *of* prostate cancer". Given that treatment of prostate cancer with either surgery or radiation therapy often cause serious side effects, it is important to distinguish which men that are in need of treatment and which men who are not.

Screening for prostate cancer, or looking for signs of prostate cancer, in the general population using a blood sample, physiological examination and radiology (x-ray) would likely decrease the number of men who die of prostate cancer. It would however be to the cost of diagnosing many men who would neither benefit from screening, let alone treatment.

In the four papers of this thesis, we try to gain more knowledge about screening for prostate cancer and to evaluate, new and old, markers that possibly can aid in deciding which men that should be treated.

In **paper I**, a screening trial launched in Stockholm in 1988 is evaluated. After twenty years of follow up, no lives were saved by screening for prostate cancer. One particular group of men, the ones that were invited to participate, but for some reason chose not to, died earlier than the ones that did participate. They died earlier both from prostate cancer but also from a variety of other causes.

We know that male sex hormones play an important role in the development of prostate cancer. Testosterone is the most common male sex hormone, but there is also dihydrotestosterone or DHT, a similar but somewhat "stronger" male sex hormone.

In **paper II** we examine if DHT promotes or protects from aggressive prostate cancer. Contrary to what one might assume, DHT protects from death in prostate cancer.

The most common way to test for prostate cancer is by a PSA test. The PSA-test, if abnormal, could indicate prostate cancer. In **paper III** we evaluate if the PSA-test, if *not* abnormal, instead can rule out prostate cancer, or at least assess future risk for prostate cancer as negligible. After 30 years of follow up, it turns out that low levels of PSA, (especially

combined with another test that measures the percentage of PSA bound to other molecules) render the future risk for aggressive prostate cancer as very low.

In **paper IV** a new marker was analyzed on old blood samples (from 1988 and 1989). The samples had been stored in a freezer with a temperature of approximately 80 degrees below zero since they were collected. The marker analyzed is called Thymidine Kinase 1 (TK1), a molecule that plays an important role in cell division. We could conclude that those who had higher levels of TK1 were at greater risk of dying from all causes, including prostate cancer.

Kort populärvetenskaplig översikt på svenska

Prostatacancer är den vanligaste cancerformen hos svenska män. Det är också den vanligaste cancerrelaterade dödsorsaken i Sverige. Ungefär 10 000 män får varje år diagnosen prostatacancer och ca 2 500 män dör varje år i sviterna av prostatacancer. En komplicerande egenskap hos just prostatacancer är att många män som får diagnosen prostatacancer aldrig under hela sitt liv utvecklar symptom av sin sjukdom. När de så småningom avlider gör de det *med* sin prostatacancer och inte *av* prostatacancer. Eftersom behandling för prostatacancer med kirurgi eller strålning ofta medför biverkningar, så är det viktigt att skilja på vilka män som behöver behandlingen och vilka som inte behöver den.

Screening för prostatacancer, eller sökande av tecken till prostatacancer i befolkningen med blodprover, kroppsundersökning och röntgen kan sannolikt minska andelen män som dör av prostatacancer men det finns en risk att man hittar många fall som inte gynnas av diagnos och än mindre av behandling för prostatacancer.

I avhandlingens fyra delarbeten försöker vi skaffa mer kunskap om screening för prostatacancer och vi utvärderar också gamla och nya markörer som förhoppningsvis kan ge oss stöd i att avgöra vilka män som behöver behandling.

I **delstudie I** utvärderar vi en screeningundersökning från 1980 talet. Efter 20 år finns ingen skillnad i andelen män som dog av prostatacancer i gruppen som deltog i screening jämfört med kontrollgruppen. En särskild grupp män, de som blev inbjudna att delta men av någon anledning inte gjorde det, hade kortare överlevnad. De dog tidigare både av prostatacancer men också av andra orsaker.

Vi vet att manligt könshormon driver utvecklingen av prostatacancer. Testosteron är det vanligaste manliga könshormonet, men det finns ett liknande, starkare, manligt könshormon som heter Dihydrotestosteron (DHT). I **delstudie II** undersöker vi om DHT påskyndar eller skyddar från aggressiv prostatacancer. Tvärt emot vad man skulle kunna anta så verkar det som att DHT skyddar från att dö i prostatacancer.

Det vanligaste sättet att hitta prostatacancer är via ett s.k. PSA prov. Om ett PSA prov är förhöjt misstänker man prostatacancer. I **delstudie III** vill vi ta reda på om man, vid *ej* förhöjda värden, kan utesluta (eller bedöma att risken som försumbar för) framtida prostatacancer. I resultaten ser vi att man vid låga värden av PSA, speciellt kombinerat med ett annat prov som mäter andelen PSA som är bundet till andra molekyler, kan konstatera en mycket låg risk för framtida aggressiv prostatacancer.

I **delstudie IV** har vi analyserat en ny markör på gamla blodprover (proverna är från 1988 och 1989). Blodproverna har förvarats i en frys som håller -80 grader C under alla år. Markören som vi analyserar kallas Thymidinkinas (TK1) vilket är ett protein som deltar i celldelningen i kroppen. Vi såg att män som hade höga nivåer av TK1 löpte större risk att dö (av alla orsaker) än de med låga värden.

ABSTRACT

In 1988 and 1989 a large screening study for prostate cancer was launched in Stockholm, Sweden. At the time approximately 27 000 men between 55 and 70 years of age resided within a defined area of southern Stockholm. 2400 men were randomly selected to participate in the trial and those accepting (n=1782), were examined with digital rectal exam (DRE), transrectal ultrasound (TRUS) and a PSA test. If DRE or TRUS indicated suspicious findings or if PSA levels were 10 ng/mL or greater quadrant core biopsies of the prostate were performed. Additionally, the screening algorithm employed stipulated reexamination with DRE and TRUS if PSA concentrations were between 7 ng/L and 10 ng/mL. The initial screening yielded 65 cases of prostate cancer. In this thesis the screening material have been assessed after 20 years (**paper I**) and 30 years (**paper II-IV**).

In **paper I** the result of the one-time screening was evaluated after linking the background population, the participants of the study and the invited but not participating cohort to the Swedish cause of death registry and the Swedish cancer registry. Estimating the possible cancer-specific mortality reduction using the Poisson regression model resulted in no difference in prostate cancer-specific mortality between the screened population and the unscreened population, IRR= 0.97 (0.71-1.23; 95% CI).

Paper II evaluated the association between the androgen DHT and prostate cancer incidence and mortality. High levels of DHT protected from lethal prostate cancer HR= 0.44 (0.25-0.77; 95% CI), p=0.004 after 30 years of follow up. The association remained significant both for men seemingly heathy at time of inclusion HR=0.25 (0.07-0.88; 95% CI), p= 0.032 and for those with a recently diagnosed cancer HR= 0.50 (0.26-0.94; 95% CI), p=0.031.

In **paper III** the threshold for PSA was examined and the proportions of its isoforms – free/bound PSA that is indicative for low, or negligible risk for prostate cancer death. The associations between both PSA and the ratio free/bound PSA and lethal prostate cancer were strong at long-term follow up. A baseline PSA of 2 ng/mL or less combined with ratio free/bound PSA of 0.25 or greater indicated a very low long-term risk for prostate cancer death and further screening in this cohort can be abstained or continued with lower frequency.

In **paper IV** thawed serum from 330 men including 36 men with lethal prostate cancer was analysed. The aim was to estimate association between elevated levels of the enzyme

Thymidine kinase (TK1), a phosphorylation enzyme important in DNA synthesis, and future risk for prostate cancer-specific mortality and overall mortality. The analyses were performed with a commercially available western blot kit. Preliminary estimates indicate that high levels of TK1 is associated with an increased risk for overall mortality irrespective of whether death occurred shortly after blood draw or after a period of follow up.

LIST OF SCIENTIFIC PAPERS

This thesis is based on the following papers

I. Long-Term Outcome of a Single Intervention Population Based Prostate Cancer Screening Study
Per-Olof Lundgren, Anders Kjellman, Ulf Norming and Ove Gustafsson.
Journal of Urology, 2018, vol 200, 82-88

II. Association between dihydrotestosterone and long-term risk for prostate cancer mortality: A prospective cohort study
Per-Olof Lundgren, Anders Kjellman, Ulf Norming and Ove Gustafsson.
The Prostate, 2020, vol 80, 777-781

III. Association Between One-time Prostate-Specific Antigen (PSA) Test with Free/Total PSA and Prostate Cancer Mortality: A 30-year Prospective Cohort Study
Per-Olof Lundgren, Anders Kjellman, Ulf Norming and Ove Gustafsson
Accepted March 17, 2021. British Journal of Urology International

IV. The role of Thymidine Kinase as a Long-Term Prognostic Marker for Prostate Cancer Mortality. A prospective cohort study.
Per-Olof Lundgren, Bernhard Tribukait, Anders Kjellman, Ulf Norming and Ove Gustafsson
In manuscript

CONTENTS

LIST OF ABBREVIATIONS

PSA	Prostate Specific Antigen
DRE	Digital Rectal Examination
TRUS	Transrectal Ultrasound
PLCO	Prostate, Lung, Colorectal and Ovarian Cancer Screening trial
ERSPC	European Randomized Screening for Prostate Cancer trial
CAP	Cluster Randomized Trial of PSA testing for Prostate Cancer
PCPT	Prostate Cancer Prevention Trial
T	Testosterone
DHT	Dihydrotestosterone
BPH	Benign Prostatic Hyperplasia
Hk2	Human Glandular Kallikrein
MRI	Magnetic Resonance Imaging
HR	Hazard ratio
NNI	Numbers Needed to Invite
NND	Numbers Needed to Diagnose
NNT	Numbers Needed to Treat
ADT	Androgen Deprivation Therapy
MCM	Minichromosomal Maintenance Protein Complex
QALY	Quality Adjusted Life Years
TK1	Thymidine Kinase 1
SNP	Single Nucleotide Polymorphism
IRR	Incidence Rate Ratio
RR	Risk Ratio
PET	Positron Emission Tomography
MSMN	Beta-Microseminoprotein
MIC-1	Macrophage inhibitory cytokine-1

LH Luteinizing Hormone

FSH Follicle Stimulating Hormone

AUC Area Under the Receiving Operator Curve

ANOVA Analysis of Variance

SEER Surveillance, Epidemiology and End Results

1 INTRODUCTION

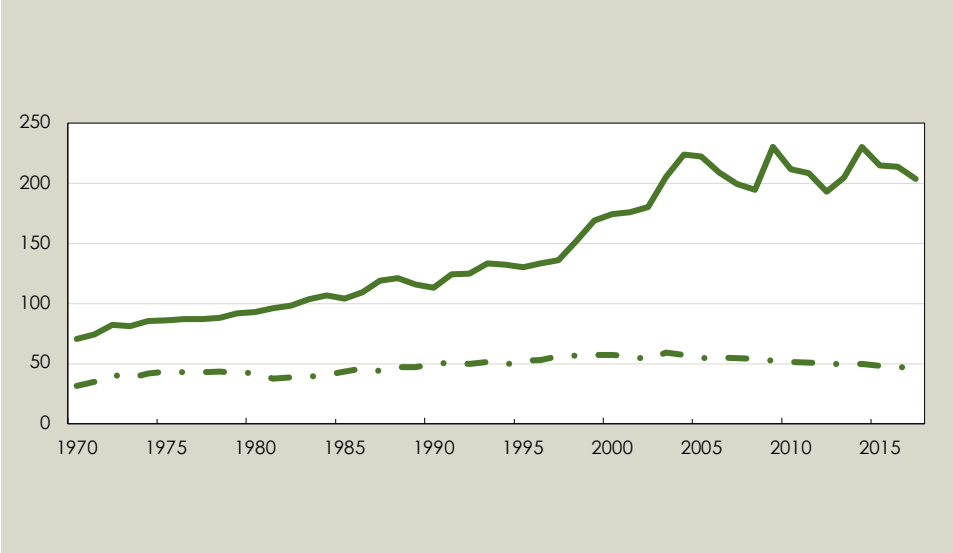
1.1 PROSTATE CANCER AND EARLY DETECTION

In 2018 more than 10 000 new cases of prostate cancer were diagnosed in Sweden, making prostate cancer the most common malignant disease in Swedish men and roughly as common as breast cancer [1].

Incidence rates have increased dramatically during the last 50 years whereas mortality rates have been stable over time. This epidemiological development prompts the discussion whether;

a) There is a true increase in incidence, but treatment regimens have improved to the extent that a much smaller fraction of men dies from their prostate cancer leaving the mortality rates unchanged, or
b) Opportunistic screening using biomarkers such as PSA have induced a much higher detection rate of low grade cancers/indolent cancers.

Figure 1. Prostate cancer incidence (full line) and prostate cancer-specific mortality (dotted line), per 100 000 Swedish citizens. From the National Swedish Board of Heath and Welfare.



1.2 DIAGNOSIS

Today most cases of prostate cancer are found after PSA levels have been determined during regular health exams. The diagnosis, however, is made after verification with histopathology. Traditionally, core biopsies to detect prostate cancer are performed systematically in order to let most areas of the prostate to represented in the specimen. In recent years, fusion technique with MRI images and images of ultrasound combined for more accurate biopsy yield [2].

1.3 PROGNOSTIC TOOLS

Contemporary tools for risk stratification of prostate cancer largely rely on the work by D'Amico et al. Based on histopathology, PSA and clinical T-stage men with localized cancer was assigned to either low, intermediate or high risk group [3]. Bratt et al added an additional risk group – the very low risk group after review of almost 1300 patients in the Swedish prostate cancer register [4]. A simplified presentation of criterions and treatment recommendations are outlined in table 1.

Table 1. Criteria and recommendations for the risk strata of localized prostate cancer.

Risk group	T-stage	Gleason-sum	Distribution of positive biopsies	PSA	Treatment recommendations
Very low risk	T1c	≤ 6	≤ 8 mm cancer totally in ≤ 4 out of 8 -12 systematic biopsies	<10 ng/mL and PSA density < 0.15 mg/mL/cm^3	Active monitoring
Low risk	T1-T2a	≤ 6	≥ 8 mm totally in more than 4 out of 8 - 12 systematic biopsies	<10 ng/mL	Active monitoring
Intermediate risk	T2b	7		10-19.9 ng/mL	Radical prostatectomy or radiation therapy
High risk	T2c-T3	8–10		≥ 20 ng/mL	Radiation therapy with neoadjuvant androgen deprivation

1.4 SCREENING

Implementation of a public screening program for early detection of prostate cancer has been extensively debated in Sweden and internationally. Screening for prostate cancer has so far been synonymous with PSA screening, i.e., men in a certain age span are invited to have PSA levels analyzed, and if elevated they are recommended to undergo systematic core biopsies of the prostate. Internationally, several large studies there have attempted to measure survival

benefits of population-based PSA screening. The PLCO (Prostate, Lung, Colorectal and Ovarian Cancer Screening trial) reported comparable cancer specific survival in the intervention (screening) and the control arm [5], whereas the ERSPC (European Randomized Screening for Prostate Cancer trial) demonstrated a decrease in prostate cancer specific mortality, RR: 0.8 after 16 years [6]. It seems that the ability to detect a survival-benefit in the American trial, the PLCO, was thwarted by opportunistic PSA-screening in the control group to such a high extent that it is therefore not regarded as solid evidence of the absence of a positive screening effect. The effect on cancer specific mortality from the European trial is generally regarded as reliable.

2 CURRENT KNOWLEDGE

2.1 THE DIAGNOSTIC DILEMMA AND RISK FOR OVER TREATMENT

Treatment for localized prostate cancer includes radical prostatectomy or radiation therapy. Regardless of therapy choice, complications and long-term adverse events are not uncommon. In a relatively contemporary effort to compare surgical approaches (open vs robot-assisted) the Scandinavian Surgical Outcomes Research group reported a proportion of men with incontinence about 20 % and erectile dysfunction of more than 70 % one year postoperatively regardless of surgical approach [7]. Radiotherapy also causes both acute and long-term side effects, including damage to the mucosa of the urinary bladder, large intestines and urethra. Different oncological approaches including dose escalation, fractioning and combination of external and brachy therapies are beyond the scope of this summary but as many as 20-30 % of patients receiving pelvic radiotherapy report debilitating symptoms from the bowel [8].

Indisputably, the risk for over diagnosis and subsequent over treatment of localized prostate cancer must be acknowledged.

In the European screening trial, *numbers needed to screen* to save one man from prostate cancer death was 570 and *numbers needed to diagnose* was 18 [6].

Our 20 years follow up of the screening cohort did not show a cancer specific survival benefit but did show an increase in cumulative incidence throughout the follow up period in the intervention arm. Given the high PSA-cut off for biopsies (10 ng/mL), one would assume that the algorithm would have a high specificity for high grade cancer, i.e., the cancers detected should to a large extent be clinically significant. Less advanced cases and low risk tumors, on the other hand, would most likely remain undetected.

2.2 MARKERS

A few decades ago, prostate cancer was commonly diagnosed at a very advanced and, more often than not, at an incurable stage. The only important blood test for prostate cancer was acid phosphatase – a test ill-suited for early detection since it is increased primarily in men with metastatic disease [9] and consequently it is rendered obsolete in clinical practice.

2.2.1 PSA

Prostate specific antigen was first isolated in semen during the 1960s, originally as a biomarker for cancer, although initially it was more commonly used for forensic purposes [10].

The arrival of PSA as useful tool for early detection of prostate cancer should likely be credited to Chu et al who isolated the antibody and rendered it usable as a biomarker [11]. Because of its inherent inability to indisputably distinguish between prostate cancer and, for instance, benign prostatic hyperplasia, it was mainly used to monitor already diagnosed prostate cancer until 1991, when Catalona et al published data regarding the use of PSA as a screening-method for prostate cancer. Published in The New England Journal of Medicine, Catalona concluded that PSA testing, in addition to clinical investigation, detected 32% and 42% percent more cancers than digital rectal exam (DRE) or ultrasonography of the prostate (TRUS), respectively [12,13]. Simultaneously, our research group conducted a trial regarding early detection of prostate cancer in Stockholm, Sweden, using a PSA cut off level of 10 ng/mL rendering the additional diagnostic value of PSA much smaller compared to that described by Catalona (cut off level: 4.0 ng/mL) [14].

Since the introduction of PSA, it has been the dominating biomarker used as methods of screening for prostate cancer, to monitor (treated or not) disease and to predict long-term prognosis.

2.2.1.1 PSA as a diagnostic/screening tool

There are cases of prostate cancer that are of very high malignant potential and display de - differentiated features, among others, loss of capability to produce PSA. These cases can consequently have low PSA values despite large volume of high-grade disease [15]. They are, however, rare and in the majority of cases the specificity for prostate cancer increases with increasing PSA, i.e., the more elevated a PSA level is, the more unlikely a benign cause for the rise in PSA is.

There are a number of factors influencing the concentration of PSA in blood apart from prostate cancer; acute urinary retention [16], treatment with 5α-reductase inhibitors [17], benign prostatic hyperplasia (BPH) [13], inflammation [13], and age [18]. The specificity and sensitivity of PSA are dependent on where the cut off level is set. A low cut off level will lead to high sensitivity and yield many cases, but there will also be a number of false positive cases. A higher cut off, on the other hand, will have a more favorable specificity but the ability to find all cancers will be decreased. Thompson et al defined sensitivity and specificity of PSA in the

control arm of the PCPT (about 18 000 men). Sensitivity for PSA cutoff of 1.1 ng/mL and 4.1 ng/mL was 83.4% and 20.5% and specificity was 38.9% and 93.8% respectively [19].

2.2.1.2 Disease monitoring

Measurement of PSA is a cornerstone of all monitoring of prostate cancer. It is widely accepted that successful surgical treatment should render PSA unmeasurable although the importance of stable low, but detectable, PSA levels is unsure [20].

PSA is a valuable tool also in the detection of recurrence after radiotherapy, but unlike after successful surgical treatment PSA levels remain measurable. Also, PSA often rises transiently after radiation without indicating relapse (PSA bounce) [21].

2.2.1.3 Long-term prediction

There is a comprehensive body of evidence suggesting that PSA can predict long-term risk for prostate cancer, sometimes defined as significant cancers and sometimes defined as lethal and/or generalized (metastatic) prostate cancer.

Marc Preston published data in 2016 using a cohort of men from the US Physicians Health study [22] and demonstrated that the cumulative risk for men aged 55 to 59 with PSA<1.02 was 0.6 at 30 years and corresponding cumulative risk for men aged 50-54 was 1.6 underlining the need for age stratification in long-term predictions [23].

Risk stratification for distant metastasis does not surprisingly follow similar patterns and Vickers et al evaluated risk using a cohort from the Malmö Preventive Project. Long-term risk for prostate cancer metastasis was 0.09% for men 45-49 years after 25-30 years of follow up [24].

More recently, an evaluation of the PLCO cohort of more than 10000 men, concluded low risk for significant prostate cancer defined as clinical stage of cT2b or higher, Gleason score >= 7 or death from prostate cancer. After 13 years the incidence of significant prostate cancer was 0.4% for men aged 55-60 years at inclusion with baseline PSA <= 0.49 ng/mL and 1.5% for those with PSA between 0.5-0.99 ng/mL [25].

2.2.2 PSA combinations

In a large trial funded by Stockholm county council (the Stockholm 3 trial), investigators aimed to find an algorithm that could decrease the number of negative biopsies and predict significant prostate cancer better than PSA alone [26]. The “Stockholm 3 panel” included, apart

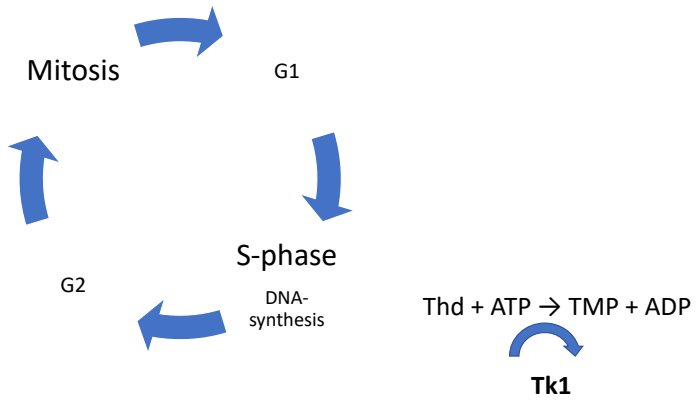
from PSA, also: isoforms of PSA (e.g., Human Glandular Kallikrein Hk2), family history, other plasma biomarkers and more than 200 SNPs (single nucleotide polymorphism). The authors concluded that the frequency of negative biopsies could be decreased by as much as 44% if the Stockholm 3 panel was used rather than employing a PSA cut-off point of 3 ng/mL for biopsies alone.

In 2015, Stattin et al published a nested case control study with cryopreserved plasma tested for concentrations of PSA, free PSA, bound PSA and Hk2 [27]. The results were compared with outcomes after 15 years and among the men with PSA> 2 ng/mL the panel could discriminate future distant metastasis better than PSA alone, Harrell's concordance-index 0.82-0.88.

2.2.3 Thymidine Kinase

Thymidine kinase is an enzyme, a phosphotransferase, playing an important role in the DNA synthesis. It is present in two forms: TK1 and TK2. TK1, the potential biomarker, is only detectable in cells that are about to go into mitosis. It mediates the transformation of Thymidine to deoxythymidine-monophosphate during the S-phase of the cell cycle [28]. Consequently, it is absent in non-proliferating cells [29]. TK1 concentration in both tissue and in plasma can be measured using the western blot technique. This means in short, that a mix of proteins are separated with electrophoresis and the protein (or part of it) of interest is marked with an antibody that produces colour or light to allow quantification [30].

Figure 2. The cell cycle and the phosphotransferase reaction mediated by thymidine kinase (TK1).



Because of its presence in cells in S-phase (with active DNA synthesis), Tk1 have been employed as a target for Positron Emission Tomography (PET-scan) substrate Fluorothymidine, revealing tumors with a high proportion of cells anticipating division.

Wu et al could demonstrate, using Western blot technique, that TK1 is much more abundant in cancerous tissue than in normal tissue [31]. Similarly, TK1 was found in higher concentrations in malignant breast cancer tissue compared to benign lesions, and normal mammary tissue [32]. The same association between breast cancer and high levels of Tk1 in tissue have also been reported in serum. A comparison between patients awaiting surgery for malignant breast lesions, benign breast lesions and healthy volunteers showed significant differences regarding s-TK1 concentration, the women with malignant lesions having significantly higher levels of TK1 in serum [33].

2.2.3.1 Thymidine Kinase and Prostate cancer

Aufderklamm et al used the specific TK1 antibody XPA-210 on core prostate cancer biopsies and found that high expression of TK1 was associated with both shorter time to biochemical relapse after treatment and shorter time to development of metastasis [34]. Again, the association has been reproduced also in serum. Shujing et al measured serum-TK1 (and PSA) in 123 patients with prostate cancer, 205 patients with BPH and 266 healthy controls. They found that Tk1 was significantly higher in serum from men with prostate cancer compared to serum from men with BPH and healthy men alike. TK1 also correlated to Gleason score in a manner that PSA did not [35].

Indications that TK1 is increased in pre-clinical cases originate from two large scale screening studies conducted in China. 11 000 and 8000 healthy persons, respectively, were enrolled and both trials were indicative of more malignant tumors in subjects with high TK1 regardless whether the tumor was found at time of screening or later on in life [36,37]. The trials lack in follow up time and could possibly be biased due to a young study population, i.e., very few of the screened participants had elevated levels of TK1.

The study of Thymidine kinase as a marker for cancer outcome is of great interest. Since it is measurable in serum it could easily be adopted in clinical practice and in oncological decision making. As far as prostate cancer is concerned, given the significantly disparate malignant potency of the disease, a marker able to predict progression and possibly cancer specific death would be highly valued.

2.2.4 Other markers

Efforts made to improve early detection of prostate cancer have prompted the search for novel biomarkers. Apart from the nowadays abandoned use of acid phosphatases and the obvious leading role of PSA and its isoforms, several candidates have been evaluated:

The β-microseminoprotein (**MSMB**), an immunoglobulin binding factor secreted by epithelial cells in the prostate (and many other organs), and the Macrophage inhibitory cytokine-1 (**MIC-1**), a growth factor cytokine, are both part of the Stockholm3 test panel. Decreased expression of MSMB (or perhaps the presence of a particular single nucleotide polymorphism causing it) increases risk for prostate cancer [38], whereas MIC-1 over-expression is associated with prostate cancer [39,40]. These two markers are not specific for prostate cancer and are, to the best of our knowledge, not routinely used as single tests.

A more specific marker is the **PCA 3**. It is based on a relatively long RNA strand and is elevated in prostate cancer tissue, but not in normal prostate tissue or hyperplastic tissue. Therefore, unlike PSA, it could be used to distinguish between prostate cancer and BPH. PCA 3 is measured in urine and should be used as an adjunct to serum PSA [41].

Genomic testing can predict aggressiveness of prostate cancer. David Olmos et al used nine unique RNA sequences and could stratify aggressiveness within the cohort of men (n=64) with castration resistant prostate cancer based on the expression of the sequences [42].

2.3 RADIOLOGY

Because of its anatomical position in the lesser pelvis, surrounded by bony structures, imaging of the prostate have traditionally dependent of transrectal ultrasound and during more resent years also Magnetic Resonance Imaging (MRI).

2.3.1 Ultrasound

The idea to visualize the prostate originates from the 1970s when Watanabe et al described the method [43]. At the time they predicted that the method would become a cornerstone of urological imaging which indeed, it has. Even though the basic fundamentals of ultrasound remain, i.e., a probe delivers a high frequency soundwave and registers the returning echo, the quality of the images have greatly improved. When all men in this cohort were screened with ultrasound, a handheld 7 MHz probe was used compared to the 3.5 MHz probe, mounted on a chair-like construction, used in Watanabes original work [43]. Apart from frequency, resolution of the images has improved greatly [44].

2.3.2 Magnetic Resonance Imaging, MRI

Blood analysis and ultrasound aside, there is growing evidence supporting the use of MRI in the early detection of prostate cancer, and possibly also for public screening [2,45]. With the exception of advanced or generalized cases MRI is recommended as a first diagnostic step and several studies have concluded its benefits compared to traditional diagnostic work up with PSA, DRE and TRUS-guided systematic biopsies [46,47]. The strongest arguments for up-front use of MRI in prostate cancer diagnosing and staging comes from the PROMIS and PRECISION trials. In short, in the aforementioned study multiparametric MRI and template biopsies on a cohort of 576 men were performed with the conclusion that MRI performed routinely can decrease biopsy rates by 27 % [48]. In the PRECISION trial men were randomized to either MRI guided biopsies or conventional template biopsies. This study, like the PROMIS trial, reported more significant cancers and fewer indolent ones were found while employing the MRI guided approach to biopsies [2]. These lines of evidence are, however, challenged based on, not so much methodology as the conclusions drawn. Vickers et al argue that the oncopathological characteristics for tumors detected via template biopsies and MRI guided biopsies are not equal even if Gleason-sums are identical and hence the value of MRI in early detection of prostate cancer is exaggerated [49]. Assuming that the same tumor is biopsied, either by a randomized template or by MRI assisted fusion biopsies, the oncopathological risk stratification will be different. If for no other reason, the second most common Gleason grade reported which means the second addend of the addition that is the Gleason sum will likely be higher in the MRI group than in the systematic biopsy group. If biopsies are aimed at suspicious lesions it is more likely that the two most common histopathological patterns are of higher Gleason grade than if a truly systematic approach has been employed when performing the biopsies.

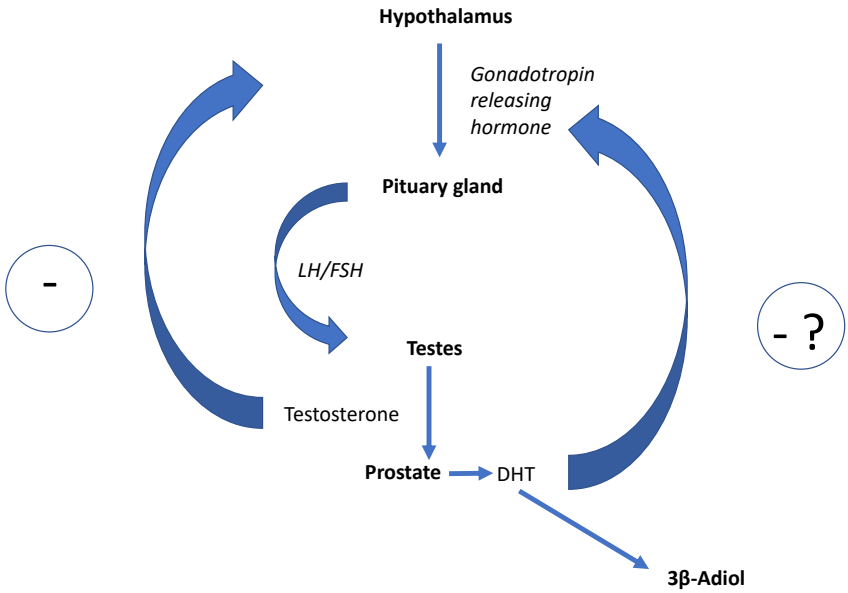
2.4 THE ANDROGENIC INFLUENCE AND DHT

It is known since the 1940s that the male sex hormone testosterone (T) promotes progression of prostate cancer. Huggins et al published the first results regarding Androgen Deprivation Therapy, ADT, of men with advanced stages of the disease who improved when T was reduced to nearly zero by surgical removal of the testes [50]. As an alternative to the surgical castration, Gonadotropin Releasing Hormones Analogues was introduced in the 1980s and in the mid-nineties the anti-androgen Bicalutamide. Even today most of the pharmacological efforts are being directed towards the testosterone homeostasis, either by decreasing production or by limiting its biological effect.

Removal of testosterone in the generalized or metastasized setting aside, the influence of androgens is perhaps more complicated than anticipated. Loeb et al compared outcomes in men who received testosterone replacement therapy and men who did not in a nested case control study [51]. Treatment with testosterone was associated with less aggressive prostate cancer and in a meta-analysis higher levels of testosterone prior to androgen deprivation therapy was associated with lower frequency of prostate cancer-specific death [52]. These finding suggest that the influence of androgens actually can promote lower grade cancers. The influence, or association, of the testosterone metabolite dihydrotestosterone is discussed below and in **paper III**.

Testosterone is mostly produced within the Leydig cells of the testes and is further metabolized to, among other metabolites, dihydrotestosterone (DHT). This transformation mainly takes place within the prostate and is accomplished by a reductive reaction mediated by the enzyme 5α-reductase. DHT has approximately four times greater affinity to the androgen receptor compared to its substrate – testosterone, thus making it a far more potent androgen. The androgen metabolism is outlined in a simplified manner in figure 3.

Figure 3. Diagram of the androgen metabolism. " - ?" represents the possible negative feedback mechanism of DHT.



Intuitively, given the high affinity for the androgen receptor, DHT should promote prostate cancer even more than T.

To the contrary, there are three lines of evidence suggesting a protective effect of DHT.

a) The prospect to eliminate DHT, being a "strong" androgen, in order to prevent prostate cancer has been tested in two large clinical trials [53,54], with the transformation of T to DHT inhibited in the intervention arms. There was indeed a decrease in total number of prostate cancer cases, but, on the other hand, there was an increase in the number of high-grade cancers in the intervention arms, rendering the idea of chemoprevention with 5α-reductase inhibitors doubtful.
b) When the Stockholm screening study was completed, 65 cases of prostate cancer had been diagnosed. All the diagnosed men and two age matched controls without any sign of prostate cancer were tested for serum levels of DHT. The hypothesis being, at that time, that high levels of DHT would promote prostate cancer to an even higher extent than T given the a priori knowledge of the androgenic potency of DHT. Instead, there was a trend suggesting the opposite [55]. At 15 years of follow up the trend had become a statistically significant difference - prostate cancer patients with high DHT have a more favorable cancer specific outcome than those with low DHT [56].
c) In 2002 Weihua et al published a description of the intraprostatic androgen metabolism [57]. The pathway includes the further enzymatic transformation of DHT to estrogen-like metabolites. Combined with the knowledge that estrogen receptors are present, both in the normal prostate, as well as in cancer tissue, it is hypothesized that the activation of the estrogen receptor (mainly the estrogen receptor-β) stabilizes the epithelium and protects from high grade prostate cancer/prostate cancer death [58].

The prostate plays an important role in the metabolism of sex hormones. Experimental studies have shown that the levels of DHT in periprostatic serum are higher than in peripheral sera. Furthermore, removal of the prostate, i.e., the conversion site of T to DHT increases the gonadotropins LH and FSH, suggesting a feedback mechanism of DHT on the pituary gland and/or the hypothalamus [59,60].

2.5 POPULATION BASED SCREENING

2.5.1 Opportunistic screening

Ever since the PSA test became generally available and its ability to detect prostate cancer was acknowledged, a widespread opportunistic screening has occurred. Recently, data regarding frequency of opportunistic screening in the UK screening trial – CAP was revealed. Around 410 000 men out of a cohort of roughly 2.8 million were screened according to definitions for opportunistic screening, i.e., PSA testing without symptoms. When the PLCO was in its screening phase, it is estimated that opportunistic screening in the control arm was as high as 46% per year [61].

2.5.2 Population based screening

Opportunistic PSA screening of asymptomatic men can be rational under certain circumstances, and it is not identical to PSA screening done without proper consideration of harms vs benefits. It is, however, frequently hypothesized that the two are more or less the same. O´Neil et al defines "low value screening", i.e., screening where harm overweighs benefits. For instance, a PSA test is done in men too young to benefit from screening, too old or to afflicted by comorbidity to benefit from it. Nearly 50% of all PSA tests could be labelled to be of low value and the strongest predictor to have an unnecessary PSA test performed was that you already have had prior PSA testing – i.e., opportunistic screening rarely ends but is rather iterated over the years [62,63].

2.5.3 PLCO

The Prostate, Lung Colorectal and Ovarian screening trial is a large, randomized American initiative. Almost 77 000 men were included between 1993 and 2001[64,65] at ten centers in the US. Men randomized to the intervention arm received a flexible sigmoidoscopy, a chest x-ray, DRE and PSA test. Participants were screened with PSA tests annually and DRE every fourth year. A PSA of 4.0 ng/mL or greater and/or suspicious findings on DRE prompted diagnostic work up including core biopsies.

10-year survival rates were analyzed by Pinsky et al and there was no difference in cancer-specific mortality between the intervention arm and the control group. A superior overall survival was observed for the hole cohort compared to nationwide data [5].

It has been argued that the improved overall survival can be attributed to a healthy volunteer effect. In short, the healthy volunteer effect is a selection bias with a larger proportion of

health-aware persons compared to the population at large accepting participation in trials [66]. The inability to demonstrate a reduction in prostate cancer-specific mortality in the intervention arm can possibly be explained by contamination in the control group. On average, men in the control arm had 2.7 PSA test performed during the 6 years the intervention took place [67].

2.5.4 The European Randomized study of Screening for Prostate Cancer, ERSPC

More than 180 000 men in eight European countries were included and followed for a maximum of 16 years. Intervals of screening and cut off for biopsies differed amongst the participating centers.

The most resent updates from 2019 show a clear cancer-specific survival benefit with a rate ratio of 0.80 (0.72-0.89). With longer follow up time, excess number of low-grade tumors have decreased. Numbers needed to screen to save one man from lethal prostate cancer was 570 [6].

Results from the ERSPC are the strongest support there is for population-based screening programs. Results vary within the trial. Only the Dutch and Swedish arms (and to some extent the Finnish arm) of the trial show definite survival benefits of screening.

2.5.5 The CAP Randomized clinical trial.

More than 400 000 were randomized either to be offered a single PSA test or nothing. When inclusion closed 64436 men were screened with PSA and approximately 219 000 men constituted the control group. At ten years of follow up there was no difference in prostate cancer-specific survival, but more low-grade tumors had been detected in the intervention arm, 1.7 % vs 1.1% in the control group [68].

2.6 ECONOMIC ASPECTS

2.6.1 Markers

Other aspects of biomarkers and the ambition to detect early and curable prostate cancer are economic and logistic. Indeed, the scientific progress is ahead of the implementation of its findings and economical barriers can slow down or impede clinical use of emerging biomarkers [69]. Limited radiology resources stand in the way of implementing, for instance,

MRI as means of screening for prostate cancer. On the other hand, implementation of novel biomarkers and advanced radiology protocols risk to outcompete other patient groups with, perhaps, more imperative need for the resources of the health care systems.

One of the more expensive biomarkers, the Stockholm 3 panel have been assessed regarding cost-effectiveness in the screening setting. The test was deemed cost effective compared to PSA screening alone by a complicated microsimulation system if PSA cut off level 2.0 ng/mL was used as threshold for the Stockholm 3 panel. Costs for the public did however increase by 0.4%. Lower thresholds resulted in significantly higher costs per unit of quality of life years gained measured in Quality Adjusted Life Years (QALY) [70].

2.6.2 Screening

Being the most common malignant disease in Sweden, prostate cancer adds considerably to society costs. Hao et al calculated costs in Stockholm, extrapolated numbers to the whole nation and thus estimated a total cost (including medical costs and productivity loss etc.) of 281 million Euros. There have been several attempts to analyze cost-effectiveness of prostate cancer screening. Naturally the cost effectiveness is dependent of the methods of screening. A more sophisticated screening algorithm, with e.g., MRI scans and the Stockholm 3 panel will yield higher costs during the screening but perhaps save on fewer biopsies and fewer unnecessary interventions. Mode of intervention should also be included in a full cost-benefit analysis. For instance, a majority of men undergoing surgical treatment for localized prostate cancer today have a robotically assisted operation performed. It is estimated that this type of surgery costs approximately 50 % more than conventional surgery [71].

An evaluation of cost effectiveness of screening for prostate cancer was performed on the cohort this thesis is based on and published in 1995. The most cost-effective strategy (including detection rates, treatment etc.) was ultrasound of all men with a PSA value of 4 ng/mL or more [72].

The Swedish National Board of Health and Welfare conducted a health economic evaluation of PSA based prostate cancer screening. Results are ambiguous. PSA testing alone is possibly less expensive compared to opportunistic screening given automatization and fixed algorithms. Nevertheless, increased costs cannot be ruled out considering more biopsies performed and more cases detected overall [73].

3 RESEARCH AIMS

I. To evaluate if a one time screening for prostate cancer decreases cancer-specific mortality and if it contributes to overdiagnosing of prostate cancer.

II. To investigate if baseline levels of dihydrotestosterone is associated with lower risk for prostate cancer-specific mortality in the long-term setting.

III. To evaluate the combination of baseline PSA and baseline free/total PSA as a predictor for future prostate cancer mortality.

IV. To investigate if the enzyme Thymidine Kinase, analysed in thawed serum samples, is associated with lethal prostate cancer and overall mortality.

4 MATERIALS AND METHODS

With the objective to evaluate best practice for detecting prostate cancer, the Stockholm screening trial was launched in the late 1980s. **Studies I-IV** of this thesis are based on the cohort and subsets of the cohort defined in the Stockholm screening trial.

4.1.1 Demographics

At the time, the area of referrals for the Stockholm South General Hospital was, almost exclusively, an urban area and, despite increasing immigration, predominantly caucasian. January 1st, 1988 about 27 000 men between the age of 55 and 70 lived in the defined part of southern Stockholm. Besides the parishes of the island of Södermalm; Katarina, Maria, Sofia and Högalid, some more rural areas like Gustavsberg-Nacka and Nynäshamn were included.

4.1.2 Randomization and inclusion

Based on contemporary census records approximately every 11th man was randomly chosen and invited to participate in the screening study. All were informed that participation included digital rectal palpation (DRE), ultrasound of the prostate (TRUS) and a blood test including sub-zero temperature bio-banking for future research. Out of the 2400 men invited, 1782 accepted the invitation and subsequently were included and signed a consent form. A response rate of, as in this case, more than 74 % is considered high. The non-responders were studied separately. The entire study cohort is outlined in table 2.

Subsets of the initial cohort that are evaluated in the papers of this thesis:

Paper I: The entire background cohort from which the study cohort was sampled (n=26 602), the participants (n=1782) and the invited but not participating men (n=618).

Paper II: The 65 cases of screening detected prostate cancer along with 130 seemingly healthy and age matched men (n=195).

Paper III: All screened men (n=1782)

Paper IV: 96 men diagnosed with prostate cancer at time of screening or during follow up and 234 non prostate cancer patients sampled at random (n=330).

Table 2. Baseline characteristics and outcomes of the study population. Outcomes are after 20 years of follow-up except for the sub-strata of the screened population marked * where 30-year data are demonstrated.

	Entire population n= 26602	Invited, not screened n= 618	Screened population n=1782	Biopsy at screening n=371	Detected cancers n=65
Age at time of screening, mean (range)	62.5 (48.1-70.3)	62.1 (54.2-70.2)	62.6 (54.3-70.3)	63.6 (54-71)	64.4 (55-71)
Overall mortality at 20 years, %	58.6	72.1	54.6	77.4*	87.7*
Prostate cancer-specific mortality at 20 and years, %	3.4	4.4	3.3	11.9*	47.7*
Prostate cancer incidence at 20 years, %	10.9	9.0	13.3	32.4	
PSA ng/mL, median (IQR)			1.8 (1.6–3.0)	2.4 (1.7–7.3)	9.1 (4.7–18.2)
Free/total PSA, median (IQR)			0.25 (0.18-0.33)	0.20 (0.14-0.29)	0.09 (0.07-0.14)

4.1.3 Examination

The physical investigations (DRE and TRUS) were performed by three experienced urologists. Any suspicious findings on either DRE or TRUS made the man eligible for core biopsies. The investigators were blinded for levels of PSA. If PSA levels were equal to or greater than 10 ng/mL, biopsies were taken and if PSA levels were between 7 ng/mL and 10 ng/mL, the man was re-examined with TRUS and DRE.

4.1.4 Yield

The study detected 65 cases of prostate cancer. Given that biopsies prompted by blood chemistry alone were performed on men with PSA levels very high (compared to modern practices) and that the participants were examined very thoroughly by the investigators, two points emerged.

1. A vast majority of cases were detected because of clinical findinds i.e. pathological lesions on DRE or TRUS rather than beacause of elevated PSA. Only 1 of the 65 cases diagnosed were subject of biopsies on account of his elevated PSA alone. As reference, in 2019 almost 60 % of new cases in Sweden were detected by means of health examination without symtoms [74]. In some cases the health examination might have included DRE but most likely the cases are almost exclusively detected by elevated PSA levels. In the context of screening for prostate cancer, this effort can not be regarded as a trial evaluating PSA-screning for prostate cancer and can therefore not easily be compared with larger and more recent trials like the ERSPC.
2. The abilty to detect less advanced cases was low. The cohort of men diagnosed originally constitues a very high risk population by todays standards. Intuitively, a screening algorithm with this design will fail to detect a number of cases that eventually progresses to lethal cases. On the other hand, the risk for over diagnosing and over treatment should be neglible. It seems, however, that detection rates regarding prevalent cancers are higher in the Stockholm trial than in other evaluations. Other trials do reach higher rates of detection as the screening algorithm progresses over time. Rates of detection and proportion of invited men accepting participation are outlined in table 3.

Table 3. Summary of included participants and rates of prostate cancer detection in the Stockholm study [14], the PLCO [64], the Swedish arm of the ERSPC [75] and the ProtecT trial[76]

	Randomized to Screening, n	**Participated, n (%)**	**Size of control group**	**Cases, n (proportion of screened, %)**
The Stockholm Screening Trial	2400	1782 (74)	25000	65 (3.6)
The Gothenburg Randomized Screening Trial, Swedish arm of the ERSPC (1st round of screening)	9945	7635(77)	9949	144 (1.9)
PLCO (1st round of screening)	38 350	34100 (89)	38355	556 (1.6)
CAP Trial	15200	8505 (56)	-	224 (2.6)

4.1.5 Follow up

Follow up started on the day of screening. Follow up ended on day of death or December 31st 2008 (paper I) or December 31st 2018 (paper II-IV).

4.1.6 Registries

4.1.6.1 The Swedish cause of death registry

All Swedish nationals are included in the cause of death registry when they die. If a Swedish national die abroad, however, it is likely that the cause of death is listed as unknown. When a person die it is up to the physician calling the death to report it to the tax authorities within 24 hours. The cause of death determined either by physical examination prior to death or by postmortem autopsy is reported to the Swedish Board of Health and Welfare within three weeks. The physician is free to state several causes of death including one as primary cause of death and one as underlying cause of death [77].

Men dying from prostate cancer are generally older than 65 years [78] and often significantly older than that. The older a population becomes one can expect more complex and multifaceted causes of death. In this setting a malignant diagnose can easily be exaggerated as primary cause of death. Indeed, when validating cause of death determined by scrutinizing medical records prostate cancer is designated as primary or underlying cause of death in an excess of three percent [79].

4.1.6.2 The Swedish Cancer registry

The Swedish cancer registry holds records of all diagnosed cancers since 1958. Attending physicians are responsible for reporting all new cases. Most commonly the diagnosis is based on histological or cytological material [80]. In these cases, the responsible physician will be reminded should he or she neglect to report the cancer. A new case can also be reported based on physical, laboratory or radiology findings alone.

A review in the late 1990s estimated that approximately 4 % of cases were not reported. Given the development with automatization of reporting and electronic forms, it is possible that fewer cases are missing today, nonetheless, since follow up in this cohort started in 1988 the risk for bias of missing cases is acknowledged.

4.1.7 Statistical analysis

4.1.7.1 Regression models

Paper I. To estimate the incidence ratio or to compare incidence ratios between the screened cohort and the not screened cohort we have counted events over person-time at risk.

Events, such as in this case, prostate cancer specific death do not assume a normal distribution but rather a Poisson-distribution meaning that events are independent of each other. In the **Poisson-regression**, time is fixed and the number of events during that period of time are counted [81]. Incidence rates are calculated for each cohort and incidence rate ratios (IRR) are presented with 95% CI as the statistical output.

Figure 4. Summary of the rationale behind Incidence Rate Ratios

$$\text{Incidence Rate } (I_1) = \frac{\text{Number of events}}{\text{Person-time at risk}} \qquad \text{Incidence Rate ratio (IRR)} = \frac{I_1}{I_0}$$

Paper II-IV

For time to event data (contrary to the above discussed event-count data) we have used the **Cox proportional Hazard model**. This model describes the underlying risk for failure per time-unit and how it changes over time [82]. The model assumes that the covariates influence on hazards are proportional, i.e., it changes or affects the Hazard and the comparative Hazard Ratio (HR) ratio equally at all times during follow up.

4.1.7.2 Other statistical estimates

Kaplan Meier estimates are used to illustrate survivor functions and time to event data. The larger a cohort is the more the Kaplan Meier estimate resembles true survivor function of that cohort [83]. Kaplan Meier estimates are useful for illustrating time to event data when persons are censored e.g., leaves the study or dies from other reasons. Examples of Kaplan Meier curves are given in figure 5 and 6. The Kaplan Meier estimates are usually accompanied by an at-risk table which gives an account of how many subjects that have been censored and an estimate of significance, most commonly the log-rank test.

Figure 5. Prostate cancer-specific survival by PSA above or below 2.0 ng/mL. Log rank p<0.0001

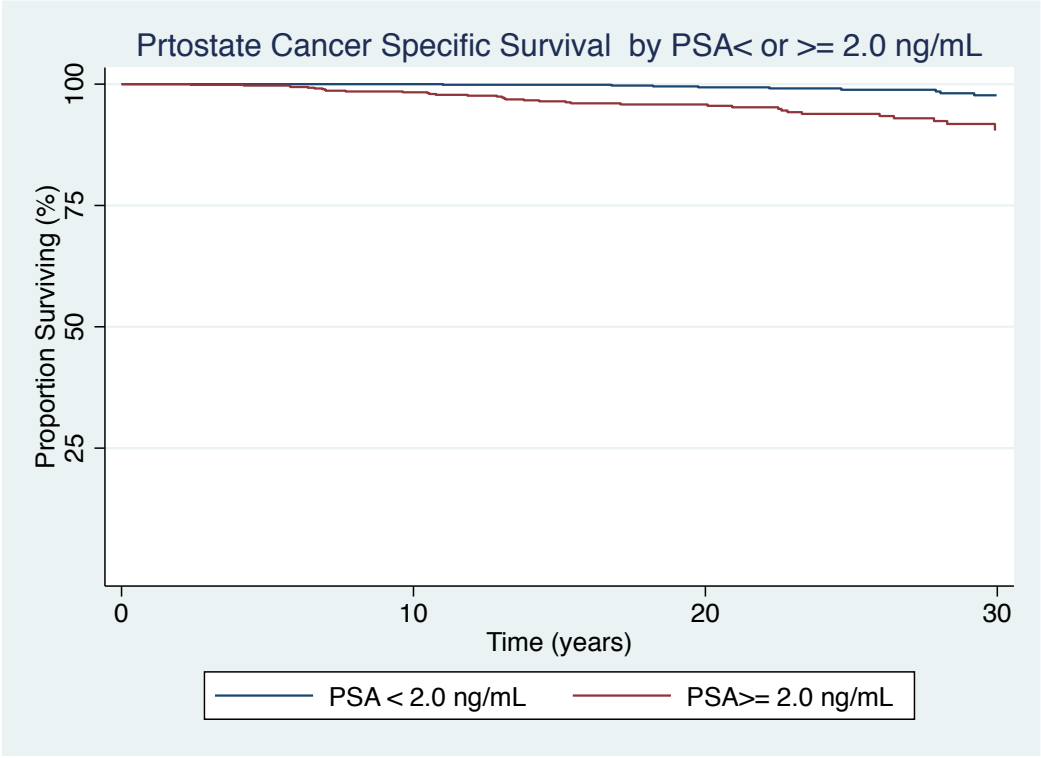
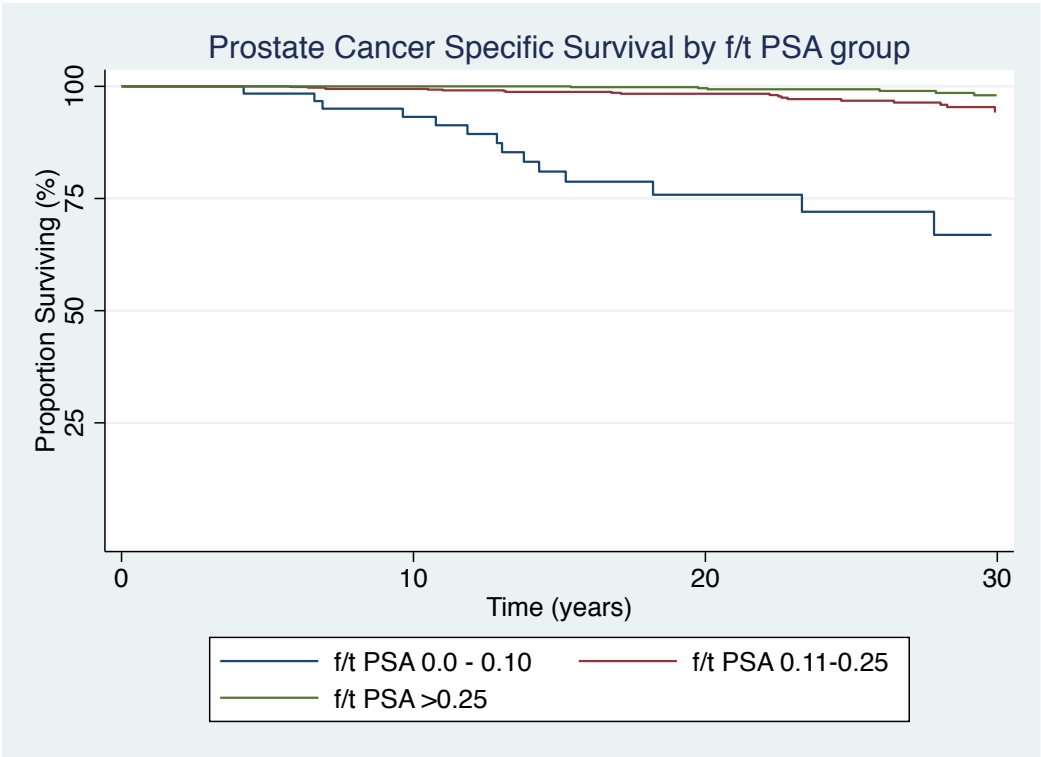


Figure 6. Prostate cancer-specific survival by f/t PSA group. Log rank p<0.0001



The Harrell's Concordance index or the Harrell's C-index resembles the Area Under the Receiving Operator Curve (AUC). Both the ROC test and the C-index evaluates a model's ability to correctly discriminate between cases and non-cases. That is, the model's ability to correctly identify true positive cases as cases. The C-index can, contrary to the ROC analysis test be applied on time to event data. A C-index of 0.5 implies that the model does not discriminate between cases and non-cases better than chance whereas a C-index of 1 suggests perfect specificity and sensitivity [84].

To estimate differences of means the **Analysis of Variance (ANOVA)** method is used. The method, in short, estimates differences in means between two or more groups [85,86] as opposed to the t-test which is limited to two groups.

The Chi Square test is employed to determine whether there is a difference in the actual frequency of an occurrence and the expected frequency of the same occurrence [87].

Both the ANOVA and the Chi Square test are typically used to describe differences in baseline characteristics of two or more substrata.

4.1.8 Laboratory analysis

4.1.8.1 PSA

Levels of PSA was twice determined for the cohort. At the time of screening PSA was analyzed using the Tandem Hybritech Tandem-R method, an immunoassay that targets two different epitopes of the molecule. The antigen used is radioactive and (after washing residual analytes) the remaining radioactivity is proportional to the concentration of PSA in the sample [88,89].

4.1.8.2 Free/total PSA

The second analysis of PSA (approximately 5 years after blood draw) included analysis of the isoforms of PSA, i.e., free vs protein bound PSA as well as total PSA. The serum samples had by then been in -80 degrees storage since shortly after blood draw and centrifugation. The method for measurement was the dual-label time resolved immunofluorometric Prostaus assay. This assay allows for simultaneous evaluation of free PSA and PSA bound to alfa-1 antichymotrypsin, the most common globulin for PSA to be bound to. The detection is done, again, by two radioactively branded isotopes. One (Eu^{3+}) binds to the PSA molecule itself and the other (Sm^{3+}) to the alfa-1-antichymotrypsin part of the complex [90]. Simanek et al published results regarding degradation and reliability for PSA stored at – 80 degrees for ten

years and concluded that PSA was stable but there were some concerns about the isoforms of PSA [91].

Figure 7. Scatterplot of baseline PSA levels and f/t PSA of the entire cohort. Red reference lines at PSA=2 ng/mL and f/t PSA 0.10 and 0.25.

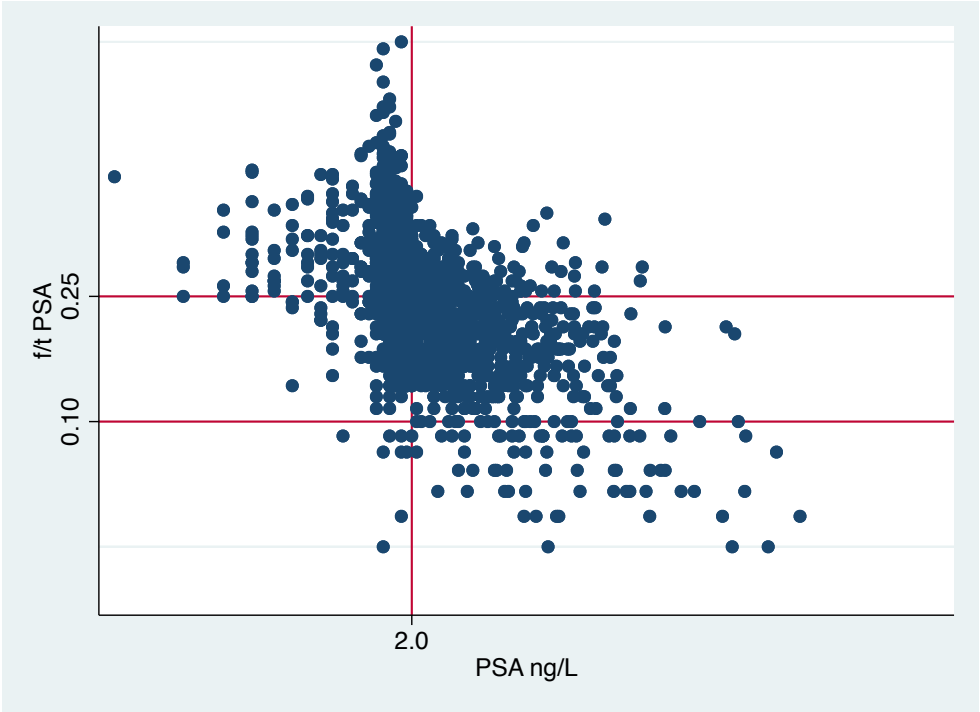
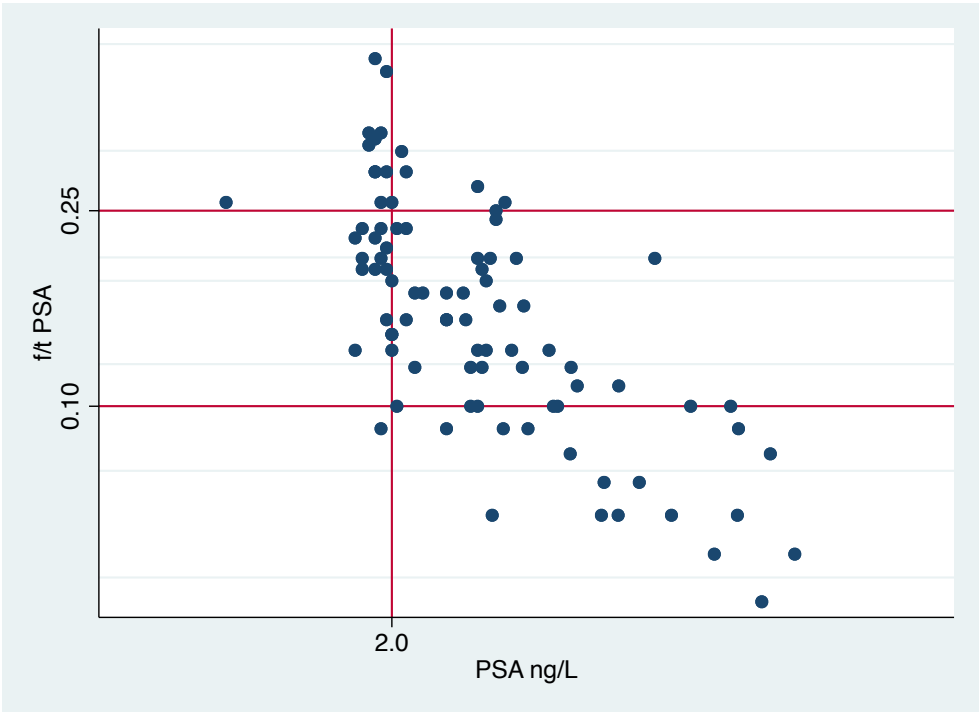


Figure 8. Scatterplot of baseline PSA levels and f/t PSA of men who had died from prostate cancer at end of follow-up. Red reference lines at PSA=2 ng/mL and f/t PSA 0.10 and 0.25.



4.1.8.3 Dihydrotestosterone

Levels of dihydrotestosterone were determined using antiserum technique, again on frozen samples three years after blood draw. A commercially available antibody, in this case the bovine 5β-carboxyethylmercapto-5α-DHT was used and DHT was quantified with radioimmunoassay [56,92].

4.1.8.4 Thymidinekinase 1

TK1 concentrations were evaluated using protein blotting or western blot technique. The commercially available antibody TK210 ELISA kit (AROCELL AB, Uppsala, Sweden) binds to two different sites of the enzyme [93]. The samples were analyzed in 2016 and 2017 almost 30 years after blood draw. There is limited knowledge about integrity of serum samples after such a long time. Ulmert et al evaluated the degradation of PSA after 20 years (at 20 degrees below zero) by comparing levels of PSA with an age-matched cohort without significant differences [94].

4.1.9 Ethical considerations

Population based screening for a disease is to a large extent a purely ethical discussion. In 1968 Wilson and Jungner established a list of criteria that has to be met in order to implement such a screening program [95]. One of these criteria states "there should be an agreed policy on whom to treat as patient". This criterion is, to this point, not completely met. Given that many cases of prostate cancer are indolent i.e., never cause any problems, the identified individuals are at risk of being treated as patients and hence, are at risk of suffering the side effects of treatment.

Large international trials have calculated NNT and with the prognostic markers available today we have to diagnose (and assumingly treat) 18 men in order to prevent one death from prostate cancer [6]. To the point – *is it worth the (perhaps lifelong) suffering of 18 men to save one man from an early death?*

Those advocating screening argue that there is already a widespread opportunistic screening in progress and therefore no additional men will be treated unnecessary, i.e., the level of overtreatment will remain. In my opinion, there is a difference.

Men actively asking to be examined today are putting themselves at risk of over treatment and they are (or should be) thoroughly informed. However, authorities reaching out to all men in a certain age-span, urging them to be examined is a completely different scenario.

The responsibility for possible unnecessary harm is in the latter case transferred to the institutions carrying out the screening.

In today's debate there are strong supporters of implementing screening, not least among the patient associations. They can, however, be argued to have a biased view of the ethics involved by default. When you have contracted an incurable illness, it is easy to assume that you would have preferred to have had it discovered when it was still in a curable state.

The men at risk of being diagnosed and subsequently receiving unnecessary and potentially harmful treatment lack this kind of representation in the debate. This is, of course, because neither of them nor anyone else knows who they are. Nonetheless they exist and deserve to be part of the ethical consideration. Maybe, one can argue that they, in fact, constitute an especially vulnerable group because they don't know that they are "at risk".

When the screening study that constitutes the base of the present projects was initiated in 1988, 2400 men were invited to undergo screening. About 600 of them did NOT participate. We don't know why they did not participate, if it was a deliberate decision or if it was just a matter of not reading the invitation letter.

In either way these men have not signed an informed consent (since they were not included). Despite this, 30 years later we study this group with particular interest. We know from other areas of medicine that people abstaining screening although invited statistically are of a lower socioeconomic stratum, have lower level of education etc. [96]. They also have worse survival, diseases-specific and overall. i.e., they die younger. So is the case for this cohort as well. These individuals can thus be considered to be of particular interest to study, but one can also argue that they are indeed (given the above) a particularly vulnerable population. The Ethical question/dilemma: *Is it ethically correct/ defendable to do research on a population that (actively or not) chose to abstain from participation?*

When presenting this to the ethical review board it was discussed at some length and the conclusion (the IRB agreed) was that the breach of integrity/disrespect of autonomy to these men was less severe than the potential gain of knowledge from actually studying the issue. It should be pointed out that the study they chose not to participate in was of a much more invasive nature (TRUS, blood test etc.) than how they actually have been studied now – by registries only.

Apart from above the projects in this thesis have progressed without difficult ethical considerations. Being a registry study, there is always the risk of sensitive information being

leaked. The follow-up data are de-personalized. Originals are kept in locked cabins. At this point in time nearly 80% of the study persons are deceased.

5 RESULTS

Paper I: A one-time screening examination for prostate cancer of men aged 55-70-years including DRE, TRUS and analysis of PSA in blood with subsequent core biopsies performed if either method of screening caused suspicion does not reduce prostate cancer-specific mortality at twenty years of follow up. A statistically significant increase in number of men diagnosed with prostate cancer does, however, remain at end of follow up. IRR for prostate cancer specific mortality was 0.97 (0.71-1.23; 95% CI) compared to the source population from which the screened population was sampled.

Paper II: DHT measured at time of inclusion influence prostate cancer mortality. Hazard ratio was 0.44 (0.25-0.77; 95% CI) for every unit of increase in DHT. The corresponding hazard ratios for men seemingly heathy at time of inclusion and for those with a recently diagnosed cancer was 0.25 (0.07-0.88; 95% CI) and 0.50 (0.26-0.94; 95% CI) respectively. There was no correlation between baseline levels of DHT and overall mortality.

Paper III: PSA and fraction of free PSA was strongly associated with long-term prostate cancer mortality. At 30 years of follow up HR for prostate cancer diagnosis was 1.05 (1.04-1.05) and 1.04 (1.03-1.06) for prostate cancer mortality adjusted for age and ratio free/total PSA. The combination of PSA< 2.0 ng/mL and free/total PSA of >0.25 indicated beneficial long-term risk stratification. Absolute risk for lethal prostate cancer in this cohort was 1.6 % (0.82-2.9).

Paper IV: Overall survival in the cohort of men with TK1 levels below 0.25 ng/mL was longer (20.9 years) than in the cohort of men with TK1 levels above 0.25 ng/mL (15.4 years), p= 0.009. In the time to event analysis TK1 above 50^{th} and 75^{th} percentile was significantly associated with prostate cancer specific mortality, HR=2.2 (1.1-4.5; 95% CI), p=0.031 and HR=2.4 (1.2-4.7; 95% CI) p=0.017 respectively.

6 DISCUSSION

6.1 SCREENING FOR PROSTATE CANCER

The question whether to launch population-based screening for prostate cancer remains unanswered. Current knowledge is largely based on PSA based screening and data in favor of screening foremost origins in results from the ERSPC. As follow-up of the ERSPC have progressed the numbers needed to screen to save one man from lethal prostate cancer have decreased and the survival benefit of screening have increased. After 22 years numbers needed to invite (NNI) to save on man from prostate cancer death in the Swedish arm of the ERPC was 217 and numbers needed to diagnose (NND) was 8. At earlier evaluations of the same cohort (after 11 and 18 years) NNI was 293 and 243, respectively, and NND was 13 and 11, respectively [75]. Risk ratio for screened men was 0.71 (0.56-0.90; 95% CI).

An almost 30 % reduction of prostate cancer mortality is, by all standards, a significant reduction. Other, already implemented screening efforts, for other diseases, does present even better outcomes. Some examples are presented in table 4.

Table 4. Survival benefits of screening algorithms for 5 different diseases. Trials selected based on impact [75,97-100]. Endpoints are diagnose-specific mortality except for cervical cancer (RR=invasive disease).

	Disease-specific mortality reduction	**95 %CI**
Prostate cancer	RR=0.71	0.56-0.90
Breast cancer	RR=0.74	0.66-0.83
Colorectal cancer (f-Hb)	RR=0.84	0.71-0.99
Abdominal aortic aneurysm	HR=0.34	0.20-0.57
Cervical cancer*	RR=0.60	(0.40-0.89)

Evidently, the disadvantage of implementing PSA based screening for prostate cancer is the risk of over diagnosing and consequently the risk for over treatment. When Ilic et al published a metanalysis of screening trials they concluded that, at best, there is a small reduction in cancer-specific mortality and, according to modelling, for every 1 000 men screened, 25 additional men will suffer from erectile dysfunction. Corresponding number for urinary incontinence (defined as the need for pads) was 3 [101]. Included reports in that meta-analysis were 13 year data from the ERSPC [102], the PLCO (also at 13 year of follow up) [103], the 20 year evaluation (**paper I**) presented in this thesis [104] and a Canadian trial presenting 11 year results. The latter published time to event data with a significant risk reduction using the cox proportional hazard model. In the meta-analysis, however, expressed as IRR, no benefit was detected regarding cause-specific mortality, IRR= 1.08 (0.82- 1.42; 95% CI).

The only evidence that really support population-based screening for prostate cancer using PSA is the ERSPC, especially the Swedish and Dutch arms. Other trials, and indeed, other sections of the ERSPC have not been able to reproduce the disease-specific survival benefits. Advances of screening algorithms including new biomarkers and new methods imaging will likely shift the balance of cost-benefit towards a situation where survival benefits are sustained and the risk for over diagnosing is reduced.

6.2 ANDROGENS AND PROGRESSION OF PROSTATE CANCER

Huggins published his data on castration, i.e. removal of the testes, in men with generalized prostate cancer in 1941 [50]. The swift improvement observed in these advanced cases with the man depleted of T have more or less, since then, dominated biological explanation models, i.e., that T induces progression of prostate cancer. Aforementioned explanation model, which is an extrapolation of Huggins results, has never been demonstrated, except for in cases where the patient is already castrated or treated with Androgen Deprivation Therapy (ADT). It is argued that the inability of the urological society to abandon the idea that T increases prostate cancer progression is in part due to group thinking and part to poorly formulated biological models [105].

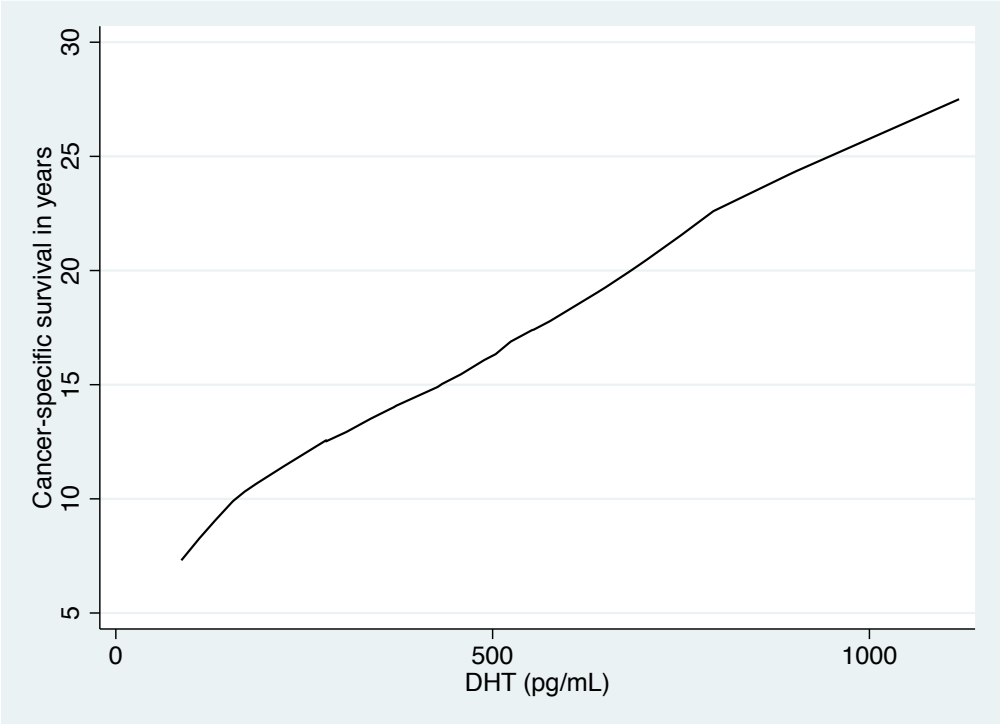
More recently, large case-control studies have been able to demonstrate the opposite. Loeb et al compared outcomes for men treated with testosterone replacement therapy. No difference in overall risk for prostate cancer was observed, OR=1.03 (0.90-1.17; 95% CI), but men with testosterone replacement therapy had a lower risk for aggressive prostate cancer, OR=0.50 (0.37-0.67; 95% CI) [51]. A meta-analysis attempting to evaluate the prognostic value of circulating testosterone acknowledged that there was no association between concentration of

T and overall survival/or biochemical recurrence in the early prostate cancer setting. However, there was a statistically significant risk reduction of death in prostate cancer in men with higher testosterone levels prior to ADT [52].

Results in **paper II** indicate that there is a strong association between DHT and cancer specific survival, demonstrated with a Lowess curve in figure 9.

There is a growing body of evidence supporting that, the generally accepted explanation-model, androgens "feeding" prostate cancer should be abandoned. The evident effect of ADT on advanced cases of cancer does not support the idea that androgens in general promote prostate cancer progression.

Figure 9. Lowess curve of cancer specific survival time by concentration of DHT for men with lethal prostate cancer.



Considering:

- The large, nested case control trial referred to above [51] with lower risk in T substituted men
- Findings from Olsson et al about the intraprostatic androgen metabolism [59,60]
- Estimations of association between DHT and disease-specific survival in the cohort of the Stockholm Screening Trial [55,56,106]
- The increased rate of high risk cancers in the intervention arms of the PCPT and REDUCE trials [53,107]
- The discovery of the intraprostatic estrogen-receptor and the further enzymatic metabolism of androgens [57]

It is reasonable, not only to re-evaluate the role of androgens in prostate cancer progression, but also to deduct that effects result from metabolites of testosterone rather than from testosterone itself.

6.3 PSA AND THE CONCEPT OF LOW RISK

The definition of low risk is, by nature, a relative concept. Statistically the definition of risk can be defined as:

$$\text{Absolute Risk} = \frac{\text{Events Occurred}}{\text{Number exposed}}$$

This simple formula gives you a crude and implicit idea of a certain risk but does not include a comparison which is often fundamental [108]. In **paper III** the absolute risks for lethal prostate cancer are demonstrated stratified by exposure, in this case different levels of base-line biochemistry. For instance, absolute 30-year risk of prostate cancer death for men with PSA< 2.0 ng/mL and ratio free/total PSA of 0.25 or more was 1.6 % (0.82 - 2.9; 95 % CI). In the cohort with even more beneficial serum levels; PSA of less than 1 ng/mL and ratio free/total PSA of 0.25 or more the corresponding risk was 1.4 % (0.034 -7.3; 95% CI). The relative risk compares the two groups of exposure with a basic assessment of risk difference:

$$\text{Relative Risk} = \frac{\text{Risk exposure A}}{\text{Risk exposure B}} = \frac{1.6}{1.4} = 1.14$$

The relative risk indicates that men with PSA< of 2 ng/mL have 14 % higher risk for lethal prostate cancer compared to men with PSA < 1 ng/mL. The confidence intervals, especially

in the exposure group with PSA< 1.0 ng/mL are very wide. The reason for this, and the intuitively modest risk increase, is the number of events in these strata. The absolute risks in the example above are calculated on 11 events in the cohort with PSA<2.0 ng/mL and only one event in the cohort with PSA<1.0 ng/mL. The low number of events make estimates of absolute risk difficult, unless study populations are very large. Other research groups with similar study questions have had the same experience. For instance, Vickers et al report a 25-year risk for prostate cancer death of 0.9 % (0.3-1.7 95% CI) for men with PSA below 1.06 ng/mL. In this case to, the output is based on one single event in the low-risk group [24]. There are challenges in describing the low-risk strata in statistically relevant terms, nonetheless it is undoubtedly safe to assume that PSA and ratio free/bound PSA are strong predictors of long-term risk for lethal prostate cancer.

The definition of low risk when it comes to prostate cancer differs between publications, usually based on statistical output. Examples are outlined in table 5.

Table 5. A selection of influential interpretations of low risk for future lethal prostate cancer

	Mortality	Incidence	Years	Participants, n	PSA (ng/mL)
Carlsson [109]	0.0%	1.9%	15	1162	< 1.0
Lilja [110]	1.5%		23	21277	<0.5
Vickers [24]	0.9 %		25	1167	<1.06
Kovac [25]		0.8 %	13	10968	<0.49

Evaluating risk is a complex area from more views than one. Besides difficulties in statistical interpretation the notion of risk itself and what actions the risk prompts vary greatly. Depending on outcome or endpoint, the interpretation of risk need to alter. In **paper III** prostate cancer- specific mortality is the designated endpoint. If instead, for instance, incidences or rate of recurrence are used the consequences of the event or failure need to be taken into account. Risk is also a highly age dependent. Therefore, the assessment of risk requires a priori knowledge about statistical methods, endpoints chosen, cohort characteristics and age stratifications.

To put data into context and perspective, a selection of lifetime cancer-risks according to the National Cancer Institute and the Surveillance, Epidemiology and End Results (SEER) program [111] are listed in table 6.

Table 6. Lifetime risks and 5-year survival for a selection of common malignant diseases, regardless of clinical stage or histopathological subtypes. Adapted from the SEER database [112].

	Lifetime risk (%)	5-year survival (%)
Prostate cancer	12.1	97.8
Pancreatic cancer	1.6	10
Breast cancer	12.9	90
Urinary Bladder cancer	2.4	76.9
Colorectal cancer	4.2	64.6
Lung cancer	6.3	20.5
Melanoma	2.3	92.7
Thyroid cancer	1.3	98.3

6.4 THYMIDINE KINASE AS A PROGNOSTIC MARKER

Because of its involvement in cell division and its absence in non-dividing cells TK1 have been labelled a proliferation marker, i.e., simply a proxy for rapid cell-division and consequently a marker for rapidly growing tumors.

Other proliferation markers fit that description better. The Ki67 is an antibody that estimates levels of an antigen in the nucleus of the cell [113]. The antigen is present in all cell-cycles except the G_0 phase, also known as the resting phase [114]. In breast cancer specimen the proportion of cells positive for Ki67 and thus are in or anticipating division is elevated. A proportion of 20 % or more is regarded as high and is associated with increased malignant potential [113,115]. Analogously, the MCM genes, or the minichromosomal maintenance protein complex are associated with cancer. For instance, in cervical cancer, expression of the MCM complex was significantly higher in tissue from squamous cell carcinoma of the cervix than

in normal cervical tissue [116]. The MCM exerts it effect during the checkpoints in the cell-cycle.

Most cytostatic drugs block the proliferation of the cells responsible for malignant lesions (and other rapidly dividing tissues as well), i.e., block cell division. It is appealing to use the proliferation model to explain why also TK1 is associated with cancer. Indeed, TK1 is elevated both in tissue and in serum in a number of malignancies [33,117,118].

Seemingly there is a strong association between baseline TK1 concentration and prostate cancer mortality in our material as well. Kaplan Meier estimates for cancer-specific mortality TK1 stratified are presented in figure 10-12.

It seems, however, that the association between Tk1 and prostate cancer is more complex. In the material, presented in **paper IV**, high TK1 is associated with overall death in general and in prostate cancer patients alike regardless if the patients were diagnosed within a year from blood draw or later during follow up. In the latter case it cannot be argued that the high level of TK1 found in serum corresponds to a prevalent and rapidly growing tumor. More knowledge about TK1 and why it is elevated in pre-clinical malignancies could prove important to future screening algorithms and nuance the perception of who is at risk.

Figure 10. Kaplan Meier estimates of prostate-cancer specific survival and baseline level of Tk1 above or below the 25th percentile. Log rank p=0.12.

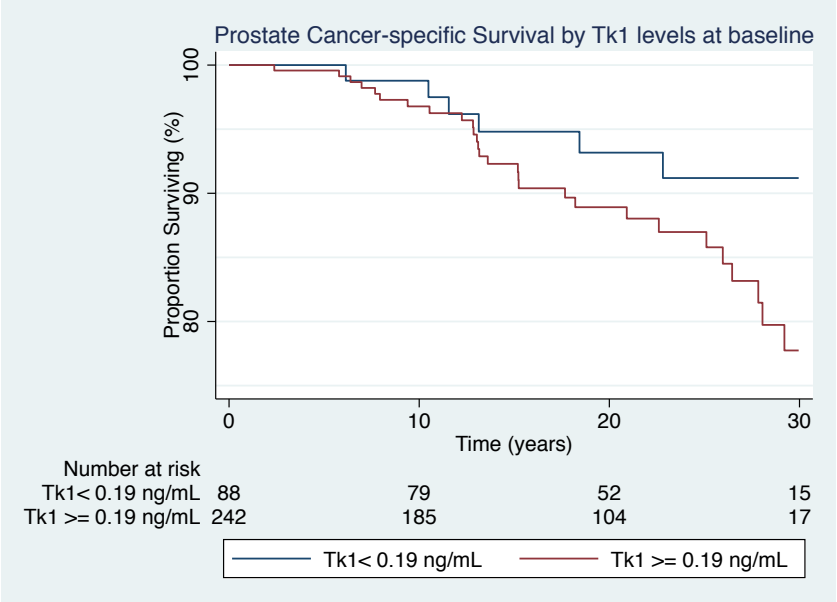


Figure 11. Kaplan Meier estimates of prostate-cancer specific survival and baseline level of Tk1 above or below median. Log rank p=0.038

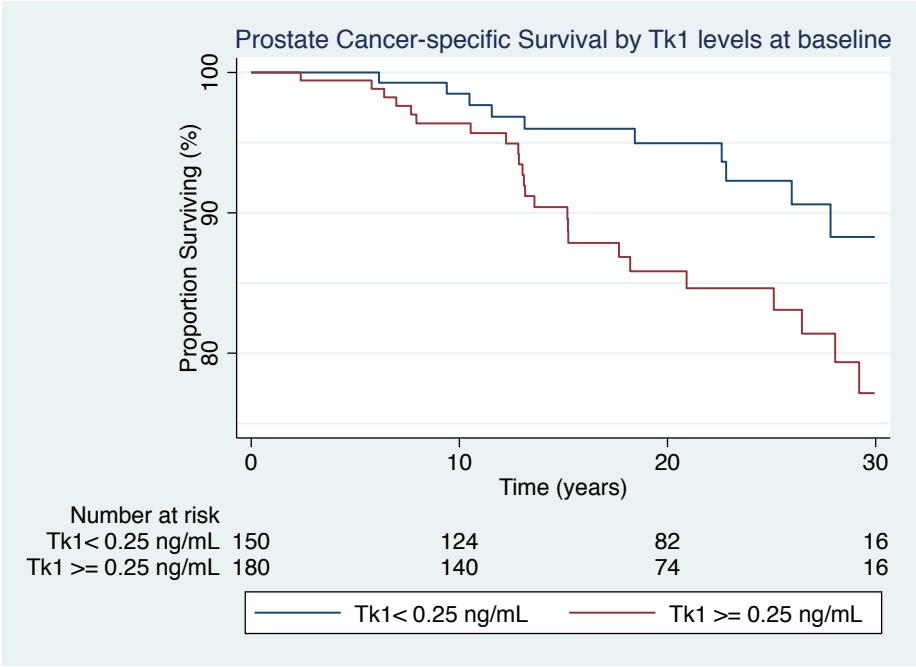
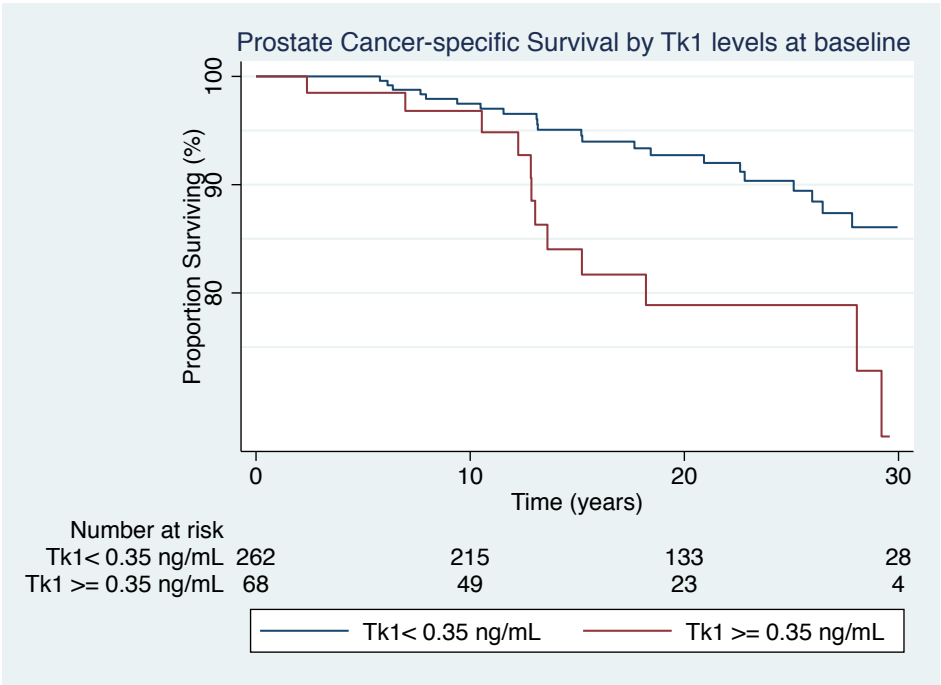


Figure 12. Kaplan Meier estimates of prostate-cancer specific survival and baseline level of Tk1 above or below the 75th percentile. Log rank p=0.009



7 CONCLUSIONS

A screening algorithm with DRE, TRUS and PSA testing does not reduce prostate cancer mortality after 20 years of follow up. The initial rise in incidence seen in the intervention arm remain throughout the study period. Population based screening, if implemented, should be designed differently.

High levels of the androgen metabolite DHT is associated with improved cancer-specific survival at 30 years of follow up. The association was significant both for men with a recently diagnosed prostate cancer at time of blood draw and for men that was seemingly healthy at the time. These data add to the large body of evidence that challenge the central doctrine that androgens promote prostate cancer.

Baseline levels of PSA and the ratio free/total PSA can be used to select men at very low or negligible risk for future prostate cancer death. Being able to exclude a large proportion of men from further screening for prostate cancer could reduce the potential harms of population-based screening.

The phosphotransferase enzyme TK1 is associated with increased overall mortality and prostate cancer-specific mortality. Since TK1, in many cases, is elevated long before cancer diagnose, the biological explanation model needs to be addressed beyond labelling it a proliferation marker.

8 POINTS OF PERSPECTIVE

8.1 FUTURE RESEARCH

The men included in the Stockholm screening trial 30 years ago were between 55 and 70 years at the time. At the last follow up, roughly one fifth of participants were alive. Prostate cancer is, with exceptions, a disease of the older man and often progresses slowly. Higher ages entail more competing risks for death. All this considered, a screening trial for prostate cancer needs to run its course and the longer the follow up the more relevant the data. We will continue to follow this cohort of men, by comparing baseline data with clinical outcomes and also as a resource for fast-track long time evaluations of new markers on frozen serum samples. Further research regarding the androgen metabolism and the possible effect of activation of estrogen receptor-beta as well as the clinical effect of DHT substitution are planned. Another aim is a prospective trial to evaluate TK1 in serum with prostate cancer outcome.

8.2 CLINICAL IMPLICATIONS

Assignment of men newly diagnosed with prostate cancer to correct risk groups has direct clinical impact. Cut-off levels in **paper III** are deliberately set to be of as much clinical relevance as possible. Besides everyday clinical considerations, results from **paper I** and **paper III** are of interest for decisionmakers planning to launch population-based screening. Results from **paper II** are of concern for a large group of men, at risk for or diagnosed with, prostate cancer with coincident hypogonadism. This group are traditionally deprived of replacement theory and results imply that they should not be. Clinical implication of the results from **paper IV** remains to be evaluated. It is possible that measurement of TK1 in blood can be part of a larger panel of markers routinely used for prediction and risk categorization.

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