Prostate Cancer Screening: Outcomes and Risk Prediction

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“Without data, you’re just another person with an opinion.”

William Edwards Deming

To my father, Ivan, with love

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ABSTRACT

The Göteborg Randomized Population-Based Prostate Cancer (PC) screening trial was started in 1995 to evaluate prostate-specific antigen (PSA) screening and its long-term impact on PC-specific mortality and PC incidence. The four papers included in this thesis present the outcomes of PSA-based screening and also describe aspects of the risk of PC at initial screening, during the 22-year follow-up of the programme, and after termination of screening.

In this trial, 10,000 men born 1930–1944 were randomized and thereafter invited to PSA screening every second year from 1995 to 2014. An additional 10,000 men were randomized to the control group (i.e., not invited). The complete incidence of PC was ascertained by linkage to the Swedish Cancer Register and the Swedish Population Register. All relevant medical documentation was retrieved continuously for every man with PC. In our first study (**Paper I)**, we investigated whether men with an elevated PSA level (≥ 3 ng/mL) and voiding symptoms were at higher risk of PC; the results showed no association between such symptoms and an increased risk of PC. Thereafter (**Paper II),** we evaluated the long-term outcome in men with an initial PSA of < 3 ng/mL. We concluded that men died from PC despite "normal" baseline PSA and regular participation in the programme. Baseline PSA was strongly associated with long-term PC risk. Free-to-total PSA had no additive value to PSA in this PSA range.

In our third study (**Paper III),** we assessed PC mortality and incidence in the screening and the control group after 22 years of follow-up, which showed that screening reduced PC-specific mortality by 29%. The absolute risk reduction has increased over the years, and the number needed to diagnose is now 9, which is an all-time low (NND=9). High risk of PC death was found in men who did not attend to the programme, men who started testing after the age of 60, and men who had a long life expectancy and terminated screening too early. **Paper IV** describes our evaluation of outcomes in men who stopped PSA screening after the age of 67–70. We found that participants with a PSA > 1.5 ng/mL (at their final screen) had a non-negligible risk of a future Gleason score of ≥ 7 cancer, and later PC death. Notably, approximately 80% of these cases could have been detected and additional PC deaths prevented, if less than half of all men in the cohort had been offered additional testing (or other diagnostics).

Keywords: prostate cancer, screening, prostate-specific antigen, mortality, prediction

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LIST OF PAPERS

This thesis is based on the following papers:

I. *The absence of voiding symptoms in men with a prostate-specific antigen (PSA) concentration of ≥ 3.0 ng/mL is an independent risk factor for prostate cancer: results from the Gothenburg Randomized Screening Trial*

Maria Frånlund, Sigrid Carlsson, Johan Stranne, Gunnar Aus and Jonas Hugosson. *BJU International, 2012, Sep; 110(5):638-43. doi: 10.1111/j.1464-410X.2012.10962.x.*

II. *Prostate cancer risk assessment in men with an initial PSA below 3 ng/mL: results from the Göteborg randomized population-based prostate cancer screening trial*

Maria Frånlund, Rebecka Arnsrud Godtman, Sigrid Carlsson, Hans Lilja, Marianne Månsson, Johan Stranne and Jonas Hugosson. Published online 21 Sep, 2018, in *Scandinavian Journal of Urology*, *doi: 10.1080/21681805.2018.1508166.*

III. *Improving Prostate Cancer Screening: 22-Year Follow-up in a Randomized Trial*

Maria Frånlund, Marianne Månsson, Rebecka Arnsrud Godtman, Gunnar Aus, Erik Holmberg, Pär Lodding, Carl-Gustaf Pihl, Johan Stranne, Hans Lilja and Jonas Hugosson *(submitted).*

IV. *Prostate Cancer Risk after Stop Age in Men Participating in a Long-Term Screening Programme: Results from the Göteborg Randomised Population-Based Prostate Cancer Screening Trial*

Maria Frånlund, Marianne Månsson, Rebecka Arnsrud Godtman, Anna Grenabo, Johan Stranne, Hans Lilja and Jonas Hugosson *(in manuscript).*

CONTENT

ABBREVIATIONS

BPH	benign prostatic hyperplasia
CG	control group
DRE	digital rectal examination
ER	expected rates
ERSPC	European Randomized Study of Screening for Prostate Cancer
fPSA	free PSA
F/T PSA	free-to-total PSA
GS	Gleason score
ICD	International Statistical Classification of Diseases
IQR	interquartile range
NND	number needed to diagnose
NNI	number needed to invite
NNS	number needed to screen
NNT	number needed to treat
NPCR	National Prostate Cancer Register of Sweden
PC	prostate cancer
PSA	prostate-specific antigen
PSA-DT	PSA doubling time
PSAV	PSA velocity
RCT	randomized controlled trial
RR	rate ratio
SG	screening group
TNM	tumour-node-metastasis (stage classification)
TRUS	transrectal ultrasound
mpMRI	multiparametric magnetic resonance imaging

1 INTRODUCTION

Testing for a disease in people who have no symptoms is called screening. This strategy can help physicians find and treat cancer at an early stage, before symptoms occur and while it is still possible to "remove" or medically treat the disease. By the time symptoms appear, malignant cells may have begun to spread and treatment may no longer be curative. However, if a tumour is detected at an early stage, the risk of death from that specific cancer can be reduced. Still, it is important to keep in mind that even if screening can be associated with many benefits for some individuals, it might cause harms in others.

Prostate cancer (PC) is a common disease that has a large impact on men, as well as on health-care providers, worldwide. The use of prostate-specific antigen (PSA) for screening is highly controversial, and organized PSA screening is not yet conducted in Sweden. Some of the difficulties lie in being able to test men who will benefit from PSA screening, and not those who may suffer from unwanted side effects secondary to diagnostics and overtreatment. A large proportion of men who are diagnosed with a screen-detected PC have no advantage of early detection, because many PCs are slow growing, and other morbidities are more likely to contribute to the cause of death.

The papers included in this thesis are all based on the Göteborg Randomized Population-Based Screening Trial, which was initiated in 1995. The 10th and final screening round was completed in 2014. This trial is unique in many ways, and it has the longest follow-up of all screening studies to date (22 years). Mortality data from this period are presented for the first time in Paper III. At the start of the trial in the mid 1990s, PSA testing was not common in Sweden. Accordingly, the studied population was previously unscreened, an aspect that is impossible to replicate today. Indeed, it is difficult to outline and investigate what is currently called "opportunistic" and widespread use of PSA (men who are PSA testing outside the programme). Another strength with the Göteborg screening trial is that adherence was as high as 77%.

The studies described in the papers included in this thesis focused on outcomes and risk assessment with the intention of improving our understanding of PSA screening and risk prediction in a screening setting. We regard PC screening as a challenging puzzle that needs to be solved!

1.1 THE PROSTATE

Symptoms from the prostate and the lower urinary tract have plagued men since the beginning of time. The origin of the word “prostate” can be traced back to ancient Greece, and literally means “one who stands before” or “protector” (1). This is a rather suitable name, considering that the gland has the location of a gatekeeper to the male reproductive tract. Some of the secretions from the prostate can help protect both the urinary and the reproductive tract from harmful bacteria that enter the urethra and can potentially damage the sperm.

The prostate gland is located below the urinary bladder and in front of (anterior to) the rectum, and it encircles the urethra. The apex of the prostate lies at the bottom of the pelvic floor, which is where the urethra enters the penile structures. The sphincters responsible for urinary control are closely connected to the prostatic part of the urethra, which is about 3 cm long. The seminal vesicles are located at the base of the prostate, close to the bladder and the ejaculatory duct, the latter of which transports the sperm from the testicles to the lumen of the prostatic urethra. The nerves mediating erection runs in the neurovascular bundles that are situated posterolaterally and symmetrically to the prostate in the space defined by the levator fascia, prostatic fascia, and Denonvilliers’ fascia. These nerves are sensitive and can be damaged during prostate surgery (2).

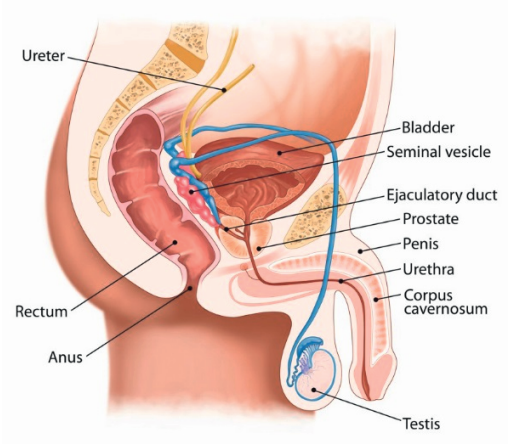

Figure 1. The prostate is surrounded by vulnerable structures: the bladder, the rectum, nerves for erection, and major arteries and veins. (iStock.com/kocakayaali)

The prostate is an exocrine gland that, together with the seminal vesicles, it produces proteins and enzymes that regulate the viscosity of the semen. Starting at puberty, the gland grows to a volume of approximately 20 cm^3 (at about age twenty). The prostate consists of glandular and fibromuscular tissue and secretes a clear, alkaline fluid that constitutes about one third of the semen ejaculate. The composition of the secretion supports sperm survival and motility outside the male body, and the fluid contains several constituents, such as citric acid, phosphatase, potassium, calcium, and zinc (3). The prostate gland is not a vital organ, indispensable for life or even for sexual function. However, after surgical removal of the prostate gland and seminal vesicles (prostatectomy), ejaculation is no longer possible.

The prostate gland is an **androgen-dependent** organ and hormones are required for its normal growth and function. This was first recognised in 1786, by dr Hunter who found that removing the testicles from young male animals prevented growth of the prostate.

In the prostate, testosterone is converted to dihydrotestosterone (DHT), by the enzyme 5alpha-reductase. Androgen action is mediated by the androgen receptor (AR), which mediates the cellular response – and testosterone and DHT activity – by promoting transcription of certain genes. The primary treatment for metastatic PC is androgen deprivation therapy.

1.1.1 Benign prostatic hyperplasia

Enlargement of the prostate gland is a very common condition in ageing men. Furthermore, if they live long enough, most men will develop benign prostatic hyperplasia (BPH), which has been found at autopsy in approximately 40% of men in their 50s and in 70% in their 60s (4). The prostate of an average 25-year-old male weighs about 15–20 g (5), and the change in weight and size of the gland over time is subject to a considerable variation. Most men already display the histopathological characteristics that define BPH by the time they reach the age of 30.

The hyperplasia in BPH is due to an increase in the number of stromal and epithelial cells in the prostate, which results in the formation of large nodules in the transition zone of the gland. Progressive enlargement of the prostate can lead to compression of the urethra and subsequent bladder outlet obstruction. It is also well known that PSA level and prostate volume are predictors of later BPH surgery (6).

1.1.2 Voiding symptoms

Symptoms from the urinary tract have often been called “prostatism”, although this term is no longer used (7). Today, we usually refer to voiding (obstructive) symptoms or storage (irritative) symptoms, and a patient can have mainly the former or the latter, or a combination of both. This can make treatment challenging. Voiding symptoms in men often indicate that BPH is the underlying cause (8). The obstructive symptoms of prostate enlargement can be secondary to an increased resistance in urinary flow, which can cause compensatory changes in the detrusor and bladder function. The initial signs of difficulties in emptying the bladder include hesitancy and straining, weak stream and intermittency. It is possible that impaired bladder contractility can successively lead to symptoms known as overactive bladder. However, symptoms such as urgency, increased frequency of micturition, reduced bladder-filling sensation, post-micturition dribble, urinary retention, and incontinence can be related to a variety of causes, for example urinary tract infections, strictures, neurological disease, or even bladder cancer (9).

Voiding symptoms are common in the ageing population. A study comprising all municipalities in Sweden and based on postal questionnaires indicated that one third of men aged > 50 years suffer from urinary symptoms (10). This finding agrees with additional investigations conducted in Sweden (11, 12) and in other European countries (8).

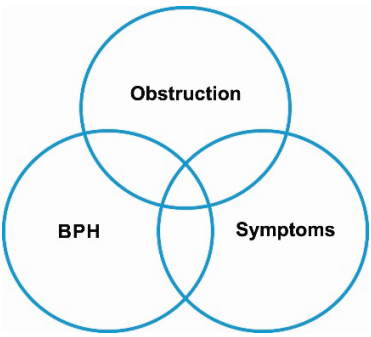

Figure 2. The relationship between prostatic enlargement, the symptomatology, and the presence of urodynamic obstruction, here illustrated by Tage Hald’s classical rings from 1989 (13), showing the complexity of interaction. The three components are not always presented together.

In most cases, symptoms from the prostate and/or bladder do not imply serious disease. However, clinicians in different disciplines often encounter patients who are anxious and experiencing discomfort and shame due to their voiding problem men also have an underlying fear of PC (14) and request urological examination and a PSA test (15), to ensure that there are no signs of cancer in the urinary tract. A worried wife or partner can also have a major impact in this context (16). Furthermore, the ongoing debate in the media regarding PSA testing has received much attention during the last decade and might have an impact on men’s urological awareness.

1.2 UROLOGICAL EVALUATION OF THE PROSTATE AND URINARY TRACT

Obtaining a **medical history** is the first step in assessing all patients, and it should include these aspects: duration and nature of symptoms, previous surgery, medication, and general health status. In some cases, **urinalysis and culture** can aid in diagnosis of an ongoing infection or inflammation in the urinary tract. In men with a confirmed infection, it is recommended to refrain from PSA testing until at least six weeks after successful antibiotic treatment (17).

Several different methods are used to examine the prostate gland. One of these is **digital rectal examination** (DRE), which is performed to evaluate the size, consistency, and shape of the prostate. Indurations and nodules can be signs of PC and often develop in the peripheral zone (18). However, the effectiveness of DRE can be questioned, because many cancers are often too small to be detected by the physician's finger, and one third of PCs are located anteriorly (18).

Transrectal ultrasound (TRUS) is the imaging modality used most often for prostate evaluation (19). It is easy to access the prostate through the rectum, where the probe is introduced. Using TRUS, the urologist can measure the volume of the prostate and, to some extent, also visualize the different zones of the gland. However, there is a significant intra- and inter-observer variation (mean 5–10%) when assessing the size of the gland (20). As previously mentioned, even if a measurement of the prostate volume is correct, it is not necessarily correlated with the degree of the symptoms (21). A definite diagnosis (PC or BPH) can be made only after histological examination of the prostate tissue.

Prostate biopsies are preferably taken under local anaesthesia and by needle aspiration. Ultrasound provides poor visualization of an actual tumour, and the TRUS-guided biopsies are focused mainly on the peripheral zone of the prostate. Thus, this approach can miss significant tumours and instead detect small, insignificant lesions. Additional tissue cores can be taken from other areas of the gland, and it can also be worth considering use of anterior sampling if the initial biopsies show benign findings with rising PSA. This invasive procedure can be associated with haematospermia (10–50%) and haematuria (10–80%) (22), as well as infectious complications. Approximately 3% of men who have biopsies develop a febrile urinary tract infection (23), and the re-admission rate has been calculated to 1–3%, depending on the healthcare system.

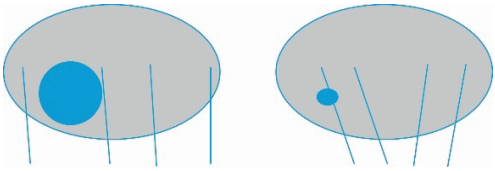

Figure 3. The current TRUS guided technique, can miss significant tumours and "accidentally" detect small indolent cancers.

Multiparametric magnetic resonance imaging (**mpMRI**) is a new diagnostic tool for evaluating the prostate gland. Studies of the use of MRI for detecting PC have indicated that MRI-based prostate-volume-adjusted PSA can improve the effectiveness of PSA for the diagnosis of men with high-Gleason-sum PC (24). MRI is superior to TRUS due to its soft tissue contrast resolution, and it has also been suggested to be useful in the management of patients with BPH (25). In the recently published PRECISION trial (26), the use of risk assessment with MRI before biopsy and MRI-targeted biopsies were considered to be superior to TRUS-guided biopsies in men with a clinical suspicion of PC. With this approach, fewer men had to undergo biopsy, and more clinically significant cancers were identified. In Sweden, MRI is often used for early detection of PC and to select patients for active surveillance, and it has been recommended in the Swedish National Guidelines since 2017 (as part of the work-up process) (27). MRI-guided targeted biopsies improve diagnosis of PC (28), and a large study has been initiated in Göteborg (G2 study) to further asses the use of this modality in a screening setting.

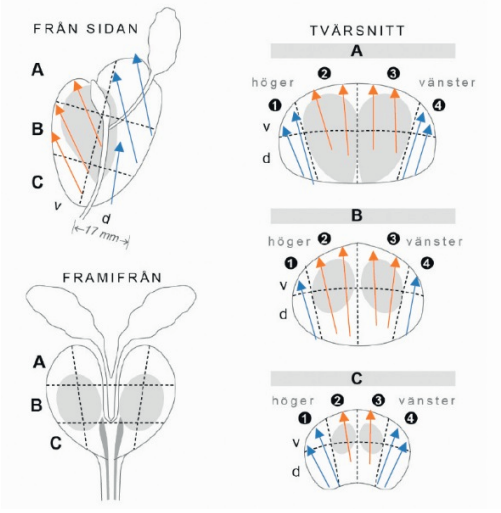

Figure 4. Template for biopsy location (targeted biopsy) after positive findings on MRI (adapted from; Nationellt vårdprogram 2017-02-28, Version 1.2 (27). Reprinted with permission. This template is presently used by urologists, pathologists and radiologists in Sweden.

A: base, B: central, C: apex, v: ventral, d: dorsal.

1.3 PROSTATE CANCER

1.3.1 Epidemiology

In Sweden, PC is the malignancy that is diagnosed most often, and it accounts for about one third of all cancers in men. Each year, approximately 10,500 new cases are reported, and 2,400 men die from PC. As of January 2018, it was estimated that the population of Sweden was 10 million, and that 108,000 men were living with the disease (i.e. the prevalence).

In a global perspective, there is considerable variation in the incidence and mortality rates for PC. In the mid 1990s, the availability of PSA testing started to increase, after which the reported incidence of PC increased in well-developed countries. This has also resulted in an apparent migration to earlier stages at diagnosis (29). PC is often described as a malignancy of elderly men, because it is rarely found before the age of 40, and most cases (> 75%) are detected in men aged ≥ 65 years (30). The average age at diagnosis in Sweden is about 70 years, and fewer than 150 men per year are diagnosed before the age of 50 (31). About one man in five is diagnosed with PC during his lifetime (32). The incidence increases with age, and thus it is assumed that, as the general life expectancy in men increases, there will be a concurrent rise in the incidence of PC. Nevertheless, there is a striking difference between the incidence and mortality curves for this disease, which implies that more men die with PC than from it.

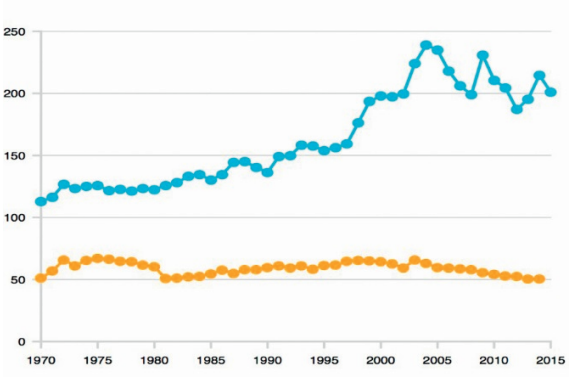

Figure 5. Age standardized PC incidence (blue) and mortality (yellow) in Sweden shown as number of PC cases and number of PC deaths per 100,000. Adapted from www.socialstyrelsen.se and NORDCAN (mortality), www.ancr.nu.

PC incidence in Sweden increased gradually over the years until it reached a major peak in 2005. In 2009, the first report from the European Randomized Study of Screening for Prostate Cancer (ERSPC) showed that PSA screening had reduced PC mortality by ~20% (33). Short thereafter, another incidence peak occurred.

The increase in incidence has been observed primarily in men aged ≤ 70 years, which is also reflected in the increasing number of patients receiving curative treatment. Several factors contribute to the elevated incidence: an ageing population, increased awareness, better access to healthcare, and increased use of PSA testing. Today, many newly diagnosed PCs that are detected have low-risk features, and the tumour is still not palpable. Data from the Swedish National Prostate Cancer Register (NPCR) show that the proportion of such indolent cancers has increased from 14% in year 1998 to 26% in 2016 (34).

PC-specific mortality has also changed in Sweden, with the major decrease (approximately 40%) noted in men aged 60–79 years. Still, the pattern in men older than 80 is different in that it shows no such decline. According to the National Board of Health and Welfare, approximately 60% of men in Sweden who have died from PC have been older than 80 when they succumbed to the disease (data from 2017).

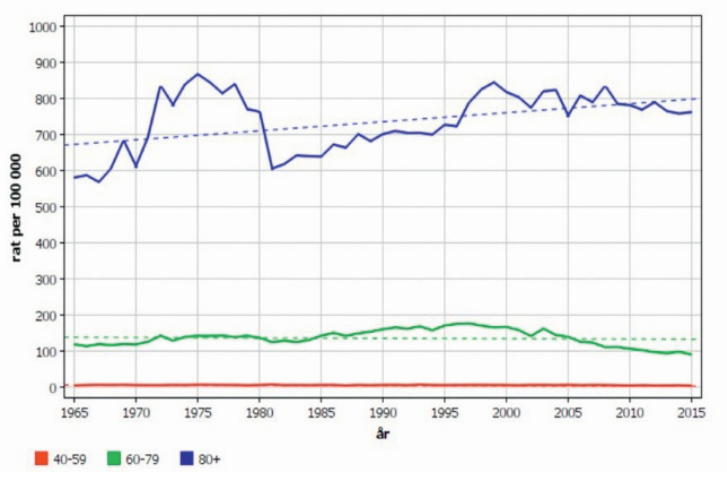

Figure 6. PC mortality in various age groups, expressed as number per 100,000. Data from NORDCAN (32) www.ancr.nu.

1.3.2 Uncertainties in aetiology and diagnosis

Slow-growing and harmless or fast-growing and aggressive

All cancers are caused by damage to the DNA. Carcinogenesis is initiated when genetic changes trigger cells in the prostate to start growing out of control. This opposes the fact that cell proliferation, adhesion, and migration must be strictly regulated to ensure maintenance of the prostatic architecture. In most cases, the abnormal cells grow slowly and remain confined to the prostate, and therefore small and **indolent cancers** are unlikely to affect men's health. The well-known autopsy study conducted by Sakr et al. (35) showed that insignificant low-grade PC is harboured by most men older than 70 years and can already appear as early as age 30 in some men. The prevalence of these clinically latent tumours is estimated to be 20–50% in men aged > 50 years (36). Men with early low-grade PC usually have no symptoms from their cancer. However, these men can have voiding difficulties attributed to BPH, and both benign enlargement of the gland and PC can coexist.

In other cases, PC presents with an **aggressive** behaviour, involving rapid tumour growth and death of the patient within a few years. Recent research has identified a genomic variant located in chromosome 19q13, that influences several genes that can potentially drive the PC to an incurable stage (37). Gao et al. (38) concluded that their findings based on manipulation of gene expression "reveal a plausible mechanism for aggressiveness of PC cells". In men with PC, the genome exhibits various genetic mutations and chromosomal aberrations, and thus it is essentially certain that PC genetics will play an important role in risk prediction and targeted therapies in the future.

Metastatic and advanced disease can cause severe morbidity such as localized bone pain, haematuria, and anaemia. Therefore, early detection is essential to increase the chance of treating high-risk PC within the "window of cure". Since PSA testing was introduced in the mid 1990s, what is called a stage-shift towards organ-confined and low-grade disease has been observed. This means that cancers are diagnosed at an earlier stage, which can help many men avoid spread of the disease. Helgstrand et al. (39) analysed data on 19,487 men in Denmark who died from PC between 1995 and 2013 and found that most of those men had advanced or metastatic disease at the time of diagnosis. During the indicated time period, the proportion of men diagnosed with metastatic cancer decreased by 15.7%, and only 0.15% of all men had low-risk disease at diagnosis. All of the findings mentioned above are primarily the result of increased diagnostic activity, but unfortunately,

this leads to overdiagnosis and subsequent overtreatment for men with an indolent PC.

Another problem, discussed in a previous section, is that the diagnostic features of a biopsy-detected PC do not necessarily represent all lesions in the prostate, and the TRUS-guided random core technique might miss aggressive cancers in the anterior part of the gland. In other words, men diagnosed with low-grade PC might also harbour a high-grade PC. This makes risk prediction on an individual basis challenging. Hence, it is important to consider age and other comorbidities when estimating the life expectancy of men with localized disease (40).

What causes prostate cancer?

Risk prediction would be less complicated, if the aetiology of PC was better understood. Studies have indicated that environmental factors and genetics play a role in PC development and progression, although the only well-established risk factors are old age, ethnicity, and family history. Cancer is caused by changes in the DNA in normal cells, and genetic alterations occur over time, thus older men are at greater risk. In other words, there are no known specific "triggers" for the disease. Inasmuch as this is a highly relevant topic, there are many innovative theories.

Family history and genetics are associated mainly with aggressive PC (41), but only a small proportion (5–10%) of men have true hereditary PC, defined as more than three close relatives who are affected. It seems that men with hereditary PC have an earlier onset of the disease, but otherwise show the same clinical characteristics and survival as observed in men with sporadic PC (42). Genome-wide association studies have identified more than 100 risk loci (43), primarily in populations of European or Asian ancestry, although mortality rates are higher in populations of African descent (44).

Inflammation and chronic infections can cause tissue damage and affect carcinogenesis in several organs of the body (i.e., stomach, penis, and cervix). It is possible that inflammatory changes and oxidative stress can cause cell alterations that promote neoplastic transformations. A study performed in Göteborg found proliferative inflammatory atrophy in tumours from PC patients treated with radical prostatectomy (45). It has been suggested that cytokines and inflammatory cells are associated with the progression of PC by facilitating angiogenesis and tumour growth (46).

Also, recent studies have found a probable relationship between human papillomavirus (HPV-16) and an increased risk of PC (47). Furthermore,

research has indicated that men with fewer sexual partners have a lower PC risk (48), which may be consistent with the HPV theory.

Lifestyle in Western countries has been suggested to influence the risk of PC (49), and discussions have focused on smoking, nutritional factors, overweight, and even alcohol consumption Unfortunately, no effective preventative recommendations have been established. Zhao et al. (51) found a significant dose-response relationship between alcohol intake and risk of PC, starting with a low-volume consumption (> 1.3 up to < 24 g per day). Dairy products have also been discussed in relation to PC, and some data suggest that milk and other dairy products are associated with increased risk of PC and even disease recurrence (50, 51). It is not clear whether this effect is due to suppression of circulating vitamin D by calcium. It has also been proposed that fried foods might be related to PC risk (52). According to the Centres for Disease Control and Prevention, tobacco smoking is the most "preventable cause of death" in the United States, whereas it appears that the association with PC is stronger for aggressive forms of the disease. Moreover, it seems that heavy smokers have a higher risk of PC-specific mortality (53). Physical activity is another factor that has been postulated to reduce PC risk (54) by four different mechanisms. (i) hormonal function (increased production of sex hormone-binding globulin, which results in low free testosterone levels); (ii) energy balance (storage of carcinogens can occur in visceral fat); (iii) immune function (effect on macrophages, lymphokine-activated killer cells, and cytokines); and (iv) antioxidant function (chronic exercise improves free radical defences by up-regulating activities of free scavenger enzymes and antioxidant levels). Physical activity may influence carcinogenesis (55) by suppressing dihydrotestosterone (DHT) activity (via inhibition of 5alpha-reductase). DHT is a promoter of BPH and prostate cells and possibly also a promoter of PC. Endurance athletes have been found to have lower basal levels of testosterone (56) known as the exercise-hypogonadal male condition. However, the epidemiologic evidence and the impact of these changes are still highly uncertain.

Hormonal effects must also be considered, and many urologists are concerned that testosterone replacement therapy (TRT) may accelerate prostate growth, both BPH and PC. If lowering testosterone levels in men can make PC regress in men, does that mean that elevated levels of this hormone can cause PC to emerge? Although, testosterone is necessary for the development of PC, a meta-analysis carried out by Boyle and colleagues (57) found that "PC appears to be unrelated to endogenous testosterone levels, and TRT (for symptomatic hypogonadism) does not seem to increase PSA levels nor the risk of PC development". Roddam et al. (58) performed a

collaborative analysis of 18 prospective studies evaluating sex hormones in serum and found no association between endogenous hormones and risk of PC.

Can prostate cancer be prevented?

Today, after years of different trials (59) no chemo-preventive method has been approved for systematic use, and many consider it rather unlikely that such approval will be granted. Earlier studies have provided results indicating that some micronutrients might protect against PC. However, the Selenium and vitamin E Cancer Prevention Trial (SELECT) (60) demonstrated that neither alpha-tocopherol nor selenomethionine offered any preventive benefit. On the contrary, the results of that trial indicated that men given only selenium, or vitamin E and selenium, were more likely to develop PC than men who were given placebo.

Other investigations have assessed the effects of 5-alpha-reductase inhibitors, which have also been in the focus for chemoprevention. The REDUCE study, evaluated the effect of dutasteride treatment (61), and the phase 3, randomized, double-blind, placebo-controlled **Prostate Cancer Prevention Trial (PCPT)** considered whether treatment with finasteride could reduce the prevalence of PC during a 7-year period (62). The PCPT assessed 18,882 men, aged ≥ 55 years, all of whom had a normal DRE and a PSA of < 3 ng/mL. DRE and PSA measurements were performed annually, and prostate biopsy was recommended for men with a PSA of ≥ 4 ng/mL. The PCPT was terminated 15 months earlier than planned. A 25% relative risk reduction in PC incidence was observed in the men treated with finasteride compared with those given placebo, although high-grade cancers (Gleason grades 7–10) were more common in the finasteride group. After 18 years of follow up, there was no difference in survival between the two study arms, and today this type of preventive strategy has essentially been abandoned.

1.3.3 Diagnosis, staging, grading and risk groups

Why are men diagnosed with prostate cancer?

In Sweden, the majority of men diagnosed with PC have no symptoms, whereas others seek medical consultation for urinary problems. Since 1998, all cases of PC in Sweden are compiled by the National Prostate Cancer Register (NPCR) including the following: quality indicators and data on diagnostics, work-up, and treatment in all six healthcare regions in the country (63). Information from the NPCR is intended to provide quality assurance and can be used for comparison of regions and hospitals, aimed at

improving PC care in Sweden. The main reasons for the initiation of the medical investigation that led to PC diagnosis are listed in the annual report from NPCR (shown below). For approximately one third of the men diagnosed with PC in this country, urological work-up was initiated due to **symptoms** from the lower urinary tract (LUTS).

Year	Health Control	LUTS	Other symptoms	Missing	Total
2004	2,800 (29)	3,473 (36)	3,107 (32)	401 (4)	9,781
2005	2,744 (28)	4,124 (42)	2,381 (24)	508 (5)	9,757
2006	2,611 (28)	3,967 (43)	2,121 (23)	486 (5)	9,185
2007	2,912 (33)	3,684 (41)	1,913 (21)	406 (5)	8,915
2008	3,078 (35)	3,658 (41)	1,793 (20)	335 (4)	8,864
2009	4,465 (42)	3,821 (36)	1,917 (18)	325 (3)	10,528
2010	4,125 (42)	3,472 (36)	1,914 (20)	255 (3)	9,766
2011	4,196 (44)	3,311 (34)	1,872 (19)	229 (2)	9,608
2012	4,178 (46)	2,871 (32)	1,699 (19)	266 (3)	9,014
2013	4,738 (49)	2,891 (30)	1,747 (18)	226 (2)	9,602
2014	5,613 (51)	3,127 (29)	1,621 (15)	571 (5)	10,932
2015	5,410 (52)	3,120 (30)	1,669 (16)	252 (2)	10,451
2016	5,623 (53)	2,995 (28)	1,748 (17)	224 (2)	10,590
2017	5,572 (54)	2,654 (26)	1,788 (17)	223 (2)	10,237

Table 1. Main reason for the initiating the medical investigations that led to the PC diagnoses shown by year of diagnosis (2004–2016). Adapted from (34) NPCR, with permission.

Recent estimates from the NPCR indicate that 54% of all men with PC in Sweden were diagnosed after PSA testing performed as part of a general **health control**. These men had no particular symptoms, and national data show that more than half of all Swedish men aged 55–69 years have had a PSA test (64). This estimate can be compared to < 35% (before 2009).

Today, in Sweden, **clinical PC** is not as common as during the pre-PSA era. Nevertheless, about 10% of newly diagnosed PC cases have metastatic disease at the time for detection, which can be compared with a rate of ~25%, 20 years ago (34).

The TNM staging system

After confirmation of a PC diagnosis, additional examinations (i.e., computerized tomography, bone scintigraphy, and MRI) can be of value for further risk classification. A **staging system** based on the tumour-node-

metastases (TNM) classification (65) is used to describe the extent of the disease (i.e., how far it has spread). The TNM system was recently updated (January 2018) (66), and it provides some key information:

- T (tumour), gives the extent of the primary cancer
- N (nodes), shows whether the disease has spread to lymph nodes
- M (metastasized) indicates whether the cancer has spread to other parts of the body (i.e., bone or lung)

Non-palpable cancers are designated (by the urologist) as clinical t-stage 1 (cT1). Palpable tumours are classified as cT2, or as cT3 if they appear to penetrate the prostate capsule. Tumours that invade surrounding organs are called T4. Pathological staging is done by histological examination after surgical removal of the prostate (pT1–4). Regional lymph node metastases are classified in N stages: NX, N0 and N1: NX (not assessed), N0 (no lymph nodes present), and N1 (lymph nodes present).

Surgeons have previously carried out explorative removal of obturator lymph nodes to evaluate metastasis and spread of disease before making a therapeutic decision. However, the role of imaging has grown in recent years, and mpMRI and computer tomography are now part of standard clinical management in many clinics, and mpMRI can aid detection and characterization of PC (67). M stages denote the following: MX, distant metastasis not assessed; M0, metastasis not present; M1, metastasis present. The stage of the disease is of prognostic value and can be useful when selecting treatment. Average survival is 2–4 years for M1 patients, whereas cancer-specific survival is 8 years for N1 patients (49).

Choline or prostate-specific membrane antigen (PSMA) positron emission tomography (PET)-CT have shown to provide high specificity in the detection of lymph node metastasis prior to curative treatment, however low sensitivity has been reported (68, 69), and these modalities are still not recommended by the EAU guidelines.

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

Table 2. TNM classification of prostate cancer. (Devised and adapted from the American Joint Committee on Cancer (70)).

Grading

Grading of a tumour is performed by a pathologist who examines tissue samples from the prostate, and this entails both intra- and inter-observer variability (71). PC is graded using the Gleason grading system (72) developed by Donald F. Gleason (1920–2008). Tumour grade specifies the degree of tissue abnormality, and thus it indicates the aggressiveness of a tumour based on the histological pattern and microscopic appearance. PCs with a higher Gleason score (GS) are more aggressive and have a poorer prognosis. The sum of the most common patterns (grades) was initially used in such classification.

In 2005, the International Society of Urological Pathology (ISUP) released an updated recommendation regarding the use of the GS grading system. The changes that were outlined led to an upgrading of PC diagnosed after 2005. The PC growth pattern is scored 1 to 5 (well to poorly differentiated), although grades 1 to 2 should rarely or never be used (73). In evaluation of biopsy materials, the most common grade and the highest (worst) grade diagnosed by the pathologist are summed to give a GS (ranging from 6 to 10), and this is highly prognostic for patient outcome. In radical prostatectomy specimens, the GS comprises the two most common patterns, and it should also be recorded if some smaller foci with high grade cells are found (estimated per cent of the extent). Tissue samples from transurethral resections of the prostate (TURP) are graded in the same manner as samples from radical prostatectomy.

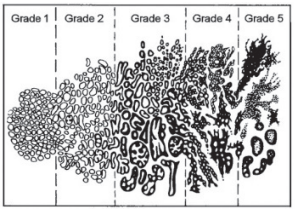

Figure 7. The Gleason grading system, introduced by D. F. Gleason in 1966. (Adapted from (72)).

It is preferable to use **risk groups** when evaluating PC treatment and prognosis (risk of the cancer being aggressive and lethal). The TNM classification system does not consider GS or PSA level for predicting prognosis. For example, a low-risk patient with T1c, GS 6, and a PSA of 9 ng/mL will be quite similar to a patient with T2a, GS 6, and a PSA of 7 ng/mL. By grouping patients with the same type, we can compare failure or success of any given treatment.

In Sweden, the definitions of groups are based on the well-known **D'Ámico** risk classification system (74). These are the main risk groups in the NPCR:

- Very low-risk: T1c, PSA < 10 ng/mL, GS 6, PSA density < 0.15 ng/mL, ≤ 4 cores with PC (at least 8 taken), cancer length < 8 mm
- Low-risk: T1–2, GS 6, and PSA < 10 ng/mL
- Intermediate risk: T1–2, GS 7, and/or PSA 10–20 ng/mL
- Localized high risk: T1–2, GS ≥ 8, and/or PSA 20–50 ng/mL
- Localized advanced: T3 and PSA < 50 ng/ml
- Regional metastasized: T4 and/or N1 and/or PSA 50–100 ng/mL, M0, or MX
- Distant metastasized: M1, bone scan with signs of metastases, and/or PSA ≥ 100 ng/mL

A similar risk stratification system is being used in the Göteborg randomized screening study (75):

	PSA		Gleason score		T stage
Low risk	< 10 ng/mL		≤ 6		T1
Intermediate	10–19.9 ng/mL	and/or	≤ 7	and/or	T2
High risk	20–99.9 ng/mL	and/or	≥ 8	and/or	T3–4
Advanced	≥ 100 ng/mL				M1 and/or N1

Table 3. Definition of risk groups in the Göteborg screening trial.

To reduce overdiagnosis and overtreatment, various measures and nomograms have been suggested for pre-treatment evaluation of patients. The **Epstein criteria** were developed in 1994 and have become a widely used tool for risk prediction in men with localized disease (76). These criteria define insignificant PC as follows: T1c, PSA density < 0.15, GS 6 at biopsy, < 50% PC in a single core, and < 3 of the cores positive for PC. However, in a review published in 2011, the same authors concluded that "the Epstein criteria have suboptimal accuracy for predicting insignificant prostate cancer" (77).

1.3.4 Treatments for localized disease

Early detection and treatment of PC is nothing new, considering that it was in 1905, that Hugh Young suggested that a DRE could detect changes in the prostate that would lead to early diagnosis and interventions (78). Men who are diagnosed with localized disease have several options. Those who have a life expectancy of more than 10–15 years can be offered curative treatment that includes surgery, external beam radiotherapy, and also brachytherapy (both alone and in combination with external radiation). Less invasive alternatives are cryotherapy and high-intensity focused ultrasound (79), although the latter is not applied in Sweden (due to lack of evidence).

Men with symptomatic and advanced PC or disseminated disease are offered hormonal and palliative treatments. Recently, novel antiandrogens (abiraterone and enzalutamide) were introduced for the treatment of metastatic castration-resistant PC. These drugs can increase survival in this group of patients, but they are very costly and less than one third of potentially eligible men in Sweden received such treatment in 2015–2016 (80). Today, these drugs are introduced at earlier stages of PC.

Expectant management

Men with a low-risk PC should primarily be offered **active surveillance** (AS), which is a management strategy intended to help avoid unnecessary treatment and adverse effects. Men with signs of advancing disease (assessed as the proportion of positive biopsies/volume of cancer) can be offered curative treatment. It should be noted that AS is not equivalent to "watchful waiting", a strategy that involves monitoring without curative intent, but rather it aims to manage symptoms in men with clinical progression.

AS has been shown to reduce overtreatment in patients with low-risk PC without compromising cancer-specific survival at 10 years (81). However, inclusion criteria and programmes for AS vary with regard to both protocol and practice. The selection criteria for most programmes are based on D'Ámico classification of low-risk PC ($\leq$ cT2a, PSA $<$ 10 ng/mL, GS $\leq$ 6) (82). The Study of Active Monitoring in Sweden (83), which was initiated in 2011, is a prospective multicentre investigation of AS for low-risk PC. The primary endpoint is conversion to active treatment, and secondary endpoints include symptoms, distant metastases, and mortality. Five hundred patients are to be included over a period of 5 years, and they will be followed for 10–15 years. Hopefully the results will increase knowledge on this topic.

Also of interest, one of very few studies to report long-term outcomes after AS was conducted by Arnsrud Godtman and colleagues from the Göteborg screening trial (84). They have concluded that some men will miss their chance of cure while on AS, and that this type of monitoring is only suitable for patients with very low-risk features.

Curative treatment

A common treatment option in localized PC is **radical prostatectomy** (RP), which entails removal of the prostate gland and the surrounding tissue (to various degree). This can be done with a retropubic or perineal approach. Terence Millin performed the first retropubic RP in 1945. The technique was further developed in the 1980s, when Patrick Walsh explained the anatomical and pathological considerations related to preservation of sexual function (2). Nerve-sparing surgical removal is important to preserve as much function as possible. The conventional laparoscopic RP technique appeared in the 1990s, but it was never fully established due to a long learning curve and data showing no advantages compared with open surgery (85). The first robot-assisted laparoscopic radical prostatectomy was performed in 2001, and since then this method has been widely established, despite the limited proof of better oncological and functional outcomes (86, 87). Nonetheless, men who undergo such robot-assisted surgery have shorter hospital stay and receive fewer blood transfusions (86). Approximately 400 operations are performed each year at Sahlgrenska University Hospital in Göteborg, and the surgical outcomes are freely available at www.npcr.se (RATTEN).

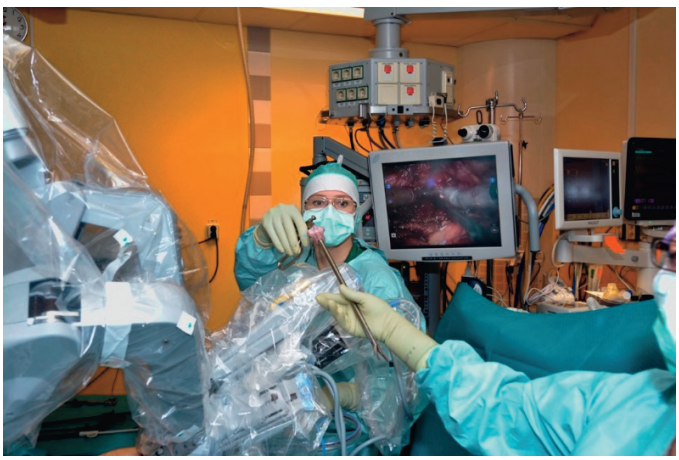

Figure 8. The author in the operating room. Robot-assisted radical prostatectomy at the Sahlgrenska University Hospital. (Photo: Lennart Wiman, 2012)

The Swedish SPCG-4 trial randomly assigned approximately 700 men with localized PC to surveillance or radical prostatectomy, with a follow-up period of 23 years. Surgery was found to be beneficial and associated with reduction in all-cause mortality (56% vs. 69%), PC mortality (18% vs. 29%), and metastatic disease (26% vs. 38%) (88).

Curative treatment also includes **radio therapy** (RT). In the 1960s, this was given as high-energy X-ray that included high doses in surrounding tissues, affecting the bladder and the rectum. Since then, this technique has been further developed, and even higher doses are used today, and the treatments can be delivered from an external beam source or as brachytherapy. With a dose-escalating regimen, 78 Gray (Gy) can be given. and more than 100 Gy can be used to treat localized disease by combining external therapy and brachytherapy. In 2009, the SPCG-7 study reported that, in patients with high-risk or locally advanced PC, adding local radiotherapy to endocrine treatment reduced the 10-year PC-specific mortality by ~50% (89).

Side effects of treatments

Early detection and treatment of PC saves lives, but the drawback is side effects in terms of erectile dysfunction and incontinence. The LAPPRO study (87) evaluated functional outcomes after both open and robotic surgery and showed that, at 12 months after surgery, approximately 20% of men were incontinent and as many as 70–75% had erectile dysfunction. Radiotherapy can induce proctitis with haemorrhage and irritative voiding symptoms.

Carlsson et al. (90) investigated the excess burden of treatment side effects in screened men, and their data suggest that 120/10,000 more men will become impotent and 25/10,000 more will need incontinence pads postoperatively among men invited to PSA screening. Studies have attempted to estimate the extent of overtreatment resulting from PC screening, but the results vary because evaluated populations differed with regard to age and comorbidities, as well as the time periods under consideration (91). Further research is needed to learn how to differentiate between men with life-threatening cancers and those who can be safely kept under surveillance. In the quest for a more selective approach, nomograms and genetic tests are rapidly emerging. New biomarkers and imaging may also guide us in the future. However, we should always consider the well-known phrase from the Hippocratic oath: *"primum non nocere"*– first, do no harm.

1.4 BIOMARKERS

The introduction of biomarkers for diagnosis and follow-up dramatically changed the practice of oncology. The term "biological marker" was introduced in the 1950s (92), and, in 1998, the US National Institutes of Health (NIH) defined a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" (93). Cancer biomarkers can be produced by a tumour or by the human body in response to a malignant process. In 2011, Shariat et al. (94) described six uses for biomarkers in the management of PC:

1. Detection/Screening: when evaluating patients/men with either symptoms of or risk factors for PC.
2. Diagnostic: Establishing the absence/presence of cancer, when standard histopathology is insufficient.
3. Prognostic: predicting the outcome in patients, in terms of their risk of recurrence, progression, or death, and thereby allowing for individualized management.
4. Predictive: predicting and/or monitoring the effectiveness of a treatment, to aid selection of the best treatment modality for an individual patient.
5. Therapeutic target: identifying the molecular target of a specific therapy and thereby establishing whether an individual patient will or will not respond; no such biomarker is currently in clinical use for any PC treatment.
6. Surrogate endpoint: as a substitute for clinically relevant endpoints when assessing a particular treatment regarding its clinical benefits and harms (or lack thereof); replacing traditional endpoints (e.g., death, morbidity, and recurrence) with biomarker-based endpoints can reduce the time and costs of clinical trials.

The use of biomarkers is a rapidly emerging field (e.g., including proteomic/genomic platforms, circulating tumour cells and urine-based analyses), and several promising biomarkers are currently being evaluated (95). There is a great potential in future profit for those who find a suitable biomarker considering that several of the new tests are associated with high costs.

This chapter specifies and discusses some of the most well-known tests (mainly blood-based markers). PSA is used as a screening tool in the Göteborg randomized population-based screening trial.

Prostate-specific antigen

Unlike many other malignancies, PC has a long history of biomarkers. As early as the 1930s, it was known that serum concentrations of prostatic acid phosphatase (PAP) are elevated in men with metastatic PC (96), and therefore PAP was used for many years as a clinical biomarker for disease progression. Research on PC biomarkers continued, and in the 1980s PAP was replaced by what is arguably still the most useful of all cancer biomarkers, namely, PSA. The introduction of PSA as a diagnostic test for PC completely changed the epidemiology and the clinical management of this disease. PSA testing has been widely used since the 1990s, not only for clinical detection and screening of PC, but also for monitoring men who have the disease and are under surveillance, before and after treatment. However, it is difficult to find a cut-off value with high specificity and adequate sensitivity (97).

The early research on PSA was conducted in the 1960s and 1970s. At that time, antigens in semen that could be associated with infertility were assessed by several groups (98). One of these antigens was the protein PSA, an enzyme that was also evaluated as a forensic marker for rape victims (99). In healthy men, PSA is most abundant in seminal fluid, where the concentration is one million times higher than in serum (100). In 1979, PSA was purified from prostatic tissue (101), and in 1987 Stamey et al. (102) published data showing that PSA was more sensitive than PAP in detecting PC. Studies performed a few years later suggested that PSA could be useful in early detection of PC. Catalona et al. (103) conducted a clinical trial including 6,630 men and found that detection of PC was improved by using a combination of PSA testing with a cut-off of 4 ng/mL and clinical examination (DRE). The United States Food and Drug Administration (FDA) approved PSA as a diagnostic marker for PC in 1994. Nevertheless, the specificity is poor when using cut-offs with sufficient sensitivity, and the optimal PSA threshold for proceeding to a prostate biopsy has been discussed intensely ever since the FDA authorization.

PSA biochemistry and physiology

PSA is a member of the kallikrein family of proteases and is also known as human kallikrein 3 (hk3). This androgen-regulated glycoprotein is secreted in high concentrations by the prostatic ductal and acinar epithelial cells. PSA is a serine protease with chymotrypsin-like activity. Its natural substrate consists of the proteins that make the seminal fluid gel-like, and it cleaves those proteins to liquefy the seminal fluid (104). Hence the physiological function of PSA is considered to be promoting sperm motility (105).

PSA is also produced by neoplastic cells originating from the prostate epithelium, although at somewhat lower levels and in varying amounts compared with benign epithelial cells (94, 106). The reason that both BPH and PC raise the serum PSA is that the architecture of the normal prostate membrane prevents PSA from reaching the circulation: only a very small proportion (one millionth) of the PSA leaks over into the bloodstream. Most prostatic diseases and traumata, including inflammation (prostatitis), BPH, cancer, and biopsy, disrupt the epithelial layer and the basement membrane (107). Although the serum PSA is usually raised in men with clinically relevant PC, some poorly differentiated PCs do not produce any PSA at all. Molecular subgroups of primary PC with characteristics similar to those of metastatic disease have been described (108).

Free/Total PSA

PSA occurs in serum in several molecular forms that can be either free (designated fPSA) or bound to protease inhibitors as stable covalent complexes. The bound forms are collectively known as complexed PSA (cPSA). Complexes with alpha-1-antichemotrypsin and α2-macroglobulin are predominant (109), and fPSA constitutes 5–30% of the PSA in serum.

The ratio of free to total PSA (F/T PSA) is known to be lower in men with PC than in men with BPH, although the magnitude of this difference varies between studies (109, 110). Hence F/T PSA, which is often expressed as the percentage of free PSA (%fPSA), may help discriminate between men with BPH and men with PC. In 1998, Catalona et al. (111) detected PC on biopsy in 56% of subjects with F/T PSA < 10% but in only 8% of those with F/T PSA > 25%. In that study F/T PSA was validated in PSA ranges of 4–10 ng/mL. The authors suggested that F/T PSA ≤ 25% could serve as the criterion for biopsy in the absence of a palpable nodule in the prostate. In Sweden, many centres routinely use F/T PSA for diagnostic decisions, and many laboratories automatically analyse F/T PSA as a reflex test if the PSA is within a certain range. A laboratory usually sets the cut-off at 18%, but as with PSA, no clear threshold can free the man from PC.

The interest in different molecular forms of PSA as biomarkers has accelerated. Various combinations of kallikrein biomarkers are often regarded as the future of PC diagnostics, because they offer increased specificity compared with PSA only and thereby reduce the number of men who require a prostate biopsy (see section on the 4Kscore).

PSA density

PSA alone is far from a perfect biomarker, but its diagnostic performance might be enhanced by analysing PSA kinetics (see below) and by relating the PSA value to the prostate volume (PSA density). Early reports concerning the usefulness of PSA density for selecting men for prostate biopsy have presented conflicting results (112, 113). Higher densities (> 0.10–0.15 ng/mL/cm^3) are more suggestive of PC, whereas lower densities are more suggestive of BPH. Nordström and colleagues (114) recently published an analysis of the utility of PSA density, which was conducted as a prospective study of 5,291 men with PSA ≥ 3 ng/mL. This well-designed investigation suggested that omitting biopsy for men with a PSA density of ≤ 0.07 ng/mL^3 would save 20% of the men from having a biopsy, albeit at the cost of missing 7% of the cancers with GS 7–10. Thus, although PSA density might provide support for decisions regarding biopsy and spare some men from the morbidity associated with this invasive procedure, the clinical guidelines issued by the European Association of Urology (EAU) (115) and the American Urological Association (AUA) (116) do not advocate the use of PSA density for diagnostic decisions.

PSA kinetics

Rising PSA levels can reflect PC progression. Different ways of measuring the rate of increase in PSA are collectively called PSA kinetics, and the two applied most often are PSA velocity (PSAV) and PSA doubling time (PSA-DT). Early research indicated a clear prognostic value of PSA kinetics. For example, the often cited study by D'Ámico et al. (117) showed a higher PC mortality after radical prostatectomy and after radiotherapy in men whose PSA had increased more than 2 ng/mL the year before diagnosis. However, later investigations (discussed below) have not obtained similar results regarding PSA kinetics as an adjunct in the diagnostic process. According to the EAU guidelines, PSA kinetics "may play a prognostic role in the treatment of PC, but they are of limited diagnostic value" (115).

PSAV is a measure of the annual change in PSA, given in ng/mL/year (as discussed in Paper IV). PSAV has been reported to provide information to aid decisions concerning biopsy and the timing of the next PSA test. In 1992, Carter et al. (118) were the first to publish data on the rate of change in PSA values over time. PSAV can be calculated in several different ways, for example, by using the first and the last value only, and by performing regression analysis using all available PSA measurements over a certain period of time. Carter and co-workers (119) also specified that at least three consecutive measurements made over a 2-year period must be evaluated.

The Baltimore Longitudinal Study of Aging showed a strong association between cancer-specific survival and PSAV 10–15 years before diagnosis (120). Based on such findings, the AUA (116) and the National Comprehensive Cancer Network (NCCN) presented clinical recommendations that men with a PSAV of > 0.35 ng/mL per year should consider having a prostate biopsy, even if they have a normal DRE and PSA below the standard cut-off. However, the results of some later prospective studies indicated that PSAV offers no additional diagnostic value compared with PSA alone (121, 122). In the PCPT (123), PSAV lost its independent predictive value after adjustment for the absolute PSA value and standard clinical variables.

In 2009, Vickers et al. (124) published a systematic review of 12 studies that compared PSAV with total PSA (tPSA) only for predicting PC on biopsy. These investigators found several methodological limitations of the 12 studies and no strong evidence supporting the use of PSAV in clinical decision-making. In 2011, the same authors reported that taking biopsies in men with a low PSA level but a high PSAV led to a large number of unnecessary biopsies (125), and that there was limited evidence supporting the AUA and NCCN guidelines recommendations regarding the use of PSAV. Vickers and colleagues explained it as follows: “It is unclear why a marker that predicts aggressive PC many years in the future should be used to suggest immediate biopsy to patients”. The interest in PSAV declined after publication of these reports. Nevertheless, many urologists have a “clinical feeling” for the importance of changes in PSA levels and use an estimate of the PSAV in clinical practice. There is much “background noise” (e.g., transient rises in the PSA level caused by BPH and biopsies detecting indolent PC regardless of the PSAV) that probably had a negative effect on the ability of the mentioned studies to assess the association between PSAV and clinically significant PC.

Data from a recent Danish study (126) of 7,455 men who had multiple PSA measurements suggested that the long-term PSA changes could help identifying men with low probability of PC mortality. In their investigation, 503 men aged 30–80 years, with and without PC, who had repeated PSA tests over 20 years (and up to 28 years before diagnosis), were analyzed. The authors concluded that *“long-term PSAV in addition to baseline PSA improved classification of risk of PC and mortality”*.

PSA doubling time (PSA-DT) is another method of measuring PSA kinetics (127). It measures the exponential increase in PSA over time.

Human glandular kallikrein 2

All members of the human tissue kallikrein gene family code for proteases. There are at least 15 such genes, and they share important characteristics, including mapping at the same chromosomal locus (19q13.4) (128). Human glandular kallikrein 2 (hK2) has been described as a valuable predictive marker for the detection of PC, in some studies as even better than tPSA REF The hK2 protease shares 80% amino acid identity with PSA, and, similar to PSA, several forms of hK2 (i.e., free hK2 and hK2-ACT) can be detected in serum. Free hK2 is associated with higher GS (129). The levels of hK2 in the prostate, semen, and serum are less than 2% of the corresponding PSA levels. It has been suggested that hK2 can be useful in predicting the outcome in PC patients treated with radical prostatectomy (130), and several studies have concluded that this biomarker has an additive role in PC detection.

Prostate Health Index

The Prostate Health Index (PHI) is a mathematical formula that combines tPSA, fPSA, and [-2]proPSA to give a single score that can be used to aid decision-making for men with PSA values of 4–10 ng/mL (131, 132). A PHI value is calculated using the formula ([-2]proPSA/freePSA) x √PSA, based on the knowledge that men with higher tPSA and p2PSA and lower fPSA more often have clinically significant PC. In 2011, Catalona et al. (133) published results from a multicentre study of PHI for PC detection in 892 men with moderately elevated PSA and benign DRE. These researchers found that the mean PHI scores were 34 and 49 for men with negative and positive biopsies, respectively. With a sensitivity of 80–95%, PHI had a greater specificity for discriminating PC than tPSA and F/T PSA. The area under the curve (AUC) was 0.70 for PHI compared with 0.53 for PSA and 0.65 for F/T PSA. PHI has been approved by the US FDA and is marketed commercially by Beckman Coulter Incorporated.

The four-kallikrein panel (4K score) adds clinical information

In some ways it seems unlikely that a single biomarker will be “good enough” to make a definite and exact decision regarding diagnosis and/or prognosis of PC. To improve the accuracy, a research group led by Hans Lilja and Andrew Vickers developed a statistical model (the four-kallikrein [4K] panel) for predicting biopsy outcomes that is based on age, DRE, tPSA, fPSA, intact PSA, and hK2. In a study of 740 men participating in the Göteborg screening trial, these investigators used the 4K model to determine whether biopsy should be performed (134). The authors report that, “using a 20% risk of PC as the threshold for biopsies, would have reduced the number

of biopsies by 57% and missed only 31 out of 152 low-grade and 3 out of 40 high-grade PCs". Adding the 4K panel increased the AUC for PC detection from 0.68 (with a base model with PSA and age) to 0.83. Similar observations were made in the French section of the ERSPC (135), which found that the corresponding AUCs were 0.63 and 0.78. Furthermore, it can be concluded that using the model in the Dutch section of the ERSPC would have saved 49% of the men from undergoing biopsy, at the cost of missing 14% of the high-grade cancers (136). The 4K panel has also been tested in men who had previously undergone biopsy during screening (137). That analysis demonstrated that applying the 4K panel to 1,000 men with persistently elevated PSA after an initial negative biopsy would reduce the number of biopsies by 712 and miss or delay the diagnosis of 53 cancers.

The 4K panel is marketed by OPKO Health Ltd. under the name 4Kscore®, and it is calibrated to identify high-grade PC on biopsy. An investigation applying the 4Kscore to participants in the Malmö Diet and Cancer Study was recently published (138). The results showed that 7.7% (one in 13) of 5,263 men aged 60–73 years with a PSA of ≥ 2.0 ng/mL died from PC within 15 years after the analysed blood sample was collected. By using the 4Kscore with a cut-off of 7.5% risk of high-grade cancer, the men could be split into two groups: a high-risk group with a 13% (one in eight) chance of dying from PC within 15 years, and a low-risk group with only a 1.7% (one in 59) chance. This showed that men in the high-risk group should have received further evaluation, such as an MRI or a prostate biopsy, whereas the men in the low-risk group could have safely avoided a biopsy. Also, monitoring the PSA in the men in the low-risk group might have further lowered their long-term risk. The 4Kscore test is included in the 2017 NCCN and the 2016 EAU Prostate Cancer Guidelines (115).

Both the PHI and the 4Kscore have been developed for predicting the outcomes of first-time biopsies. It has also been shown that these strategies perform better as diagnostic tests compared with PSA alone. Moreover, a study directly comparing the PHI and 4Kscore constituted similar performance of these two tests (139). However, it should be noted that the cited investigation did not include men with PSA < 3 ng/mL, and significant cancers can be found in as many as 25% of men with PSA in the range 2–3 ng/mL (140).

The STHLM3 model: adding clinical information and genetic markers

The Stockholm3 (STHLM3) model is a new PC diagnostic test that combines the analysis of five serum biomarkers (tPSA, freePSA, hK2, MSMB and

MIC1) and 254 genetic markers (SNPs), risk factors (age, previous biopsies and family history), and clinical variables (DRE and prostate volume) (141). It seems that performance of this model is similar to that of the 4Kscore and the PHI tests, but to date only three studies of the STHLM3model have been reported, one of which assessed this model in combination with MRI (142). The STHLM3 test predicts the risk PC with a GS of ≥ 7 PC on biopsy. Single-nucleotide polymorphisms (SNPs) are single-nucleotide (A, T, C, or G) alterations in the genome. SNPs normally occur in the DNA, most often in the DNA between genes. Genome-wide association studies have identified at least 150 SNPs associated with the risk of PC (143), and approximately 30% of the familial risk is due to theses variant mutations.

The first study of the STHLM3 model was published in 2015, and invited 145,905 men for evaluation of PC risk (141). In a stepwise logistic regression analysis, the risk factors (i.e., age, family history, and biopsy history), the combined genetic score, all individual plasma protein biomarkers, and the clinical variables (i.e., prostate examination and prostate volume) all contributed significantly to the multivariable model. In a second analysis of the STHLM3 model, Ström et al. presented results obtained using an updated version of the model (144), in which intact PSA was removed and analysis of the G84E mutation in the *HOXB13* gene was included (shown below).

Risk factors	AUC (bivariate) 95% CI	AUC (cumulative) 95% CI
Age	0.59 (0.57–0.61)	0.59 (0.57–0.61)
Digital rectal examination	0.63 (0.61–0.64)	0.63 (0.61–0.65)
Previous biopsies	0.61 (0.59–0.63)	0.65 (0.63–0.66)
Prostate volume	0.67 (0.66–0.69)	0.71 (0.69–0.73)
Family history	0.59 (0.57–0.61)	0.71 (0.70–0.73)
Free PSA	0.65 (0.63–0.67)	0.72 (0.71–0.74)
Free/total PSA	0.65 (0.63–0.67)	0.73 (0.71–0.74)
Intact PSA	0.58 (0.56–0.60)	0.74 (0.72–0.75)
hK2	0.59 (0.57–0.61)	0.75 (0.74–0.77)
MIC1	0.59 (0.57–0.61)	0.75 (0.74–0.77)
MSMB	0.60 (0.58–0.62)	0.76 (0.74–0.77)
HOXB13	0.59 (0.56–0.60)	0.76 (0.74–0.77)
Genetic score	0.61 (0.59–0.63)	0.76 (0.74–0.77)

Table 4. Performance in predicting GS ≥ 7 disease for different variables included in the STHLM3 model. MSMB denotes microseminoprotein-beta, MIC1 macrophage inhibitor cytokine-1 and HOXB13 homeobox B13 gene.

The new model was fitted to data from the STHLM3 training cohort, and the authors reported that this adjustment "slightly improved the AUC" compared with previous results (0.75 vs. 0.74). The AUC for PSA alone was 0.58 (95% CI 0.57–0.60). However, genetic testing requires well-informed men, and there are several ethical considerations with this method (145). Furthermore, the additive value of the genetic score in the STHLM3 model has been questioned (146) and freePSA, intact PSA and hK2 are also incorporated in the model constituting the 4Kscore® (mentioned above).

A urine test for prostate cancer antigen 3 (PCA3)

The PCA3 gene is expressed only in the prostate, and in 1999, scientists reported that this gene was highly overexpressed in PC compared with normal and BPH tissue (147). A diagnostic method using polymerase chain reaction (PCR) is applied to detect PCA3 mRNA in urine, and this test has been evaluated in several clinical investigations. In 2012, the US FDA approved the PCA3 assay, which is called PRoGensa®, and studies have provided evidence that it might be useful in reducing the number of negative biopsies (148, 149). The PRoGensa assay is indicated for use in combination with other clinical data in men aged ≥ 50 years who have had previous negative biopsies. Prior to urine collection, a DRE should be performed, and pressure should be applied on the prostate. Although the brochure given to physicians does explain that such pressure should not entail a "prostatic massage", it is highly possible that the procedure will result in some discomfort for both the patient and the doctor.

The ERSPC risk calculator (RC1–6)

Over the last 10 years, there has been extensive development of nomograms and risk calculators for the prediction of PC-positive biopsy. Prediction tools are intended to support physicians in clinical decision-making by helping to avoid unnecessary biopsies. The most well-established of these tools is the PC risk calculator from the ERSPC, which is used for men aged 55–74 years and is based on data from the Dutch section of the ERSPC. This method was developed using multivariable logistic regression analysis (150), and external validation has been performed using data from the Swedish (Göteborg) and Finnish sections of the ERSPC (151). This risk calculator has six levels (designated RC1–6) created using different logistic regression models, and it is available online for both doctors and patients (www.prostatecancer-risk calculator.com). RC1 considers family history, age, and voiding symptoms. RC2 uses PSA levels to predict the necessity of future investigation. RC3 and RC4 include prostate volume and DRE findings (which can be used for men with no previous biopsy or men with a benign result); inclusion of prostate

volume enhances the estimation of the risk of significant PC (152). A possible inclusion of the PHI blood test has also been evaluated (153) and seems to slightly improve the predictive capability. RC5 calculates the probability to have an indolent PC. RC6 is the most recent one, calculating the future PC risk over the next four years (based on family history, age, PSA, DRE and previous biopsy status).

Genomic testing for localized PC

Several genomic tests are available for men who have an established PC diagnosis, and these analyses are not diagnostic tests. Prolaris®, Decipher®, and OncotypeDx® can all be used for prognostic risk stratification and to aid treatment decisions (154). All three of these tests can be used to analyse prostate tissue (tumour cells in prostate biopsy). The Prolaris and Decipher tests can be performed after radical prostatectomy in cases in which secondary therapy can be considered. OncotypeDx provides an individual risk score that can be used in combination with other clinical data, and it is marketed as "the only test developed specifically for men deciding between active surveillance or curative treatment" (155). Unfortunately, these test are rather expensive; OncotypeDx costs approximately $4,200, although some researchers claim that substantial savings can be made as a result of the increase in uptake of active surveillance (156).

1.5 SCREENING

The concept of screening entails the use of a simple and preferably inexpensive test that can classify a large number of individuals as either likely or unlikely to have the disease that the test is intended to detect. The main purpose of screening is to reduce mortality, although it can have other favourable effects as well. The use of screening as an approach to cancer control is controversial, and various benefits, costs, and potential adverse effects of screening programmes have been discussed for years (157). There are chiefly three types of screening: testing that can be applied to individuals requesting such evaluation (opportunistic testing); testing of high-risk individuals (selective screening); testing of the community as a whole (population-based screening). All individuals who participate in organized screening programmes are offered the same services and information.

In 1968, Wilson and Junger (158) listed a number of criteria for evaluating screening tests and the effectiveness of a given screening strategy. Twenty years later, in 1988, the criteria for justifying a mass screening project were further developed by Hulka et al. (159) and can be summarized as follows:

1. The disease investigated should represent a substantial burden at the public health level, and the early stages of the disease should be prevalent in the population.
2. The early phase of disease should be detectable by a screening test.
3. The screening test should have good performance with respect to sensitivity, specificity, and positive predictive value.
4. Individuals with the disease who are diagnosed at an early stage should be more amenable to curative treatment than those diagnosed at more advanced stages.
5. Early diagnosis and early curative treatment should reduce cause-specific mortality.

Thus it is assumed that early diagnosis and treatment will improve prognosis and reduce the risk of severe symptoms and/or the risk of dying due to the disease, and thereby affect the long-term outcome for those who participate in screening. However, even if a screening test can detect disease at an early phase/stage, that does not necessarily mean that all individuals subjected to screening will benefit. Those who participate and undergo the test with false-positive results will obviously not gain from participating, and indeed might suffer from adverse effects such as anxiety (160), the embarrassment and discomfort associated with diagnostic procedures, or adverse sequelae.

Also, many malignancies are discovered when signs and symptoms have become rather severe (and when cure is beyond reach), and thus the favourable outcomes of screening can only be achieved in asymptomatic participants. The optimal test should only detect cancers that lead to morbidity and mortality if untreated (and thus are still curable when diagnosed at an early stage). It is also possible that detecting cases that never develop symptoms can lead to overtreatment and the need for costly monitoring and active surveillance (161). This is particularly relevant for PC, in which the lead-time from diagnosis to symptoms can be very long.

In addition to considering quality of life aspects, it is essential that the screening is simple to perform, cost-effective, and acceptable for both the subjects and the individuals performing the procedures (safe, acceptable and accurate). These aspects play a major role in the decision whether population-based screening should be introduced. The number of individuals that have to be screened to prevent one death is defined as "the number needed to screen" (NNS) and is a measure of the effectiveness of the screening programme.

Screening for other cancers

Cervical cancer has a long period of pre-clinical disease. Screening with a relatively simple and cheap cervical (Pap) smear test (162) has been offered to women in Sweden since the late 1960s. The cervical screening programme represents a successful model of preventive care that has reduced the cancer incidence and cancer specific mortality dramatically (approximately 60–75%) in some countries. Primary prevention in the form of vaccination against HPV infection has been available in Sweden since 2012, and it is now part of the national vaccination programme offered to girls aged 11–12 years. Since 2017, the screening guidelines call for a tests every third year between the ages of 23 and 49 years (HPV detection is an additional analysis in women 30-49 years). For women between 50 and 64 years, screening takes place every seventh year. Approximately 700,000 cervical smears are taken in Sweden every year, and 150 deaths per year occur secondary to this disease. Studies have found that non-attenders constitute a high-risk group, and efforts have been made to improve participation rates and self-testing for HPV in Sweden (163).

Lung cancer screening with chest X-rays is another example of efforts to identify disease at an early stage. However, there is no evidence that this strategy can detect the disease early enough to improve prognosis. One study showed no initial reduction in lung cancer mortality as the result of screening (164). Over the last few years, screening with low-dose chest computed

tomography (CT) has shown some mortality benefit, and CT screening programmes have been applied in some clinical settings (165). Tobacco cessation is an essential part of such a programme and is currently the only intervention that can prevent the development of disease.

Breast cancer screening with mammography is conducted to find a tumour before a lump can be felt. The rates of overdetection and overtreatment associated with such screening are similar to those noted for PC screening. Many women will receive a diagnosis even if their cancers would not have led to death or aggressive disease. The authors of the Cochrane review from 2013 found that screening resulted in very little or no reduction in the incidence of aggressive breast cancers (166). Those researchers stated the following: "If we assume that screening reduces breast cancer mortality by 15% after 13 years of follow-up and that overdiagnosis is at 30%, it means that for every 2000 women invited for screening throughout 10 years, one will avoid dying from breast cancer and ten healthy women will experience anxiety and uncertainty for years because of false positive findings". It has been proposed that advances in treatment and improved awareness among women have diminished the mortality reduction found in previous screening trials. Overdiagnosis is problematic, and little or no reduction in the incidence of advanced disease has been reported.

Colorectal cancer (CRC) screening can be performed by faecal occult blood testing. Such screening has been reported to reduce CRC mortality by approximately 30%, but only when a positive test leads to more invasive procedures, such as colonoscopy, a method that enables examination of the entire colon and removal of pre-cancerous polyps. Other potential methods for early detection include CT colonography, video capsule colonoscopy, stool DNA testing, and double contrast barium enema. Most guidelines recommend that CRC screening for average-risk individuals be initiated at the age of 50 years, although several European programmes start at around age 60 (167). Most CRC patients are diagnosed between the ages of 65 and 74 years, and individuals of African descent are reported to be at higher risk of this disease. Many guidelines recommend an upper age limit for screening varying from 70 to 75 years, because the associated harms may potentially exceed the benefits after this age. As for other screening programmes, adherence is crucial, and a less invasive method makes it easier for individuals to participate. Screening intervals, as well as the rank order of different tests/procedures, are more uncertain.

1.5.1 Screening for prostate cancer, a dynamic process

The aim of screening men with PSA is to reduce morbidity and mortality from PC by detecting the disease at an earlier stage when it still is curable. To understand the concept of PSA screening, we must consider different phases of disease progression, as has been clearly illustrated by Törnblom et al. (168).

First, there is **a nondetectable preclinical phase (A)** when cells transform into malignant cells and start dividing. Eventually, this transformation process will lead to an elevated PSA level, which, in a screening setting, will trigger intervention (biopsy). At this point, the cancer is in a preclinical, but **detectable phase (B)**, also known as the sojourn time. The **delay time (C)** can be described as the period spanning from when the cancer is in a detectable preclinical phase up to diagnosis (in this case the delay time is shorter as an effect of screening).

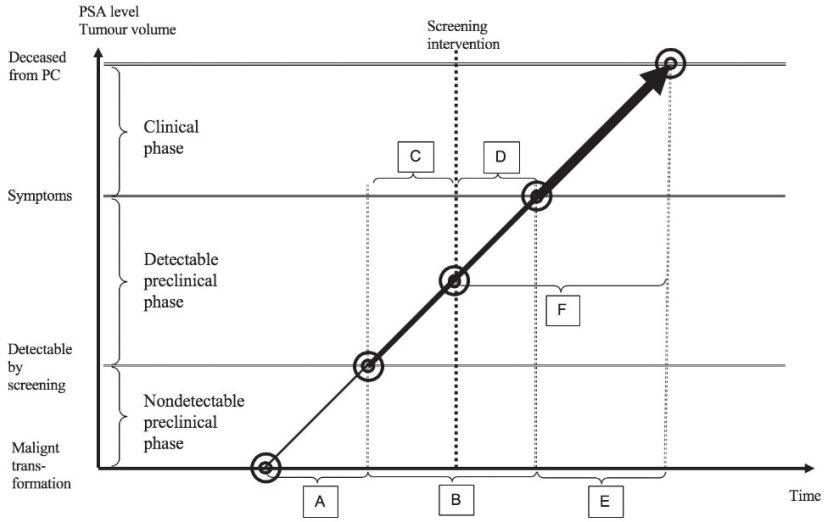

Figure 9. Prostate cancer progression is a dynamic process that occurs over time. Illustration by Magnus Törnblom, reprinted with permission.

Thus, the screening regimen can detect the tumour in an earlier phase (compared with diagnosis based on clinical symptoms), and the time gained by such early detection is often referred to as **lead time (D)**, whereas **E** represents the time period from diagnosis (due to clinical symptoms) until death and censoring. When interpreting the results of screening, it is necessary to be aware of an aspect called ***lead-time bias*** (if survival is

estimated). Detecting PC at an earlier stage can result in what seems to be an **extended survival (F)** after diagnosis. For the individual, this means that the PC is diagnosed earlier, but the time of death might not be affected (no actual survival benefit exists). Lead time can be estimated by comparing the time difference in PC incidence in screened men and controls (ratio of detection rate at screening to the expected incidence), and such estimates are essential for defining an optimal screening interval. However, lead time is not a fixed value, because different types of PCs have very different growth rates. The most common approach is to estimate the median lead time, although the optimal would be to understand the distribution of lead time in the cancers detected by screening. Longer lead times indicate higher risk of overdiagnosis (at least in an elderly population), whereas shorter lead time implies low sensitivity of a screening programme. PC screening has shown that many cancers are incurable long before they are clinically detected (169). Figure 10 illustrates plausible characteristics of an unscreened population.

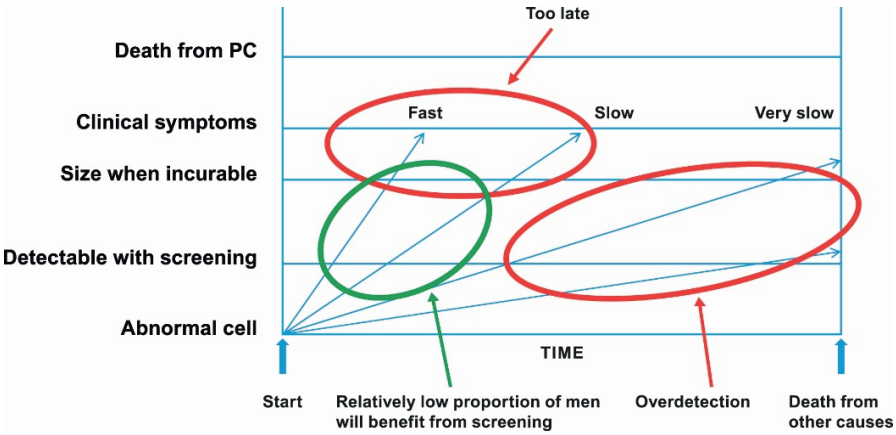

Figure 10. Prostate cancers detected during the first screening round can be heterogeneous. Some are already beyond the "window of cure", and others have a very slow growth rate and can be regarded as overdetected.

Cancers detected during the first screening round (prevalence screening) can be very heterogeneous. Some cases are already incurable, and others have a very slow growth rate and can be regarded as overdiagnosed. However, the situation immediately after conclusion of an ideal prevalent screening will have no cancers in the detectable phase. As time proceeds, new non-detectable cancers will grow and become detectable, but their features will differ. Fast-growing cancers will reach the detectable phase more quickly than slow-growing cancers, and the distribution between fast and slow-

growing cancers will differ from the situation seen in prevalent screening (Figure 11). This model outlines some important features of PSA screening.

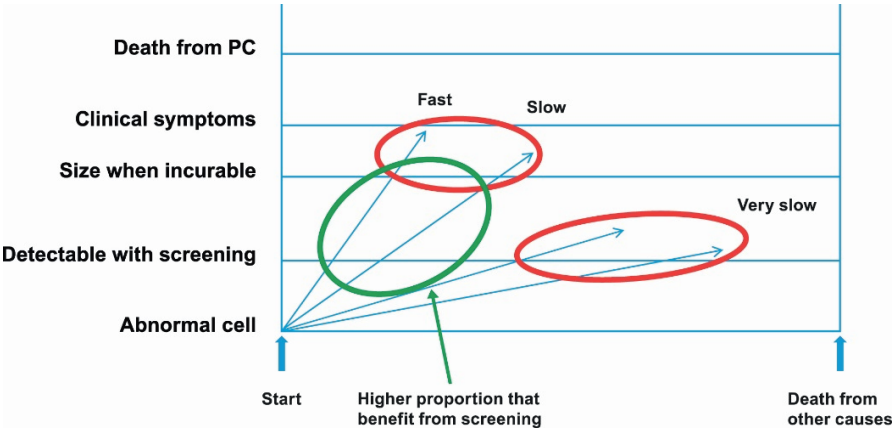

Figure 11. Cancer detected at repeat screening rounds are more homogeneous, few are incurable, and the proportion of slow-growing PCs is lower.

- The risk of detecting slow-growing and probably insignificant cancers is largest during the initial screening round.
- Many incurable cancers are detected during the initial screening round.
- The proportion of fast-growing cancers in a curable phase is larger in repeat screening rounds.
- Every screening round detects new fast-growing PCs (i.e., potentially lethal cases) that are still in a curable phase.
- The lead time of clinically important cancers is unknown but is probably much shorter than estimates based on all PCs detected during screening.

The weakness of this model is that the first PSA screening round does not detect all PCs in the curable and preclinical phase. Indeed, it appears that it takes approximately three screening rounds to detect all prevalent cancers (170). In any case, this model can explain many of the discrepancies found between different screening studies. This model also implies that PSA testing on only one occasion will offer little benefit but still entail a high risk of overdiagnosis. In short, in programmes limited to a single testing, the small proportion of fast-growing cancers that are cured will "drown" in the large population of cases that are either overdiagnosed (slow-growing) or detected too late. There is no biological reason to hypothesize that PSA screening only

once would be particularly beneficial in reducing PC mortality. On the contrary, a programme with only one screen may be harmful as a large proportion of very slow growing PCs will be detected (and result in overdiagnosis) (171). To catch the fast growing and potentially lethal cancers in a curable stage, PSA screening has to be repeated. Thus screening men with PSA is a dynamic process, not a static once-in-a-lifetime intervention.

1.5.2 Randomized screening trials

To evaluate the effect of screening, two large-scale population-based studies were designed in the 1990s: the **ERSPC** in Europe and the **PLCO** screening trial in the United States (172). The ERSPC was started in 1994, and it represents the largest trial for screening of PC, performed in eight European countries: the Netherlands, Sweden, Finland, Belgium, Italy, Spain, Switzerland and France. Although the protocols applied in the ERSPC differed between the eight countries, in generally men were randomly allocated either to a screening group that offered PSA testing every 2–4 years, or to a previously unscreened control group with no invitation to PSA testing. The control group was exposed to opportunistic screening (173) (contamination 23–40% after 13 years).

The core age group in the ERSPC was 55–69 years, and in Göteborg we also included men aged 50–54 and used a 2-year screening interval. Our centre in Göteborg and several other ERSPC centres independently publish results that are available at www.erspc.org. As mentioned above, the ERSPC is the largest screening trial for PC, randomizing 73,000 men to screening and 90,000 to serve as controls (all men 50–74 years of age). Men included in Italy, Finland, and Sweden, were identified from population registers and randomly assigned to the screening or control arm before giving informed consent. In the other countries, men first provided consent and the men who provided consent were later randomized to the screening or the control group. The primary outcome was the rate of death from PC. In 2014, data from 13 years of follow-up in the ERSPC showed a 21% relative decrease in PC mortality in favour of the screening arm (173).

Such a reduction was not seen in the US **PLCO** screening trial (174), most likely due to the very high levels of PSA testing in the control arm in that study. PSA testing was introduced in the US in 1988, and by 1994 most men over the age of 65 years had been tested. Between 1993 and 2001, men were invited to participate in the PLCO initiative, and those assigned to the screening group were offered annual PSA testing for 6 years and DRE for 4 years. Self-reported PSA testing rates in the control arm ranged from 40% to

52% (during the first and the last year, respectively). Participants in both study arms were pre-screened with PSA before entering the study. However, after adjusting for differences in implementation and setting, the data from both the ERSPC and the PLCO do not indicate that the efficacy of screening (relative to no screening) differed between these two trials (175).

The **Norrköping study** was initiated in 1987, and data on 20-years of follow-up in that trial were published in 2011 (176). A total of 1,494 men were invited to screening, and 7,532 were allocated to the control group. The interventions were screening every 3 years versus control (i.e., not invited). The first and second screening rounds included only a DRE (i.e., no PSA test), whereas the third and fourth rounds included both a DRE and a PSA test. Biopsies were performed if the DRE was abnormal or if the PSA level was ≥ 4 ng/mL. After 20 years of follow-up, the death rate from PC did not differ significantly between the men in the two arms. The Norrköping study has been criticized for both its statistical presentation, low power and the use of DRE as a screening tool (177). Furthermore, information on the investigation was distributed via television, radio, and newspapers, which might have had an impact on men's decision to participate in the study.

Trial	Size of study population (screening + control)	Target age group (years)	Screening tests used	Participation rate	Follow-up (years)	No. of prostate cancer deaths (screening + control)	RR for prostate cancer mortality (95% CI)
ERSPC	72,891 + 89,352	55–69	PSA	83%	13	355 + 545	0.8 (0.7–0.9)
PLCO	38,340 + 38,343	55–74	DRE, PSA	85–89%	15	255 + 244	1.0 (0.9–1.2)
Norrköping	1,494 + 7,532	50–69	DRE, PSA	70–78%	20	30 + 130	1.2 (0.8–1.7)
Stockholm	2,400 + 24,772	55–70	DRE, TRUS, PSA	74%	20	86 + 857	1.1 (0.8–1.3)
Quebec*	31,133 + 15,353	45–80	PSA, DRE	24%	11	153 + 75	1.0 (0.8–1.3)
CAP	189,386 + 219,439	50–69	PSA	40%	10	549 + 647	0.9 (0.7–1.3)

Table 5. Randomized prostate cancer screening trials. Note: in the Quebec study the authors reported a reduction in PC mortality that could not be found when data were re-evaluated according to the intention-to-screen principle (178).*

The **Stockholm study** was initiated in 1988 and recently published a report presenting data on its 20-year follow-up of PC outcomes (171). Men aged 55–70 years and living in the Stockholm area were included in a screening arm (n = 2,374) or in a control arm (n = 24,772) for comparison. The intervention was **"one screen only"** versus no screening, and the screening consisted of DRE, PSA testing, and TRUS. Biopsies were performed if a

participant had abnormal DRE findings and/or an abnormal TRUS. A repeat ultrasound was performed if the PSA level was ≥ 7 ng/mL, but the PSA threshold demanding biopsy was high (≥ 10 ng/mL). With this study design (i.e., a single intervention in a previously unscreened population), many of the PCs detected were in an advanced stage at diagnosis, referred to as "prevalent cancers". Hence no effect on PC mortality was found in the Stockholm study (RR 1.05, 95% CI 0.83–1.27), but an excess incidence of 10–20% remained after 20 years. This investigation was initiated primarily to evaluate a PC detection strategy, not mortality. Neither the screening procedure nor the treatments used were state of the art, and the external validity can be considered low (not generalizable to the modern setting). A null result from a one-time screening is not unexpected, even with such a long follow-up period.

The **Quebec study** of PC screening recruited men aged 45–80 years who were identified in electoral rolls and allocated 2:1 to a screening and a control arm. Annual screening with the combination of DRE and a PSA test was offered. The cut-off for further biopsy was set at PSA ≥ 3 ng/mL and/or abnormal DRE. Follow-up screening rounds included a PSA test, and TRUS biopsies were performed if the PSA level was ≥ 3 ng/mL (for the first time), or if PSA increased > 20% compared with the previous screening round. The outcomes in participants after 11 years of follow-up were reported in 2018 (179). Only 24% of the 31,133 men who were invited to undergo screening actually participated, and this low adherence rate reduces the power of the study. The Quebec trial has also been criticized for the long time lag between randomization and screening (on average 3 years), and also for the crossover between groups (180). More than 7% of the men in the control arm underwent PSA screening, and the results were not analysed according to the intention-to-screen principle.

Data from the **Cluster Randomized Trial of PSA testing for Prostate Cancer (CAP)** was recently published in the Journal of the American Medical Association (181). CAP can be described as a large and well-designed cluster-randomized study conducted in the United Kingdom. In short, primary care units were randomized to invite men to a **single PSA test**. PC specific mortality was used as end-point, and more than 400,000 men, aged 50–69 years were recruited between 2001 and 2009. After a median follow-up of 10 years no difference was found between men in the screening arm and men in the control arm (RR 0.96, (95% CI; 0.85–1.08). More than 50% of the PC deaths in the intervention arm occurred during the first 7 years of the study. Two important aspects should be mentioned. First, it is highly unlikely that PSA screening would have had an effect on these cases, given

the biology of the disease; many of these cancers were already "beyond the window of cure". Second, the follow-up was too short, considering the long natural history of PC. Our data from the Swedish section of the ERSPC (presented in **Paper III**) show that screening had no major effect on PC mortality during the first 10 years; a reduction in mortality was observed after approximately 12–14 years.

Both the CAP trial and the Stockholm study used a single PSA measurement, which, as argued before, is a very ineffective way to screen men for PC. Our findings (Paper III) indicate that the first screening round detects more advanced and incurable PCs. So, screening men at only one occasion result in either a benign biopsy or detection of a prevalent cancer (which often is too late to cure). Such cancers "dilute" the difference between screened men and controls, and thus the true effect of screening is diminished (discussed in Paper III). Therefore, it is irrational to assume that a single PSA measurement can be beneficial in reducing PC mortality. Although this approach does identify advanced and incurable cancers, it misses the chance of later detection of progressive disease (as the men in the cohort grow older). On the other hand, a single PSA test in midlife is a valuable predictor of future lethal PC (182) and can be used in conjunction with other factors (age, comorbidities) to stratify risk and intervals for re-testing (183, 184). Commentators discuss the evidence as to whether PSA-based screening actually reduces PC-specific mortality and some base their arguments on the findings from studies where PSA was used as a single intervention.

In September 2018, Ilic et al. published a systematic **review and meta-analysis** (185) of five major trials (all mentioned above): the ERSPC, the PLCO trial, the Quebec study, the CAP and the Stockholm study. Ilic and colleagues pooled data from these very different and heterogeneous trials and concluded that "PSA screening yields, at best, only a small benefit in PC-specific mortality, but does not reduce overall mortality". These authors also emphasized that "this small benefit should be weighed against the potential complications related to the biopsy and long-term side effects from treatment". Indeed, weighting the benefits of screening against the potential harms, is of the outermost importance, when discussing PSA testing with men, but the main conclusion drawn in this review can be questioned. Guidelines for conducting systematic reviews and meta-analyses mandate that only studies that are conceptually similar should be included in a quantitative synthesis, otherwise the findings will not be applicable to clinical practice and may misinform lay audience. The Cochrane Handbook (186) advises against combining 'apples with oranges' and states: "if studies are clinically diverse, then a meta-analysis may be meaningless and genuine

differences in effects may be obscured" and "meta-analyses of studies that are at risk of bias may be seriously misleading." The five studies that were assessed did not address the same scientific question. Furthermost, using overall mortality, which has a power that is too low to detect a difference between trial arms, is not a valid end-point. A trial of cancer screening with such endpoint would require well in excess of 500,000 individuals, which is why no such study has ever been initiated. An article by Carlsson et al. (187) discusses a number of important weaknesses related to this type of meta-analysis, and several of the errors mentioned by these authors can also be applied to the above-discussed review by Ilic et al.

In summary, evaluating screening studies and the outcomes of PSA screening is perplexing and results are often misinterpreted. Besides the actual effect on the population, consequences on health economy must be considered when discussing screening strategies. Nevertheless, negative effects of not having a strategy (as is the situation of today with the on-going opportunistic testing) are difficult to overlook and neither a good option.

1.5.3 Negative and positive effects of PC screening

Negative effects of PC screening

Screening with a PSA test can generate false-positive results, because the level of PSA can be elevated due to any disease of the prostate (i.e., BPH and prostatitis. False-positive findings lead to additional PSA tests and unnecessary biopsies. This **can be very stressful** for the individual, and some men will also suffer from unwanted side-effects from diagnostic procedures (infections, haematuria etc). The main objective of screening is to reduce mortality and "the benefit-versus-harm balance" must be continuously assessed, both on a group and individual level.

Both the ERSPC and the Göteborg study found that PSA screening resulted in a significant reduction in PC-specific mortality, but no trial has shown that men undergoing screening have an advantage with respect to overall survival. As discussed before, **overall mortality is the wrong endpoint** and a reduction in PC-specific mortality is a more appropriate outcome measure, which should be weighed against the harms of screening.

Many men harbour a localized and indolent PC, and it has been estimated that up-to 25–50% of all screen-detected cancers are overdiagnosed (91, 188). **Overdiagnosis and overtreatment** are the major concerns, because they are associated with negative aspects that reduce quality of life, such as impotence and incontinence. The term overdiagnosis is usually applied to

specify detection of a tumour that never would have caused any symptoms. Overdiagnosis and overtreatment constitute the main reasons why PC screening is not generally recommended (189). Concentrating screening to those who benefit the most is therefore something we strive for.

With the emergence of many novel biomarkers (and biomarkers combined with clinical examination and imaging), it is also necessary to consider the **costs of screening and PC treatment**. Choosing what biomarker to use and selecting the "optimal" target population can be challenging tasks. Today, urologists and clinicians are not always familiar with the financial impact that their recommendations have. In an editorial published in 2016, Eggener et al. wrote the following: "*Nowadays, it is possible for a man to undergo a parade of shockingly expensive tests, a MRI-based fusion biopsy, tissue-based genetic evaluation of the cancer, robotic surgery or proton-beam therapy, immunotherapy, multiple novel androgen-blocking therapies, multiple chemotherapy regimens, radium-based bone-targeting agents, and bisphosphonate or receptor activator of nuclear factor-kappaB (RANK)-ligand supportive bone care.*"(190). This statement describes the direction in which PC care is being developed. There are many options for patients with PC, associated with uncontrollable costs.

On a population level, gaining quality-adjusted life years (QUALYs) might favour implementation of a screening programme, but many men will also experience adverse effects and harm following treatment. For the individual, this can lead to a miserable life with years of suffering. A study using microsimulation models found that the positive impact of screening was diminished by loss of QALYs resulting from long-term side effects (191).

Positive effects of PC screening

PSA screening increases the chance of detecting PC, while the disease is still curable. Early detection of PC also results in a **reduction of metastatic disease**, which was found to be approximately 40% in men who were screened in the ERSPC (169). Bone metastases are often very painful for the individual and causes a number of clinical problems. Furthermore, diagnostics and treatments are also very costly and have side-effects.

Large RCTs have demonstrated that PSA screening **reduces PC specific mortality** by 25–32% (75, 192), findings that are confirmed by the results reported in Paper III. The ERSPC trial has shown a decline in the NNI and the NND over time (173); the number of men who need to be **i**nvited and

diagnosed to save one man. With a longer follow-up time the absolute number of avoided deaths from PC increased.

PSA testing is easily accessible in Sweden and the public awareness contributes to unorganized and widespread opportunistic testing. More than 50% of all PC cases in this country are diagnosed this way (193), even though it has been shown that an **organized screening is more effective**. According to results from the Göteborg screening trial opportunistic PSA testing in the control arm only resulted in an absolute reduction in PC-specific mortality of only 0.2% (compared with 0.73% in the screening arm) (194). Men who are tested outside the programme are recommended different follow-up regimens, and some with high PSA levels are not followed at all.

Another aspect of PSA testing is that some men prefer to know if they have PC. Studies assessing anxiety levels in men offered PSA screening have noted that **"seeking peace of mind"** was the motivation mentioned most often by those who were anxious but still underwent screening (195).

1.5.4 Screening approaches

Routine PSA-based screening of asymptomatic men is one of the most controversial topics in urology and preventive medicine. The recommendations are confusing both for men who themselves request testing, and for physicians who rely on current guidelines. Even guideline groups and other experts are unable to agree on, nor decide, what strategy to use (196). In 2012, the US Preventive Services Task Force provided a statement with a very stringent recommendation **against PSA screening** (197), which they later changed to a shared decision making process. Other guidelines have focused on a more **individual approach**, considering when to terminate further PSA screening (i.e., a stop age) and on other ways to reduce harm.

Today, Lithuania and Kazakhstan are the only two countries in the world that offer an organized population-based screening programme for PC (198, 199). The programme in Lithuania was initiated in 2006, and it was originally suggested that all men aged 50–74, as well as men aged 45–49 with a family history of PC, would undergo PSA testing once a year; in 2008, the screening interval was changed to every second year due to logistical problems and high costs. In Lithuania, the PC incidence increased dramatically in 2005–2009, when more than 15,000 men were diagnosed with the disease (compared with 2,200 cases detected during the period 1990–1994 (200). The full effect on PC mortality is still to be awaited, but it is obvious that, in general, overdetection is a huge problem with their screening strategy.

The Swedish National Board of Health and Welfare does not recommend a countrywide population-based PSA screening programme for men. However, there is an ongoing investigation that is being performed to analyse and make suggestions regarding whether screening should be **organized in regional projects**, and if so, how that can be accomplished. Both the AUA (116) and the EAU recommend that PSA testing should be offered to "well-informed men" who have insight into the benefits and possible harms of screening (115). The EAU guidelines also recommend the use of what are known as risk calculators to evaluate the individuals' risk of PC and thereby avoid biopsy in some men.

In 2016, Carlsson and Roobol published a summary on currently available recommendations on PC screening (201). They conclude that "new biomarkers, multiplex screening and PSA based risk stratification at early age can shift the ratio of benefits and harms". A "one-size-fits-all" solution might no longer be the right approach. Age, health status and PSA level are therefore important when considering if and when to screen.

Crawford et al. (202) suggested that the effect on PSA testing depends on other comorbidities: Men who have other illnesses (e.g., heart disease and diabetes) in addition to PC are more likely to die from those conditions, than from PC. Theses authors came to the following conclusion: "Selective use of PSA screening for men in good health appears to reduce the risk of PC-specific mortality with minimal overtreatment". Furthermore, the man needs to be fully informed and comprehend what consequences screening might have. Most guideline groups recommend **shared decision-making** where the patient and doctor discuss pros and cons of screening. To aid in this dialogue, a risk-stratified approach aimed at primary detecting lethal PC has been suggested.

One approach is to use a **baseline PSA** for risk-stratification. Vickers and colleagues have assessed data from the Malmö Preventive Project cohort to study outcomes in 20,000 men (203). PSA levels in these men were analysed more than 25 years after enrolment in the project and associations between initial PSA and later PC were calculated. It was possible to define a baseline PSA (specified by age) and to approximate future risk. Men who were in their 40s had a 10-fold risk of metastatic disease if their initial PSA level was > 1.6 ng/mL (compared with a level of > 0.6 ng/mL). The majority of men aged 40–45 years had low PSA values (< 1.0 ng/mL) and a very low risk of PC death within 25 years. Vickers and co-workers concluded that having a PSA test in early midlife can identify a small group of men at increased risk of developing aggressive disease several decades later, and these men need to

be closely monitored. Furthermore, these investigators deduced that men at very low risk, with PSA values of < 1.0 ng/mL could wait a few years and return for screening in their early 50s, and again at age 60 (if PSA was unchanged). Others have proposed that when PSA exceeds 1.0 ng/mL, testing every 2 or 4 years should be recommended (204). The mentioned suggestions indicate that the main focus should be on men who are in the highest 10% of PSA levels at around the age of 50.

In 2015, a report from the Swiss branch of the ERSPC proposed the use of a "PSA pyramid" for men with initial PSA levels below 3 ng/mL (205). This suggestion emanated from an evaluation of data on 4,300 men aged 55–70 years who participated in a screening programme with PSA testing every 4 years. In that study, baseline PSA was found to be a strong predictor of future PC and aggressive disease. Half of all the men who were assessed had an initial PSA of < 1.0 ng/mL, and these men also had a very low risk of developing aggressive PC within 8 years (0.21%). The authors proposed the following: men with an initial PSA of < 1.0 ng/mL can be safely retested after 8 years; men with PSA 1–1.99 ng/mL can be retested after 4 years; and men with PSA in the range 2–2.99 ng/mL require needs further risk stratification.

Five simple and straightforward "golden rules" for PSA screening have been proposed by Vickers and colleagues (206):

1. Get consent. Do not take a PSA test without discussing it with the patient. A PSA test is sometimes added when doctors order blood work as part of a routine health check-up. Information about harms and benefits should always be given.
2. Do not screen men who won't benefit. Men who are age 80 and have multiple comorbidities or men who have a life expectancy less than 10 years are very unlikely to benefit from taking a PSA test. Most recommendations use age 75 as a stopping point.
3. Do not biopsy without a good reason. A majority of men with slightly elevated PSA do not have PC and few men with low PSA have aggressive PC (207).
4. Don't treat low-risk disease. Many screen-detected PC have low-risk features (GS < 7) and can be managed with active surveillance (208).
5. If you have to treat, do so at a high-volume centre. Experienced surgeons have higher cure rates and fewer complications. Also, they have better outcomes for urinary and erectile function (209).

The authors conclude that *"the benefit-harm ratio of PSA screening can be improved by avoiding screening, biopsy and treatment in men who are unlikely to benefit"*

1.5.5 Public attitude towards screening: what do men want?

In 2012, the US Preventive Services Task Force recommended against PSA screening, which caused a public outcry, and the new guidelines were met with resistance by many men in the United States (210). Likewise, the field of breast cancer screening has provided findings suggesting that even if people are informed that screening is associated with potential overdetection and possible harm, they are willing to take the chance that screening can be beneficial (211).

People are often prepared to take part in activities that have (or might have) a positive impact. Such behaviour can also result in irrational decisions and a desire to "take action", regardless of the consequences. In psychology, this is called "action bias" or "error of commission" (212) and it is discussed at length in a paper recently published by Scherer et al. (213). These authors explored the public enthusiasm for cancer screening, and they surmised that the extensive interest in PSA screening might be due solely to lack of knowledge about the risks. Participants in that study were told the following: *"There is a new test to screen for prostate cancer, and this test uses diagnostic technology to look for abnormalities that may be early stage cancer"*. The subjects were also given this information: *"One problem with the test is that it may detect cell abnormalities that will never develop into dangerous PC. This might lead to unnecessary treatment and harms (physical, emotional, and financial). The test can also fail to detect dangerous cancers*". A majority of the men who were asked if they were willing to take the test said that they would have done so, even knowing that the results obtained could neither help prevent death due to PC nor extend length of life. Scherer and colleagues concluded that many people perceive a possible advantage of receiving health information, even if it provides very uncertain or no survival benefit.

Another, recent evaluation of the preferences of screening subjects was conducted by Vernooij et al. (214) as a systematic review of 11 studies, and the results revealed considerable variation in men's values and preferences. Several of the vetted studies reported that men were willing to accept considerable risk of harms (e.g., unnecessary diagnostics, impotence, and incontinence) to accomplish even a small reduction in risk of PC death. Thus, Vernooij and co-workers came to this conclusion: *"The variability of men's*

values and preferences, particular to the degree that their information needs are met, reflect that the decision to screen is highly preference sensitive".

Regardless of whether we are proponents or opponents of screening, it is time to admit that PSA screening is something that men request. As mentioned previously, many men in Sweden have a PSA test on their own initiative (opportunistic testing), even if there is no national recommendation to guide them in this decision. Such ambiguity makes it difficult to allocate the right resources to PC care. Therefore, politicians and healthcare providers must have a better contingency plan to ensure correct handling of this issue.

2 AIM

The overall objective of the studies underlying this thesis was to assess the long-term outcome of a PSA-based PC screening programme in Sweden. The specific aims and research questions were as follows:

PAPER I

To investigate whether voiding symptoms in men with an elevated PSA (≥ 3 ng/mL) are associated with the risk of PC.

PAPER II

To evaluate the risk of PC in men with an initial PSA level below 3 ng/mL and determine whether the free-to-total PSA ratio can be a useful prognostic marker in this PSA range.

PAPER III

To compare differences in PC incidence and PC-specific mortality after 22 years of follow-up, and also to consider how PC screening can be improved.

PAPER IV

To evaluate the future outcomes in men terminating the screening program at stop age (≥ 67 years) and to ascertain whether some of these men might need prolonged PSA screening.

3 PATIENTS AND METHODS

3.1 STUDY POPULATION

The Göteborg Randomized Screening Trial

The four papers included in this thesis (designated I–IV) describe our investigations based on data from the Göteborg randomized population-based PC screening study, which was initiated in 1994 and was approved by the Ethics Committee of the University of Göteborg, Sweden, and registered with *controlled-trials.com* (no. ISRCTN54449243).

According to the Swedish Population Register, as of December 31, 1994, 32,298 men born between 1930 and 1944 (i.e., aged 50–64 years, median 56 years) were living in the city of Göteborg (total population 440,000). Of these 32,298 men, 20,000 were randomly assigned to either a screening or a control group (in a 1:1 ratio). The randomization procedure was performed at the Department of Statistics, University of Göteborg. No informed consent was needed for men in the control group. Men in the screening group were invited for biennial PSA testing; men in the control group were not invited (although they did have access to opportunistic PSA testing). A total of 106 men were excluded (55 in the screening arm and 51 in the control arm) for the following reasons: 55 were already diagnosed with prevalent PC; 35 were deceased; 10 had emigrated; five had moved away from West Sweden (these men were still in the Population Register at the time of randomization); and one man assigned to the control arm was not willing to participate.

Men were invited to join the study starting in January 1995. In 1996, the investigation became connected with the large ERSPC, but no changes were made in the Göteborg protocol when this association was established. Figure 12 shows the screening algorithm used in the Göteborg Randomized Screening Study.

Every second year, men in the screening group received a letter describing PSA testing and its advantages/disadvantages, together with an invitation to participate. The upper age limit for invitation was 67–71 years (median 69 years). The oldest men received three invitations before reaching the upper age limit, whereas the youngest men had 10 invitations. The last screening round took place during 2013–2014. No invitations were sent to men in the control group.

Initially, the PSA cut-off that led to further urological assessment was set at 3.4 ng/mL, but this level was changed twice to be consistent with other ERSPC sites and to deal with issues related to calibration of the PSA assay (Prostatus Total/Free PSA-Assay, Perkin-Elmer, Turku, Finland). The actual cut-off was 3.4 ng/mL during screening rounds 1–2 (1995–1998), 2.9 ng/mL during rounds 3–5 (1999–2004), and 2.5 ng/mL during rounds 6–10 (2005 and onward). DRE, TRUS, and prostate biopsies were recommended for men with a PSA value that exceeded the cut-off level. Sextant biopsies were performed up to 2009, after which a 10-core biopsy was used in the study protocol. Men with a benign biopsy or with a PSA of < 3 ng/mL were re-invited for PSA testing after 2 years. Men who were identified as having PC were offered consultation and treatment by physicians working at the Urology and/or Oncology Department at Sahlgrenska University Hospital. Some men were diagnosed with PC outside of or after leaving the study (interval cancers or after stop age). These cases are referred to as non-screen detect PCs. Available and relevant medical information regarding tumour stage, treatment, PSA relapse after treatment, and metastases was continuously entered into the database, and every third month information and data were linked to the Regional Cancer Register and the Swedish Population Register. Since 2009, data have also been linked to the Swedish Cancer Register, which has very high completeness (> 96%) (215). Thus, information on PC diagnosis, mortality, and emigration has been added continually to the study database in this manner. For all deceased men, a copy of the death certificate was obtained. The cause of death (COD) in men with PC was determined by an independent COD committee, according to a flow chart (216). Three members of the committee reviewed the medical information on deaths in a blinded and independent manner.

The 10th and final screening round was completed 2014, at which time all age cohorts had reached the upper age-limit for invitation. Nevertheless, the study database continues to be regularly updated on PC diagnoses and deaths (in both study groups). Cases in which information on deaths is lacking and the COD is uncertain are labelled as “pending” in our database.

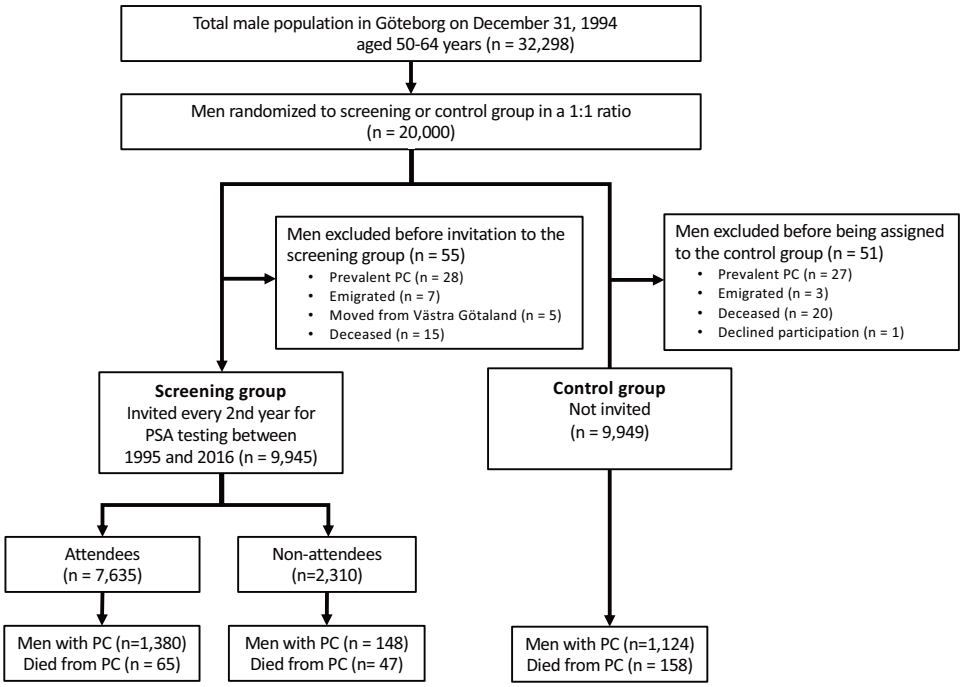

Figure 12. Consolidated Standards of Reporting Trials (CONSORT) diagram showing the screening algorithm of the Göteborg randomized population-based prostate cancer screening study. Last follow-up on December 31, 2016.

Paper III presents the outcomes after 22 years of follow-up. For all men who had moved away from West Sweden before their last invitation was sent, the latest test results were excluded from the analysis. Thus, any cancers that were diagnosed after that timepoint were not included in the assessment.

3.2 REGISTERS

Sweden has a long tradition of collecting epidemiological data. Use of the unique personal identity number enables researchers to link data from studies obtaining information from several registers at the same time.

The Swedish Cancer Register (SCR)

The SCR is the oldest health register in Sweden (established in 1958). It covers the entire population and is now divided into six regional cancer registers, administrated by the Regional Cancer Centres (RCCs). Quality assessment is performed by the Swedish Board of Health and Welfare. It is mandatory for all healthcare providers to report new cancer cases to the SCR, which covers 96% of all cancers (215). About 64,000 malignant tumours are diagnosed every year. The register contains data on the patient (personal identity number, age, sex, place of residence) and medical aspects (tumour location, histological type, TNM stage, date of diagnosis, reporting hospital/ pathology department, and identity number for the tissue specimen), as well as follow-up data (date of death, cause of death, and date of emigration).

The Swedish Cause of Death Register

Since the early 1960s, the Swedish Cause of Death Register has compiled the following data on deceased individuals: sex, date of death, age at death, place of death, and cause of death coded according to the International Classification of Diseases (ICD). The data in this register are updated on an annual basis. When a person dies in Sweden, a death certificate is first completed by a physician (or a nurse if the death was expected) and thereafter sent to the Swedish Tax Agency within 2 workdays. An additional COD form is also filled in (in some cases after an autopsy) and sent to the National Board of Health and Welfare within 3 weeks. The register contains data on ~98% of all deaths. A study conducted in Göteborg found that Swedish death certificates for men with PC are highly accurate (217). Death certificates can therefore be used for endpoint evaluation in screening studies for PC.

The Swedish Population Register

The population register in Sweden was managed by the Church of Sweden up to 1991, and since then it has been administered by the Swedish Tax Agency. This register contains information on personal identity number, place of birth, civil status, children/adoptions, address, citizenship, immigration to Sweden, and death and place of burial. Furthermore, valuable information about migration within Sweden and emigration to other countries can be obtained from this register when conducting screening studies.

3.3 STATISTICAL METHODS

Several different statistical methods were used in the present investigations. In the Göteborg screening trial, data were collected prospectively and analysed longitudinally. A short overview of the methods applied is presented below.

Survival analyses: time-to-event assessment

In PC studies, survival analyses can be performed to achieve the following:

- Visualize survival curves and estimate cumulative incidence (e.g., Kaplan-Meier [KM] and Actuarial methods).
- Describe the effect of covariates on survival (e.g., Cox proportional hazard regression).
- Compare survival curves of different groups (e.g., log-rank test).

The survival analyses discussed in this thesis focused on the time between entry into the study and a subsequent event (e.g., PC diagnosis or PC death). However, this event will not have occurred in some of the men within the study period, but these subjects can be **censored** for other reasons, for example:

- Lost to follow-up during the study period (moved from West Sweden or emigrated to another country).
- Death from other causes.

The **Kaplan Meier (KM) method** (218) was applied in two of our studies (Paper II and IV). The KM estimator of the survival function is a non-parametric statistic used to estimate the survival function (i.e., the probability of being event free at a certain time). The survival probability is calculated as the number of men who are event free divided by the number of men at risk. The KM analysis uses exact times for each outcome in contrary to the actuarial method (see next page). Men who are censored are no longer at risk. KM curves and estimates of survival data have become "the standard way" of reporting patient survival in cancer research. Many studies deal with different survival times (times to event), particularly when some subjects do not remain in the study until its conclusion. With the KM method this might be a problem, if men in a PC screening study die from other causes (competing even) (219). If the KM method is used to estimate the event-specific survival, competing events are censored in the same way as other censored events (i.e.,

those “lost to follow-up”). For example, men who die from a cause other than PC cannot also die from PC.

When using the KM method, these men would be censored and removed from the “at-risk” set. In other words, we assume that these individuals would have had the same risk (as those who were not censored). This might lead to an overestimation of the probability of dying from PC, especially because elderly men with PC often die from other diseases. Many epidemiologists prefer to use the **competing-risk analysis** (219) rather than the KM method to correct for such differences. KM rely on the assumption of non-informative censoring (when time to event and time to censoring are independent conditional on the level of covariates). Here, (**Paper III**), competing risk estimates of cumulative incidence were calculated as described by Choudhury et al. (220)

The actuarial life table method is similar to the KM approach but does not require exact time of the event. Instead, time is treated as “intervals”. To perform this type of calculation we had to have information on the number of men at risk (who entered the 1-year time interval), the number of events, and the number of men who were censored during the time interval. The life table analysis described in **Paper III** was performed to estimate the observed cumulative PC incidence and PC-specific mortality (in the screening and the control group) calculated as 1 minus the actuarial survival estimate.

In **Paper III**, we wanted to use historical data from 1990–1994 to compare the screening and the control group with regard to an estimated PC incidence and PC-specific mortality in the absence of PSA screening. During this period, which represents the pre-PSA era, PSA was not an established screening tool in Sweden. We used what is called Ederer II method (221) to assess expected survival rates in a cohort of men from the general population, so that the men in our investigation could be considered to be at risk until the corresponding men (in the general population) with PC died or was censored. Cumulative PC incidence and mortality were estimated as 1 minus the Ederer II estimator. To match the age distribution in the screening and the control group when calculating the expected estimates, we used 1-year age stratum. These calculations were based on the entire male population in Göteborg.

Cox proportional hazards regression analysis: This model estimates the effects of several explanatory covariates (continuous or categorical) on the hazard of an event. The hazard can be explained as the instantaneous risk of having an event (assuming the man to be event free up to this time). In contrast to the survival function, which is focused on not having the event,

the hazard function is focused on the event actually occurring. The hazard ratio is the **ratio of the hazard rates** and can also be regarded as a measure of how frequently a certain event happens in one group compared to another group. The hazard ratio is presumed to be constant over time (the "proportional hazard assumption") and, in two of our investigations (**Papers II** and **IV**), we used the Cox model and defined the event as PC, GS ≥ 7 disease, or PC death (Paper IV). In the study reported in **Paper IV**, it was used to explore the relationships between multiple covariates (age, PSA, F/T PSA, PSAV, prostate volume, previous biopsy and the presence of voiding symptoms). The strength of association was presented as Harrell's concordance index (c-index; discussed below) (222).

Discrimination and calibration in predictive models

Risk prediction models are valuable tools for cancer prevention and management. When assessing the performance of a risk algorithm, the most important component to consider is the ability of the algorithm to distinguish between individuals who will and those who will not develop a disease (an event), or between those who have the disease and those who do not. This is known as **"discrimination"** and refers to how well the model differentiates those at higher risk of having the event/disease from those at lower risk. It depends on the distribution of individuals' characteristics within the population in which the model is being applied. The performance of a screening test in discriminating affected from unaffected individuals is usually evaluated in terms of detection rate – or sensitivity – for a given false-positive rate (specificity). The relationship between sensitivity and specificity is often illustrated as a plot called a receiver operating characteristics (ROC) curve. The area under this curve (AUC) is also referred to as **concordance (or c) statistic** which is commonly used as a summary estimate of test performance – or predictive accuracy – in terms of AUC. It can take values from 0.5 (not a good test) to 1.0 (the ideal discriminatory test). In other words, the c-statistic is a unitless index applied to estimate the probability that a randomly selected individual/man who experienced the outcome (PC or equivalent) will have a higher probability of having the outcome compared with a randomly selected individual/man who did not experience it. AUC was not calculated in the present studies. Instead, as discussed in **Paper II** and in **Paper IV**, we used the **c-index proposed by Harrell** et al. (223, 224), which can be described as a measure of the predictive ability of a survival model. In short, this index can be seen as a generalization of the AUC for time-to-event data, and, comparable to as for ROC curves, c=0.5 stands for "random predictions" and c=1 for "a perfectly discriminating model" (222).

Discrimination alone is not sufficient to evaluate a model's predictive capability, and hence the model must be calibrated. **Calibration** or "goodness of fit" is a very important property of a model, because it reflects the extent to which the model correctly estimates the absolute risk, and whether the values that are predicted agree with the observed values (225). A calibration plot illustrates the relationship between observed and predictive values and shows how closely the risks predicted by a certain model agree with the individual's true risks. Discrimination and calibration are both important and should be considered when evaluating the performance of a model. Calibration is often underreported in medical studies. In this thesis, we have focused on predictive ability of different variables rather than the individuals' risks, hence no calibration has been carried out.

Characterizing risk predictiveness

The variation in risk of disease in the population under investigation can be illustrated using a graphical tool, called the Lorenz curve. In economics, this curve is used for representing inequality in the distribution of wealth. A recent paper by Mauguen et al. (226) describes the utility of the Lorenz curve in public health research. As outlined in **Paper IV**, we used a similar curve to illustrate the proportion of men that required further examination (Y-axis) versus the proportion of men with GS ≥ 7 disease (X-axis). In this analysis, we wanted to identify many high-grade cancers but examine only a few men after stop age. The optimal model in this context would identify men in the lower right corner.

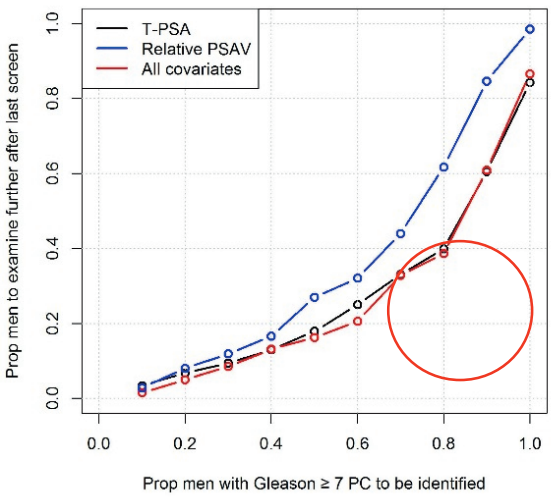

Figure 13. The proportion of men that must be further examined to identify fixed proportions of Gleason score ≥ 7 PCs. We aim to "detect many high-grade cancers, but investigate few men".

Risk assessment

When discussing "risk" in epidemiology, the aim is to measure the probability of a certain outcome during a specific period of time. In a cohort study of cancer, *risk ratios* and *rate ratios* are commonly used to compare two groups (e.g., a screening and a control group, or attenders and non-attenders) with regard to the frequency of the disease, and the term "*relative risk*" is often used to encompass both. These measures indicate the "strength of association". A **risk ratio** is calculated by dividing the cumulative incidence in the exposed group (CI^e) by the cumulative incidence in the unexposed group (CI^u): risk ratio = CI^e/CI^u.

	PC deaths	No PC deaths	Total	Cumulative incidence
Screening arm	112	9,837	9,949	112/9,949 = 1.13%
Control arm	158	9,787	9,945	158/9,945 = 1.59%

Risk ratio = 1.13/1.59 = 0.71.

In the study reported in **Paper III**, we found that men in the screening group had 0.71 the times the risk of dying of PC compared to men in the control group.

Rate ratios (RRs) are related to risk ratios, but differ in that they represent a proportion related to time of exposure. In PC screening, the RR can be calculated as the ratio of the incidence rate in a screened group divided by the incidence rate (cases per person-years) in a non-screened group (controls). The RRs in **Paper III** were calculated as follows:

	PC deaths	Person years	Rate
Screening arm	112	177,091	112/177,091
Control arm	158	177,152	158/177,152

The PC mortality was 112/177,091 = 0.63 per 1,000 person-years in the screening arm and 158/177,152 = 0.89 per 1000 person-years in the control arm, which gives a rate ratio of 0.63/0.89 = 0.71.

Men in the screening group had 0.71 times the rate of PC deaths compared to the men in the control arm. In other words, PC mortality is reduced by 1–0.71 = 29% for those who underwent screening. In this example, risk ratio and rate ratio agree, but this is not always the case. For instance, assume that

the same proportion of men would die from PC in both groups. Then the risk ratio would equal 1. If, however, the men died earlier in the control arm then in the screening arm, then there are fewer person years in the control arm compared to in the screening arm. This would lead to a higher rate of PC deaths in the control arm and lower rate ratio for screening versus controls.

Rate ratio reflects the event-free follow-up time for each man and gives a better estimate in this type of investigation. However, both rate ratios and risk ratios can be used to calculate risk.

Risk can also be estimated in terms of cumulative risk/incidence, because that refers to the occurrence of risk events (e.g., PC diagnosis or PC death). **Survival analyses** are therefore common in randomized controlled studies. These methods assess the time it takes for a certain event to occur, called "time to event". An example of an event is PC diagnosis or death, and study participants can be followed for various lengths of time. Observations are censored if information regarding the participants' survival time is incomplete. For instance, if a man in such a screening study emigrates before he experiences the event (PC), he is censored so that his survival time is calculated up to the time of emigration, knowing that this man was not diagnosed with PC while he was under observation in the screening study. Censoring can also be done for drop-outs and men who die from other causes, because these individuals are no longer "at risk" of the event studied. Censoring is an important issue in survival analyses, and it represents a certain type of "missing data". However, individuals that are censored do contribute information on time at risk, even if they have not been diagnosed with PC during the observation period. The various types of survival analyses that are used are discussed in chapter 3.3. Non-parametric estimations, such as the Kaplan-Meier (product-limit) method and the actuarial approach (221) are often used in epidemiology and medicine.

Number needed to screen (NNS) and number needed to treat (NNT): NNS can be defined as the number of men that must be screened during a given period of time, in order to prevent one PC death (227). This represents a measure of the effect of the screening programme. In **Paper III**, all men in the screening arm were included in the calculations (even if they were non-attendees and did not participate in the programme), and therefore it is more correct to describe NNS as "number needed to *invite*" (NNI).

NNI is calculated as 1 divided by the absolute risk reduction of the given endpoint between the two study arms. In the Göteborg screening trial 10,000 men were randomized to each arm (screening and control arm, respectively).

NNS or NNI = 1 / (158 - 112 PC deaths) per 10,000 men = 217

NNT is often used in epidemiological studies to describe the effect of an intervention or treatment (228). NNT is affected by the follow-up time and type of study population, and can therefore be difficult to compare between studies. However, many men with screen-detected and low-risk PC are not given immediate treatment (some are offered surveillance), NNT represented the NND, that is, the number of men that had to be *diagnosed* to prevent one death. We calculated NND as 1 divided by the absolute PC mortality reduction multiplied by the excess rate/incidence:

1528 PC (diagnosed in the SG) - 1124 PC (diagnosed in the CG) = 404 (excess incidence) per 10,000 men

NND or NNT = 0.0404 x 217 (NNI) = 9

3.3.1 Methods and statistical considerations; Paper I–IV

This section briefly summarizes the materials and methods used in each of the four studies underlying this thesis (**Papers I–IV**). The strengths and limitations of the methods applied are also discussed.

PAPER I

The aim of the first study was to investigate whether men with PSA ≥ 3 ng/mL and voiding symptoms were at increased risk of being diagnosed with PC (at the time of the actual examination/biopsy). Long-term outcomes were not evaluated. All men with a PSA level of ≥ 3.0 ng/mL were offered consultation and additional biopsies. Men with PSA below the threshold were not further evaluated, but they were invited for assessment again after 2 years. The study cohort consisted of the men who had an elevated PSA (≥ 3.0 ng/mL) at least once during the study period (1995–2010). For men who were biopsied on more than two screens, only the first biopsy result was included in this analysis.

In the waiting room, directly before the examination, the attenders completed a self-administered study-specific questionnaire. This instrument included one question which concerned obstructive voiding symptoms: *"Do you have voiding symptoms in terms of weak stream or difficulty emptying the bladder?"* The answers were ranked on an ordinal scale from 1 to 3: 1, no symptoms; 2, minor/moderate symptoms; 3, major/severe symptoms. The data obtained were dichotomized so that men who answered "2" or "3" were

regarded as having voiding symptoms, and those who answered "1" were considered asymptomatic.

Statistics

Logistic regression models are often used to determine the association between several explanatory variables and a dichotomous variable (e.g., cancer or no cancer). In the present research, multivariable logistic regression was performed to assess the impact of the different covariates on biopsy outcome at a particular time point (first biopsy).

Differences between the groups (benign vs. cancer) and (asymptomatic vs. symptomatic) were evaluated. The impacts of the covariates age, prostate volume, tPSA, F/T PSA ratio, and voiding symptoms were assessed by univariate logistic regression analyses, and variables with a statistically significant impact were retained in the final multivariable model. Age, tPSA, F/T PSA ratio, and prostate volume were used as continuous variables, and voiding symptoms were set as a dichotomous variable (symptoms present or absent). Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for all covariates. The level of statistical significance was set at $p < 0.05$.

The ***chi-square test*** can be used to compare variables for randomly sampled data, and to determine whether a significant relationship exists between two categorical variables. For example, in the present setting, the chi-square test was used for comparison of voiding symptoms in men with benign biopsy versus those with PC.

The nonparametric ***Mann-Whitney U test*** is used to analyse the difference between medians of different groups. Unlike the **t-test**, the Mann-Whitney method does not require normally distributed data.

Methodological considerations

In this study, we had no information on non-attenders (men who were invited but did not participate in the screening programme). The data later presented in **Paper III** showed that these men had a higher risk of PC, but offered no data on their symptoms and comorbidities. A healthy selection bias can be the result of asymptomatic men being more likely to reject invitations to PSA screening and prostate examinations. Avery et al. (229) interviewed men concerning their decisions regarding PSA testing and found that many men felt that a PSA test was unnecessary because they lacked voiding symptoms,

thus identifying themselves as being at lower risk of PC and therefore declining further investigations.

No validated questionnaire for assessing voiding symptoms was available for use in our investigations. When the Göteborg screening study was planned during the early 1990s, the International Prostate Symptom Score (IPSS) questionnaire (230) was not being applied in Sweden, whereas today it is recommended for all men seeking consultation for urinary problems. If we had used IPSS, a better survey could have been made. Furthermore, we had no information on symptoms in men with PSA < 3.0 ng/mL, and thus no conclusion in this regard can be drawn for men in this PSA range.

PAPER II

The second paper reports our study of 5,174 participants in the first round of screening (1995–1996), who had an initial PSA level of < 3.0 ng/mL. The aim was to evaluate the long-term outcome in these men, but also to assess whether the F/T PSA ratio can be useful as a prognostic marker in this PSA range. The men were stratified into subgroups according to baseline PSA level, and the follow-up time was 19 years.

Statistics

Kaplan Meier estimates were used to calculate cumulative incidence, and differences between men in the various PSA strata were tested for significance by the log rank test ($p < 0.05$). We assumed that, at any given time, men who were censored would have the same prospects as those who continued to be followed in the programme. We assumed that PC probabilities were the same for men recruited early and late in the study, and that the events occurred at the specified time. Harrell's concordance index (222) and the likelihood ratio test were used to determine whether F/T PSA ratio added predictive value to tPSA. Models that included age and tPSA were compared with models that included age, F/T PSA ratio, and the interaction between F/T PSA ratio and tPSA.

Methodological considerations

Paper II describes a cross-sectional test result, and the outcomes might not be directly transferable to other populations with different age distributions or underlying PC prevalence. Chiefly six-core biopsies were performed in the Göteborg screening study, whereas 10–12 cores are more common today, and MRI-targeted biopsies are also emerging (26). Undersampling of the prostate leads to clinically significant cancers being missed on initial biopsy (231), and it has been suggested that a repeat biopsy should be performed when

using traditional sextant sampling. However, our study has a very long follow-up, which enables cancer detection, with repeated screening.

PAPER III

This study analysed the main endpoints PC-specific mortality and PC incidence according to the intention-to-screen principle: "Once randomised, always analysed". This means that all outcomes were analysed according to the group to which the men were randomized, regardless of whether they were allocated to the screening group, and did not attended, or to the control group, in which they could have been tested outside the programme.

In this way we "preserved randomization", because the aim of randomization is to equalize prognostic factors. Inasmuch as the men in the control group had access to opportunistic PSA testing (mainly during the last 10–15 years), the actual difference between the two study arms is more representative of a comparison between organized screening and opportunistic PSA testing than between screening an no screening.

Observed versus expected data

The observed cumulative PC incidence and PC mortality were calculated by life-table analysis (in the screening vs. the control group). Follow-up time was calculated from start of the study (January 1, 1995) to the date of the event (PC diagnosis or PC death). The men who did not experience an event were censored at date of emigration, death, or last follow-up (December 31, 2016).

Incidence and mortality data from the screening study were also compared with expected rates (ERs), by using widely available data from the years before PSA testing in Sweden. ERs were calculated based on historical PC incidence and mortality data from Statistics Sweden, for the period 1990–1994. At that time, PSA testing was virtually non-existent in Göteborg. This calculation allowed us to extrapolate how the outcomes might have been with no PSA testing available to the study population. This type of estimation has previously been described by our research team (194). We used 1-year age-group incidence and mortality rates to reflect the actual distribution of events in all men (all ages), who were randomized. To adjust for exclusion of prevalent cancers, we subtracted the observed mortality rate for prevalent cases from the expected mortality (from the pre-PSA era). Expected cumulative mortality and incidence were estimated as 1 minus the Ederer II survival estimate (221). The validity of using historical data could be considered high, since PC mortality has been fairly constant and the natural

history of PC unchanged (during the last 25 years). The observed cumulative PC incidence and mortality (for screened men and controls) were calculated from start of the study (January, 1, 1995) to the date of the event (PC diagnosis or PC death). Those who had no event, were censored at time of emigration, death from other causes, or December 31, 2016, whichever occurred first. The Greenwood method (232) was used to estimate standard errors.

Comparisons between groups were performed using rates (number of events divided by the total number of person-years) but also using cumulative incidences calculated by the actuarial life table approach. ERs were estimated using historical data (as described above). Competing risk estimates of cumulative incidence were calculated as described by Choudhury et al. (220). Adjusted rate ratios (RRs) for PC mortality were calculated with correction for non-participation (233).

Secondary endpoints were identifying and analysing subgroups with higher PC mortality, formed on the basis of adherence to the programme and the time of diagnosis. Three subgroups were identified and defined as listed here:

- Prevalent cancers: PCs diagnosed at the time of the first invitation/screening occasion.
- Cases detected after the screening period (after stop age).
- PC in non-attenders (men who were invited but never participated in the programme).

Methodological considerations

A competing event is defined as any event that prevents the event of interest from occurring. For example, if a man dies from a heart attack during the study period, he cannot die from the cause of interest (i.e., in our study PC). Expected rates (ERs) calculated by the Ederer II and life table methods do not consider competing risks (the same as for the KM method), so these censored observations (men dying from other causes) are removed from being “at-risk” and thereby assuming that these men would have had the same risk of PC death as those who were not censored. In other words, such calculations can result in overestimations. This is a common problem in clinical research, especially because study populations often consist of elderly individuals. Koller et al. reviewed 50 clinical studies performed on individuals susceptible to competing risks (and published in high-impact journals), and found issues affecting competing risks in 70% of all these articles (234). By comparison,

the observed cumulative PC mortality reported in Paper III was estimated using both life table and competing risk methodology.

Even if as many as 20,000 men were randomized in the Göteborg screening trial, it was not as large as the PLCO trial or the whole ERSPC. On the other hand, this is compensated for by the long follow-up and the large number of events (270 PC deaths). In the PLCO study the corresponding number of PC deaths was 303 cases (235).

PAPER IV

The study cohort described in this paper was based on men who were attenders and left the Göteborg screening trial with no previous PC diagnosis. Those who were younger than 67 years at their final screen were not included in the analysis, because men who left the trial before the age of 67 were actually invited to one or more screens, that they did not attend (even if they were "attenders" on at least one occasion). Thus choosing not to participate in the final screening rounds was considered to be a different issue.

Men in the cohort were stratified by their PSA level at the time of their final screen. Men with PSA < 3 ng/mL and no biopsy; men with PSA ≥3 ng/mL and no biopsy (due to comorbidities or refusal); men with PSA ≥ 3 and a benign last biopsy, and men with PSA < 3 ng/mL and a benign biopsy at last screen. The fourth of these groups consisted of those who were biopsied secondarily to the MRI pilot study (in which 1.8 ng/mL was used as a cut-off) (236). The first consort diagram was constructed as shown on the next page (Figure 14).

The main end-point was GS ≥ 7 PC during follow-up (after final termination of screening). The four subgroups were reduced to two main groups (designated A and B), because men with PSA ≥ 3 ng/mL and no biopsy and men with PSA < 3 ng/mL and a benign biopsy, could be regarded as deviant from the protocol.

For men in group A, our aim was to evaluate whether the covariates age, tPSA, F/T PSA ratio, PSAV, and/or previous biopsy could predict risk of GS ≥7 disease after the end of the study. For men in group B, we also had information on prostate volume and voiding symptoms, because these individuals underwent urological evaluation at their final screen (same as for men with PSA ≥3 ng/mL at first screen, in Paper I). We evaluated whether a model including one or several covariates could add predictive value to PSA, when identifying those who could benefit from continued screening.

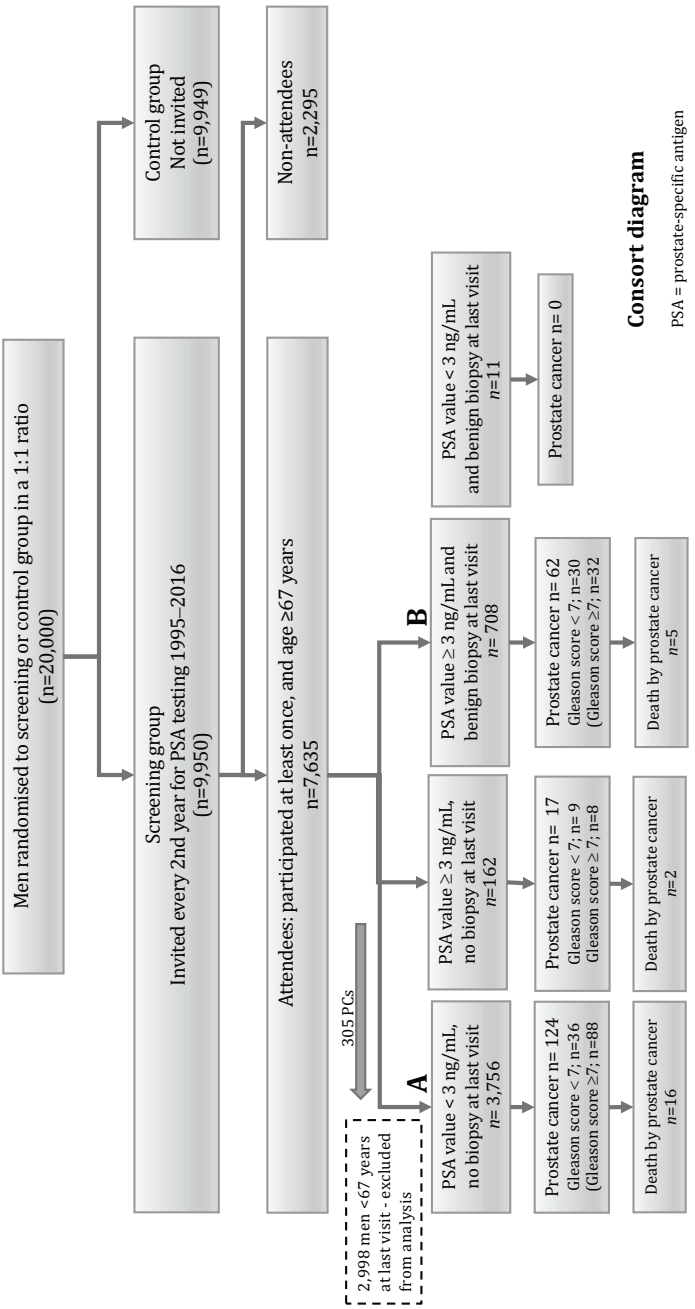

Figure 14. Initial flow-chart of study cohort in Paper IV.

Statistics

Cumulative PC and GS ≥ 7 incidence were analysed as 1 minus the KM estimates. Follow-up time was calculated as time from the date of last PSA in the screening trial to the date of diagnosis or censoring (date of emigration or death, or December 31, 2016). The covariates mentioned above was examined by comparing increasingly larger Cox regression models. Variables were added one at a time in the order of increasing univariable c-indices. The predictive ability was measured using Harrell's c-index, and the models were compared using the likelihood ratio test.

PSA velocity (PSAV) was calculated as the slope in a linear regression model based on all available PSA measurements for each man. Log-transformed PSA values were used. Relative long-term PSAV (percent per year) was also calculated. In addition to velocity based on tPSA we examined velocity of F/T PSA, and we used restricted cubic splines to investigate potential non-linear relationships. Neither F/T PSA velocity nor non-linearity improved the models, and hence these results were not reported in the paper.

It was also our intention to use one or more covariates to identify a subgroup of men who would benefit from continued screening and estimate how many of the men in the cohort (i.e. the size of this subgroup) would benefit from continued screening after stop age. This was illustrated by plotting a curve, similar to a Lorenz curve (described in statistical methods, page 60).

Methodological considerations

The median follow-up period was 8.6 years (after the final screen in the study), and the median age at last follow-up was 80.2 years. As the estimated age at time of PC death is 82 years (in Sweden), we are expecting additional PCs and PC deaths with longer follow-up. It is therefore too early to evaluate the "true" PC-specific mortality in this study population. So far, 21 men had died from PC. This number will be higher with a longer follow-up period.

Another limitation in this paper is that some men (n = 43) only had two PSA values within the study. Outlying values due to short-term variation of PSA could bias these estimates. PSAV is a measurement of how fast PSA levels increase over a time period. Some recommendations for the use of PSAV include collection of multiple PSA values (over a period no less than 24 months) and a minimum of three values.

4 RESULTS AND COMMENTS

PAPER I

Of the 7,625 men who attended the screening study, 34% (n = 2,590) had a PSA level of ≥ 3 ng/mL on at least one screening occasion (1995–2010). The majority of these men (n = 2,353) answered the questionnaire and accepted TRUS and prostate biopsy. The median prostate volume was 37.8 cm^3 (interquartile range (IQR) 30.0–48.6). Men with a benign biopsy (n = 1,720) had larger prostates and reported voiding symptoms more often than men with PC (50% vs. 40%; p < 0.001). A total of 633 PCs were detected.

	Benign, n = 1,720	PC, n = 633
Median age (IQR)	63.0 (59.8–65.8)	64.3 (60.8–66.8)
Median volume cm^3 (IQR)	40.0 (31.6–50.8)	32.6 (26.6–41.6)
F/T PSA ratio in % (IQR)	20.6 (15.7–26.5)	16.8 (12.0–23.3)
tPSA in ng/mL (IQR)	3.8 (3.3–4.6)	4.1 (3.4–5.6)
Voiding symptoms (%)	867 (50%)	255 (40%)

Table 6. Clinical characteristics of the subjects. Benign vs. prostate cancer at time for the first prostate biopsy.

In the multivariable logistic regression, age and PSA level were significant predictors of PC detection, whereas prostate volume, F/T PSA, and voiding symptoms were correlated with a lower odds of PC detection:

	Odds ratio (OR)	95% CI	P value
Age at time of biopsy	1.10	1.07–1.12	< 0.001
Prostate volume (cm^3)	0.96	0.96–0.97	< 0.001
F/T PSA ratio in %	0.97	0.96–0.99	< 0.001
tPSA in ng/mL	1.09	1.06–1.12	< 0.001
Voiding symptoms	0.78	0.63–0.98	0.032

Table 7. Multivariable analysis of the impact of different covariates on biopsy outcome in men with a tPSA level of ≥ 3 ng/mL.

In this study 378 PCs (31%) were detected in 1,230 asymptomatic men, and 255 PCs (23%) were detected in men who reported voiding symptoms (n = 1,123). When the incidence of an outcome is low (< 10%), the adjusted

odds ratio (OR) derived from the logistic regression is similar to the risk ratio (237). Incidence of the outcome in this analysis was > 10%, and based on our data, the risk ratio was 0.84 (this was not reported in the Paper I).

PAPER II

This study evaluated long-term outcome in men with a baseline PSA below 3 ng/mL (n = 5,174). F/T PSA ratio was also assed as a prognostic reflex test in this PSA range.

The median age at initial screening round was 57.2 years (IQR 53.8–61.4). During a median follow-up of 18.9 years (IQR 18.1–19.3), 754 PCs were diagnosed, which corresponds to a cumulative incidence of 17.2%. Median age at time of diagnosis was 66.8 years (63.7–69.1). In total, 224 cases (30%) were GS ≥ 7 cancers, and 108 out of these 224 (48%) were non-screen detected and diagnosed after the men terminated the screening programme (after stop age, 67–71 years).

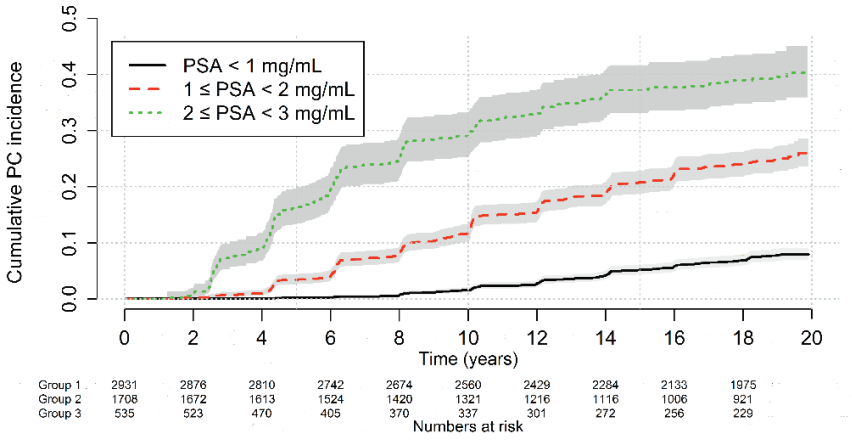

Figure 15. Cumulative prostate cancer incidence in men with an initial PSA < 3ng/mL.

At baseline, 57% (n = 2,931) of the men had **PSA levels of ≥ 0.99 ng/mL**. Among them, the cumulative risk at 20 years was 7.9%, and the cumulative risk for GS ≥ 7 cancer was 3.7%. Seven men died of PC (five of them were diagnosed after stop age).

About one third of the men (n = 1,708) in this cohort had a PSA level between **1 and 1.99 ng/mL** at initial screen. The cumulative PC risk at 20 years was 26.0% (9.7 % for GS ≥ 7 cancers). Fifteen men later died of PC

(ten of them after stop age). For the remaining 535 men (10%) with **PSA 2–2.99 ng/mL,** the cumulative risk was 40.3% (10.9% for GS ≥ 7 PC), and three out of six PC deaths were diagnosed after stop age. Of the total study population, 74% represented complete attenders (e.g., the invitation lead to a PSA screen).

In all, 28 men died of PC, which corresponds to a cumulative risk of 0.7% (95% CI: 0.5–1.0). Most of these cases (n=18) were diagnosed after stop age.

Free-to-total PSA

Adding F/T PSA did not improve PC prediction as assessed by Harrell's c-index (base model 0.76 vs. 0.76), or considering the likelihood of the model (p = 0.371). The corresponding figures for men with GS ≥ 7 disease, were 0.68 vs. 0.67 and p = 0.079.

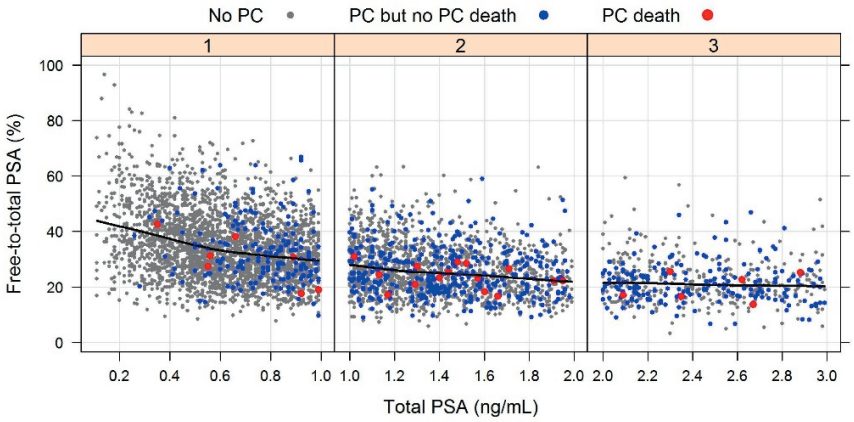

Figure 16. Scatter plot of F/T PSA vs. PSA at baseline. Blue indicates men who are later diagnosed with PC, and red stands for men who died of PC. Most of the men had PSA levels of < 1.0 ng/mL at first screen.

The scatter plot above illustrates distribution of the men in the study population with regard to PSA level and F/T PSA ratio. Among the 28 men who later died of PC (red in the plot), 17 had F/T PSA below and 11 F/T PSA above the median PSA. Adding F/T PSA did not provide more accurate prediction of which men who would develop PC or die from the disease.

PAPER III

This paper describes the main study presented in this thesis, and it was performed to compare the screening group and the control group with regard to PC mortality and incidence. We also compared the findings of these assessments with expected incidence and mortality rates based on historical (pre-PSA) data.

Follow-up time was calculated up to December 31, 2016. The final invitation to the screening study was sent in 2014, and the attendance rate at 22 years was high (77%). Of the 7,635 men who attended the screening programme, 2,672 (35%) men had an elevated PSA (≥ 3 ng/ml) at least once, and 2,525 (33 %) men underwent at least one prostate biopsy. This resulted in 1,046 cancers being diagnosed within the screening trial (screen-detected PCs). An additional 482 cancers were diagnosed in men in the screening arm, albeit outside the programme (non-screen-detected PCs). Thus, a total of 1,528 men randomized to screening were diagnosed with PC. The corresponding number of PCs diagnosed in the control group was 1,124.

Cumulative PC incidence at 22 years was 18.7% (95% CI 17.9–19.6) in the screening group and 14.4% (95% CI; 13.6–15.2) in the control group. This can be compared with an expected rate of 10.3%, according to data from the pre-PSA era. Expected rates (ERs) were similar to PC incidence found in non-attenders.

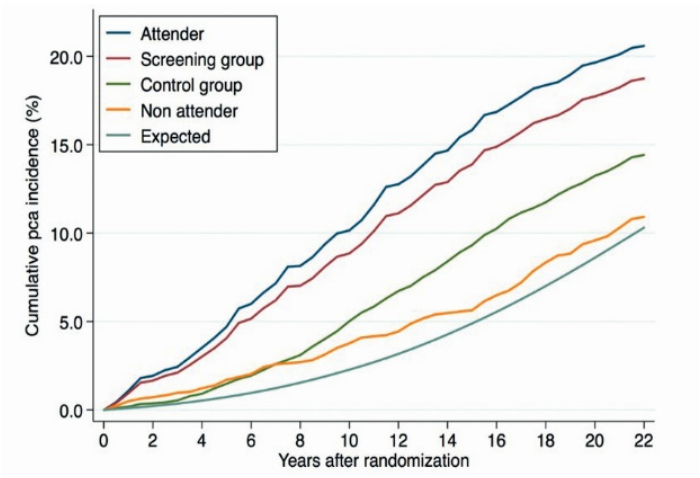

Figure 17. Observed and expected PC incidence up to December 31, 2016. Expected values are based on PC incidence in Göteborg 1990–1994 (the pre-PSA era) and extrapolated to data from the screening study.

	CONTROL ARM	SCREENING ARM			
		Prevalent cancers	PCs detected during round 2-10	PCs detected after stop age	PCs detected in non-attenders
Number of PCs	1,124	144	1,023	213	148
Number of PC deaths	158 (14%)	15 (10%)	24 (2%)	26 (12%)	47 (32%)
Md age at diagnosis	68.8 (64.8–72.9)	60.5 (57.4–63.7)	65.8 (62.6–67.7)	76.1 (73.2–78.8)	68.8 (64.1–73.7)
Md PSA at diagnosis	8.6 (5.1–19.0)	6.2 (4.0–11.2)	4.3 (3.5–6.0)	10.0 (6.3–22.5)	19.0 (7.6–61.0)
Clinical stage					
T1	545 (48%)	86 (60%)	805 (79%)	95 (45%)	46 (31%)
T2	377 (34%)	48 (33%)	187 (18%)	64 (30%)	48 (32%)
T3/T4	155 (14%)	10 (7%)	22 (2%)	46 (22%)	49 (33%)
Tx	38 (3%)	–	9 (1%)	8 (4%)	5 (3%)
Unknown	9 (1%)	–	–	–	–
Gleason score					
≤ 6	494 (44%)	110 (76%)	815 (80%)	63 (30%)	43 (29%)
7	407 (36%)	22 (15%)	176 (17%)	92 (43%)	44 (30%)
≥ 8	186 (17%)	12 (8%)	32 (3%)	57 (27%)	47 (32%)
Unknown	37 (3%)	–	–	1 (0.5%)	14 (9%)
Risk group					
Low	269 (24%)	62 (43%)	616 (60%)	28 (13%)	17 (11%)
Intermediate	434 (39%)	59 (41%)	326 (32%)	88 (41%)	37 (25%)
High	207 (18%)	19 (13%)	55 (5%)	57 (27%)	43 (29%)
Advanced	136 (12%)	4 (3%)	16 (2%)	28 (13%)	41 (28%)
Unknown	69 (6%)	–	10 (1%)	12 (6%)	10 (7%)
Lymph node MET (N1)	25 (2%)	3 (2%)	7 (1%)	2 (1%)	8 (5%)
Distant MET (M1)	101 (9%)	–	8 (1%)	26 (12%)	32 (22%)
Treatment					
Prostatectomy	329 (30%)	66 (46%)	428 (42%)	24 (11%)	31 (21%)
Radiation therapy	118 (11%)	17 (12%)	68 (7%)	15 (7%)	18 (12%)
Surveillance	388 (35%)	52 (36%)	493 (48%)	89 (42%)	28 (19%)
Endocrine treatment	268 (24%)	7 (5%)	26 (3%)	82 (38%)	69 (47%)
Unknown	11 (1%)	2 (1%)	8 (1%)	3 (1%)	2 (1%)

Table 8. Descriptive statistics of PCs detected in the control arm vs. the subgroups in the screening arm of the Göteborg Randomized Population-Based PC Screening Trial. Last follow-up was December 31, 2016.

The table presented above outlines the tumour features and treatments in different groups. In general, most of the cancers detected in the screening group were early-stage disease. Considering PSA at the time of diagnosis and number of men with advanced disease, the levels observed in the screening arm (5.1 ng/mL and 89/1,528 PCs [6%]) were lower than those noted in the control arm (8.6 ng/mL and 136/1,124 PCs [12%]). This is also reflected by the treatments given: endocrine therapy was recommended for 12% of men with PC in the screening group and 24% of the men in the control group.

However, treatments with curative intent were similar in the two study arms: 447/1,124 (40%) vs. 667/1,528 (44%).

Non-attenders in the screening arm and men in the control arm were diagnosed with PC at the age of approximately 69 years, whereas attenders (diagnosed in round 2–10) were about 66 years. Non-attenders had more advanced cancers at diagnosis, and 69/148 (47%) of them received endocrine therapy.

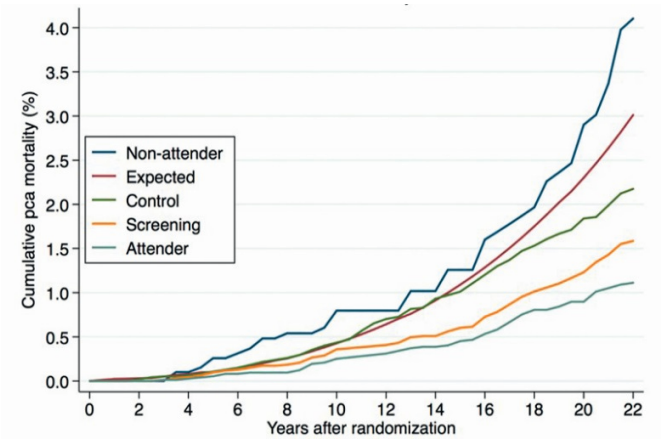

Figure 18. Observed and expected PC mortality up to December 31, 2016. Expected values are based on PC mortality in Göteborg 1990–1994 (the pre-PSA era) minus PC mortality due to prevalent cancers.

There was no discrepancy in all-cause mortality between the screening and the control group (RR 1.02, CI 0.97–1.07). However, the number of PC-specific deaths was higher in the control group: 158 vs. 112, corresponding to a RR of 0.71 (95% CI 0.56–0.90). The cumulative risk of dying from PC at 22 years in the trial was 1.59% (95% CI 1.32–1.90) in the screening arm compared with 2.17% (95% CI 1.86–2.54) in the control arm; the estimated rate for the pre-PSA era corresponded to 3.01%. As noted for the incidence curve, expected values for PC mortality were similar to the mortality rates for non-attenders.

At 22 years in the trial, the NNI to prevent one PC death was 217 and the NND was 9. The corresponding rates at earlier time points (as reported in previous publications) were, respectively, 293 and 13 at 14 years, and 243 and 11 at 18 years.

Three groups of men with PC who were at higher risk of death due to this disease were identified in Paper III:

- Men who were diagnosed at first screen (prevalent PCs)
- Men who were diagnosed after stop age
- Men who were non-attenders

Among the 144 men who had a **PC detected at first screen**, 15 (10%) men later died of PC. Eleven of these 15 men were diagnosed with GS ≥ 7 disease, and nine were aged ≥ 60 years at the time they were invited to participate in the study. Men who died of PC in this group had, at time for diagnosis, a median PSA level of 10.7 ng/mL (IQR 6.2–28.7), and F/T PSA was 10.6% (IQR 6.1–13.2). Median age at the time of PC death in these 15 men was 71.8 years (IQR 70.0–72.9).

A total of 213 men were diagnosed with **PC after stop age** (67–71 years), and 26 of those subjects (12%) later died of PC. The median age at time of diagnosis for these 26 men was 78.2 years (IQR 73.1–81.6), and age at time of death was 80.4 years (IQR 76.6–82.9). Twenty-five of the 26 men had GS ≥ 7 disease.

Most deaths (47 of 148 cases) occurred in the group designated **PCs in non-attenders**, which consisted of men who were invited to screening but did not participate (n = 2,310). The cumulative risk of PC death at 22 years was much higher for the non-attenders than for the attenders (4.1% vs. 1.1%).

	Rate ratios for non-attenders vs. attenders	***Non-attenders vs. men in the control group***
PC incidence		
Rate ratio:	0.45 (95% CI: 0.38–0.53)	0.71 (95% CI: 0.60–0.84)
PC mortality		
Rate ratio:	3.23 (95% CI: 2.22–4.70	1.63 (95% CI: 1.18–2.25
All-cause mortality		
Rate ratio:	2.34 (95% CI: 2.19–2.50)	1.92 (95% CI: 1.80–2.04)

Table 9. Rate ratios in non-attenders vs. attenders and vs. controls.

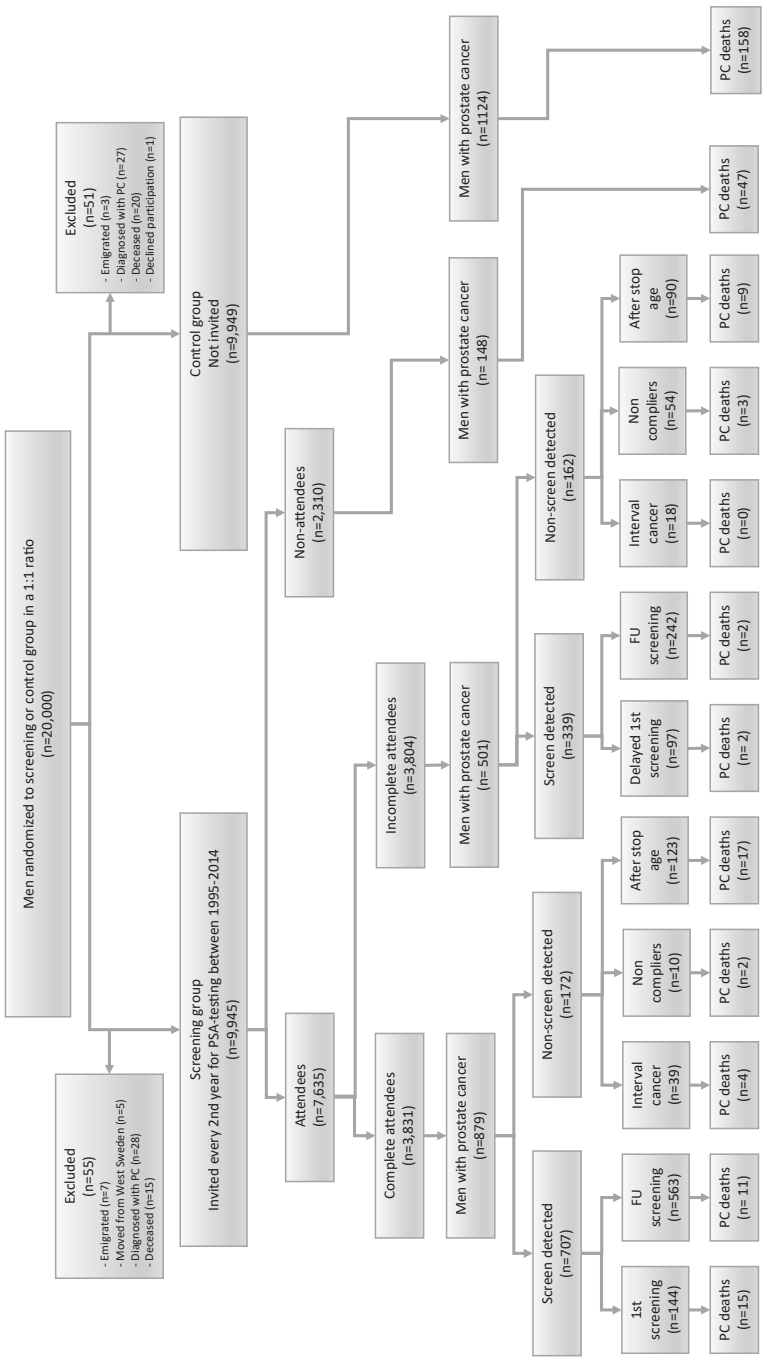

Figure 19. Consort diagram showing the number of PCs, and PC deaths.

PAPER IV

In this study, we investigated the outcomes in men who participated in the Göteborg screening trial and had their final screen at stop age (67–71 years). These men were free of PC at last screen and had a median of five (IQR 3–7) screens during the trial.

In this study population, most men (n = 3,756) left the screening trail with a PSA level of < 3 ng/mL and were therefore not recommended prostate biopsy at the time of the final screen. The majority of these men (n = 3,492, designated group A) had never been biopsied at previous screens. During a median follow-up of 8.6 years (IQR; 5.2–11.7), 124 of the men in group A (3.3%), were diagnosed with PC, and 88 (2.3%) of those cases were GS ≥ 7 cancers. Group B consisted of the men who had an elevated PSA (≥ 3 ng/mL) and a benign/negative biopsy (n = 708); 62 (8.8%) of those subjects were later diagnosed with PC, and 32 (4.5%) of those cases were GS ≥ 7 disease.

All men were stratified by PSA level, and cumulative risk of high-grade (GS ≥ 7) PC was estimated by the KM method. At 15 years of follow-up, men with PSA < 1 ng/mL were found to be at very low risk (~2%), whereas men with PSA ≥ 10 ng/mL were at high risk (~20%). Those with PSA 2–3 ng/mL were at higher risk compared with the men with PSA in the range of 3–10 ng/mL, who had been biopsied at the final screen (~13% vs. ~8%).

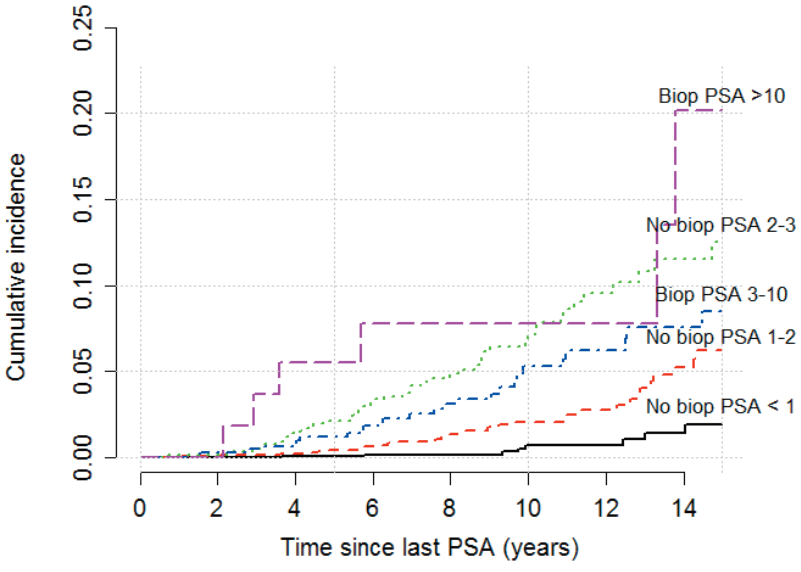

Figure 20. Cumulative risk of Gleason score ≥ 7 PCs within 15 years of follow-up after final PSA test in the screening trial.

A total of 21 men died of PC during the follow-up period (median 8.6 years). At 15 years, the cumulative risk of PC death was highest in men with PSA ≥ 10 ng/mL. The number of PC deaths in each PSA stratum varied between two and seven cases.

Men in the ranges 1–1.99 ng/mL and 2–2.99 ng/mL had a 1.5–2% risk of PC death within 15 years. This can be compared to 2.1% for the whole control group, at 22 years (results in **paper III**).

The predictive accuracy of the Cox regression model for men in group A; tPSA had a c-index of 0.77 for GS ≥7 cancers. The predictors were evaluated one-by-one and cumulatively (as shown below). Compared with tPSA, none of the other variables added predictive value to PSA.

Group A: PC (GS ≥ 7) in men with PSA < 3 ng/mL at final screen

	c-index	c-index cum.	LR-test
tPSA	0.77	0.77	0.000
PSA velocity	0.67	0.78	0.067
F/T PSA ratio	0.66	0.78	0.217
Age at final PSA	0.50	0.77	0.727
Previous biopsy	0.50	0.77	0.463

Table 10. Concordance index for different covariates (Group A). 3554 men had values for all variables and these men were included in calculation of the models.

For men in group B, PSAV had the strongest predictive value:

Group B: PC (GS ≥ 7) in men with PSA ≥ 3 ng/mL at final screen

	c-index	c-index cum	LR-test
PSA velocity	0.70	0.70	0.011
Prostate volume	0.66	0.71	0.051
F/T PSA ratio	0.63	0.69	0.095
tPSA	0.55	0.71	0.073
Voiding symptoms	0.53	0.69	0.517
Age at final PSA	0.53	0.69	0.368
Previous biopsy	0.49	0.69	0.412

Table 11. Concordance index for different covariates (Group B). 651 men had values for all variables and these men were included in calculation of the models.

The other variables did not add predictive value to PSAV in the PSA range ≥ 3 ng/ml. A low predictive value of tPSA (c-index 0.58) has also been shown in the STHLM3 population (144). One explanation could be that men with elevated PSA had been previously biopsied at one or several occasions, and thus leaving a selected population where the predictive value of tPSA has declined.

All men in the cohort PSA cut-offs*	No. of men in cohort n = 4,464	No. of PCs n = 186	No. of GS ≥7 PC n = 120	No. of PC deaths n = 21
PSA ≥ 1.0 ng/mL	2,985 (67%)	176 (95%)	112 (93%)	18 (86%)
PSA ≥ 1.2 ng/mL	2,627 (59%)	167 (90%)	106 (88%)	17 (81%)
PSA ≥ 1.4 ng/mL	2,294 (51%)	161 (87%)	104 (87%)	17 (81%)
PSA ≥ 1.6 ng/mL	1,982 (44%)	152 (82%)	**96 (80%)**	**16 (76%)**
PSA ≥ 1.8 ng/mL	1,700 (38%)	142 (76%)	88 (73%)	11 (52%)
PSA ≥ 2.0 ng/mL	1,493 (33%)	133 (72%)	80 (67%)	11 (52%)
PSA ≥ 2.2 ng/mL	1,278 (29%)	121 (65%)	72 (60%)	11 (52%)
PSA ≥ 2.4 ng/mL	1,098 (25%)	106 (57%)	59 (49%)	9 (43%)

Table 12. Detection rates at different PSA cut-offs.

We also performed an assessment to estimate the proportion of men in this age group who require additional follow-up to increase the possibility of detecting and preventing ~80% of high-grade cancers and PC deaths.

Most men (n = 2,482) had a PSA level of < 1.6 ng/mL at final screen. Subjects with PSA below this level were considered to be of "low-risk" of future GS ≥ 7 disease, whereas "only" 24 out of 120 GS ≥ 7 cancers and 5 out of 21 PC deaths were detected during the follow-up period. To determine the proportion of all men that would need to be investigated further we evaluated different PSA cut-offs to describe the distribution of later cancers being detected. This was also illustrated by the Lorenz-curve (illustrated on page 60, in *Statistical Methods*).

Among the 44% of the men in the cohort with a PSA level ≥ 1.6 ng/ml at time for final screen, 80% of all GS ≥ 7 cancers were detected and 76% of all PC deaths were in men above this cut-off. These results indicate that only men above this PSA level need continued screening. However, we require better tools for selecting these subjects, and the usefulness of PSAV needs to be explored more closely.

5 DISCUSSION

Widespread PSA testing has had a marked impact on the epidemiology of PC. The incidence of this disease in Sweden has increased considerably, mainly among men in midlife. However, patients, doctors, and even guideline groups find it difficult to know whether screening should be performed, and, if so, how it should be done. PC screening is multifaceted, and there is an urgent need for improved screening strategies. The research leading to this thesis has analysed and discussed outcomes and predictive factors with the aim of improving our understanding of PSA-based screening for PC.

5.1 RISK ASSESSMENT BASED ON URINARY SYMPTOMS

More than 50% of all men over 50 years of age report problems with urinating (238), which is why many men seek medical consultation and advice regarding additional PSA testing. Data compiled by the NPCR have shown that approximately one third of men diagnosed with PC had their urological work-up initiated due to symptoms from the lower urinary tract.

In the first of the present studies (**Paper I**), we asked men with elevated PSA levels (> 3 ng/mL) about their symptoms, and we found that the absence of voiding symptoms was an independent risk factor for PC. However, this observation can only be applied to men with elevated PSA levels, and hence the true association is unknown. In an investigation of the the HUNT 2 cohort in Norway (239), PC incidence and PC mortality were recorded during 9 years of follow-up, whereas the biopsies in our initial study were performed in close connection with completion of the questionnaire in the waiting room. That is, we did not analyse long-time data. In 2013, a population-based cohort study in the United States (240) found that men treated for lower urinary tract symptoms (LUTS) were more likely to undergo a prostate biopsy, and this increased diagnostic activity among symptomatic men had an impact on how they were selected for further investigations. Notwithstanding, a man who complains of increased frequency of urination or a poor stream and has a PSA level of > 3 ng/mL is not at higher risk of being diagnosed with PC than a man who has a PSA of > 3 ng/mL and no apparent bothers. Patients need to be informed that voiding symptoms are often due to benign conditions, and urological work-up should follow the same routine in these men. Importantly, our findings do not imply that men with symptoms should be withdrawn from investigation or PSA testing.

Today, there is insufficient evidence to recommend PSA testing in men who have voiding symptoms but no other risk factors. We concluded that men with symptoms are less likely to be diagnosed with PC if their PSA level is ≥ 3 ng/mL. This is an important finding, because it contradicts the common belief that urinary symptoms are signs of PC, and thus indicates that all men should receive advice based on the same guidelines (symptoms or no symptoms). Furthermore, prostate volume is highly relevant when evaluating PC risk.

Future research could, for example, be designed as an interventional study aimed at determining whether men with urinary complaints are overdiagnosed with PC. It appears that gaining such knowledge can be useful, because it can help us avoid unnecessary and untimely PSA testing in elderly men who are not amenable to treatment of PC with curative intent. Such information might also make it possible to circumvent diagnosis of clinically insignificant cancers.

5.2 RISK ASSESSMENT IN MEN WITH PSA < 3 NG/ML AT INITIAL SCREENING

The aim of a PC screening program is to detect potentially lethal disease, so that curative treatment can be offered before the cancer is too advanced. The optimal cut-off value for further diagnostics (i.e., biopsy or MRI) is a matter of debate, and in 2004 Thompson et al. (140), reported having demonstrated that there is no lower PSA limit at which PC cannot be found.

The results reported in **Paper II** are based on a rather large cohort of men who participated in a long-term screening programme with a high attendance rate and few men were lost to follow-up. We evaluated the outcome in men with an initial (at first screen) PSA of < 3 ng/mL, because this level is commonly used as a threshold for recommending prostate biopsy. Most of the PCs detected were low grade (GS < 7), and thus the benefit of detection could be questioned. We did not assess any negative impact on quality of life (caused by treatments or PC-induced anxiety).

We found that the baseline PSA level was strongly predictive of the future risk of being diagnosed with PC and also the risk of dying from this disease. Few men with a baseline PSA level below 1.0 ng/mL later died of PC (cumulative risk of 0.3%). These findings are consistent with several other reports (207, 241, 242). However, it is clear that, even with biannual PSA testing, some cancers are missed. Our analysis showed that in the PSA range of 1–3 ng/mL, the cumulative risk of GS ≥ 7 disease was ~10% at 20 years, and as many as 28 men in the study population died of PC. This is an important finding suggesting that the current screening algorithm has pitfalls. Interestingly, approximately half of all GS ≥ 7 PCs (108/224) were not detected by screening (i.e., were interval cancers or PCs diagnosed after stop age).

It has been proposed that use of a baseline PSA and a risk-adjusted PSA testing strategy might minimize the effect of overdetection (205). A study conducted in the United States (242) found that men with an initial PSA of < 1 ng/mL had a 10-year PC risk of ~3%, and it was suggested that men with this PSA level could safely be re-tested every 10 years. Such an approach could reduce both the cost of screening and the overdetection of indolent PCs, but still detect tumours that can be treated to lower PC mortality. Retesting intervals were not considered in **Paper II**, but it does seem rational to apply a restrained approach in men with PSA levels of < 1 ng/mL.

Extrapolation from our data indicates that the risk of GS ≥ 7 disease is very low the first 8–10 years of screening (in men with PSA <1 ng/mL), which supports the conclusions drawn in the cited study.

5.2.1 Risk assessment with F/T PSA ratio

The second of the present investigations (**Paper II**) also tested the value of adding F/T PSA ratio to a predictive model. Several other studies have demonstrated that such an approach can be a useful (adding F/T PSA as a reflex test to tPSA) in the PSA range 2–10 ng/mL (243-245). The Finnish arm of the ERSPC has reported results showing that a low F/T PSA was of prognostic value even in men with a baseline PSA of < 3 ng/mL (246), although this observation could not be confirmed by our data. In the Finnish study, men with a PSA of ≥ 4 or 3–3.99 ng/mL and a F/T PSA of < 16% were selected for biopsy. The cumulative risk of PC associated with F/T PSA was analysed in quartiles based on the distribution in men with PC. The adjusted relative risk for those in the lowest quartile (F/T PSA< 14.2%) was 6.9 compared with those in the highest quartile (F/T PSA > 23.7%), and thus it was concluded that a low F/T PSA is a strong predictor of PC diagnosis in men with low tPSA. Considering that the screening algorithm in the Finnish study called for biopsy at a low F/T PSA, a higher cumulative incidence of PC could be expected for the men identified in this manner. Many men with PSA < 3 ng/mL in the first screening round in the Finnish analysis had an elevated PSA in the second screening round (4 years later); the authors received comments from other researchers regarding this potential bias (247), and, after exclusion of these PC cases, the impact of F/T PSA was not significantly altered.

It seems that F/T PSA offers advantages primarily to men with elevated PSA. It appears that this ratio is more useful when applied in the PSA range of 4–10 ng/mL, a strategy that can reduce the number of unnecessary biopsies while retaining a high PC detection rate (248). At a PSA level of < 1.0 ng/mL, the free PSA is close to its detection limit. Furthermore, the test results provided by assays from different manufacturers can vary by as much as ~10% (249). Such calibration issues might lead to over-or underestimation of the true diagnostic performance, and this aspect must be taken into consideration when comparing the results of different studies. In any case, we can conclude that the use of F/T PSA ratio should not be recommended for men in low PSA ranges.

5.3 EFFICACY OF THE SCREENING ALGORITHM: OUTCOMES AFTER 22 YEARS

Paper III presents the results of the longest follow-up to date in a PSA-based screening study. During the last decade, PC incidence in the control arm has slowly been approaching the level in the screening arm. At 22 years, a 36% excess incidence remains in the screening arm (1,528/9,945 vs. 1,124/9,949), although the incidence curves have become more parallel. The absolute reduction in PC mortality is still increasing to some extent, while the relative risk is decreasing with longer duration of follow-up and a larger number of PC deaths. These observations demonstrate that differences in both incidence and mortality between the two arms are stabilizing. The RR for PC mortality is now 0.71 (95% CI 0.56–0.90). The NNI for screening to prevent one PC death was improved, from 243 at 18 years compared to 217 at 22 years, and the NND dropped from 11 at 18 years to 9 at 22 years. Our data confirm that an organized screening programme reduces mortality by ~30%, a level that is similar to evidence from the ERSPC and the PLCO trial (showing a 25–32% lower risk) (175).

5.3.1 Improving prostate cancer screening

It is clear that screening reduces PC-specific mortality, but how should a screening programme be designed to optimize such benefits and at the same time reduce the harms of overdiagnosis? This is a complex question, and it is necessary to start by identifying the pitfalls associated with the current strategy, because there are some **protocol-related issues** that need to be considered.

When screening starts too late. In the study reported in **Paper III**, 144 men were diagnosed with a prevalent PC (at the initial screening round), and 15 of them later died from this disease. Most of these men (n = 80) were over 60 years of age when they had their first PSA screen. Men diagnosed in screening rounds 2–10 had more favourable prognostic features. Similarly, the Dutch section of the ERSPC (250), found that men in the second round were at 2.9-fold lower risk of dying from the disease, compared with those diagnosed in the first round). Initiating screening at ages older than 60 years is apparently too late for many men, possibly because the window of cure might be missed and numerous men will already have aggressive high-grade cancers at the time of diagnosis. Earlier publications have proposed that screening should be initiated for men between the ages of 50 and 54 years (251), a strategy that would reduce the number of PC deaths and also lead to

fewer cases of metastatic disease. One concern with that approach is the risk of overdiagnosis when PSA testing is started in midlife. A previous study by our group found no difference in PC incidence at termination of screening, regardless of whether start age was 50, 55, or 60 years (170). In that study, the cumulative risk of PC was ~7%, at age 60, ~14% at age 65, and ~22% at age 70, irrespective of start age. These findings indicate that the rate of overdiagnosis is not correlated with the age at which screening is initiated.

Recommendations for screening younger men differ among guideline groups. One strategy has been to offer screening to men at high risk, as an individual option, starting at 50–55 years of age. The AUA guidelines suggests that screening can be considered at an earlier age for men who are younger than 55 years and have a positive family history or are of African descent (116). In the Swedish national guidelines for PC care, men at higher risk are defined as those from families with hereditary PC (i.e., men who are BRCA2 positive or have at least two close relatives diagnosed with PC at age < 75 years). Others, have suggested that a baseline PSA at ages 45–49 years can provide risk stratification superior to that based on race or family history (252).

When screening is terminated too early. Overdiagnosis increases with age (253), and older age and comorbidities should always be considered before PSA testing. The study outlined in **Paper II,** showed that 28 men died of PC despite screening, and most of these cancers (n = 18) were detected after stop age (post screening). It seems rational to assume that some of these deaths could have been avoided if PSA testing had continued after the age of 70 years, or if additional diagnostics had been offered to selected individuals. In our subsequent investigation (**Paper III**), we mapped all cancers in the screening group, which revealed that 213 men were diagnosed after stop age, and 26 of these men died of PC. The majority of these deaths (20 of 26) occurred in men who started screening when they were aged 60 years or older. Most men had a PSA level of < 3 ng/mL when they left the study, and, although they were approximately 80 years old when they died of PC, some of them might have been "saved" if the cancer had been detected in the PSA range of 2–3 ng/mL. This observation concurs with reports showing that elderly men are more likely to develop high-grade cancers and/or die of PC (254, 255).

The data in **Paper III** also showed that the stage distribution of cancers detected after stop age was shifted towards more advanced PC (only 13% were low-risk PC). It has been suggested that in Sweden it is less likely that curative treatment will be given to men who are in their seventies and have non-metastatic high-risk PC but are otherwise healthy, than to younger men

with the same life expectancy (256). A possible explanation for this difference is that physicians underestimate the life expectancy of many elderly men. The Göteborg screening trial stopped inviting men after the age of ~70 years. Considering that 26 out of 112 deaths in the screening group occurred in men diagnosed post screening, it seems logical to assume that this was too early for men in apparent good health. We have previously published data showing that the protective effect of screening disappeared after 9–10 years (257).

When screening does not apply to all men. Almost a quarter (n = 2,310) of all men who were invited to the programme never participated (non-attenders). Notably, these men had both a higher all-cause mortality (RR 2.34) and higher PC-specific mortality (RR 3.23) than attenders. They also had higher mortality compared with men in the control arm (RR 1.63), in which opportunistic screening increased over the last decade (258). However, there is insufficient knowledge concerning the characteristics of non-attenders in PC screening. Studies of cervical cancer screening have suggested that non-attenders have less contact with their general practitioner and that they were more often live alone (259), and also that people from ethnic minority backgrounds are more likely to be unaware of screening (260). Although the features of non-attending men in PSA screening have not been identified, this is a group that might require extra attention if a PC screening programme is introduced in Sweden. We found that more than 40% of all deaths in men randomized to screening occurred in non-attenders.

This investigation reveals that the men at high risk of PC death were those who were invited, but did not participate in the program, those who started screening after age 60, and those who had a long life-expectancy and terminated screening too early. To improve a future screening programme these findings must be regarded.

5.4 RISK PREDICTION IN ELDERLY MEN CONCLUDING SCREENING

All men in the Göteborg screening trial who terminated the programme at the upper age limit of ~70 years had received a letter notifying them that no further PSA tests were recommended, and that they had a low risk of future PC. To the best of our knowledge, this was based on the information available at the time the screening trial was initiated (in 1995). As described in **Paper IV**, we found that being free of PC at time of the final screening is no guarantee that GS ≥7 disease will not occur, and as many as 21 men in that study-population later died of PC. So, the question is, can we safely refrain from prolonged PSA screening in men older than 70 years? From the findings in our study, this seems questionable.

In Sweden today, the estimated age at PC death is 82 years. The average 70-year-old man has a life-expectancy of ~15 years, (Figure 21), and men in good health live even longer. Guidelines (116, 261) recommend against screening in elderly men due to the high rate of overdetection. However, studies have found that outcomes of surgery for localized PC are similar for older and younger men (262), and there is even some evidence that active treatment has a survival advantage in the age group 65–80 years (263).

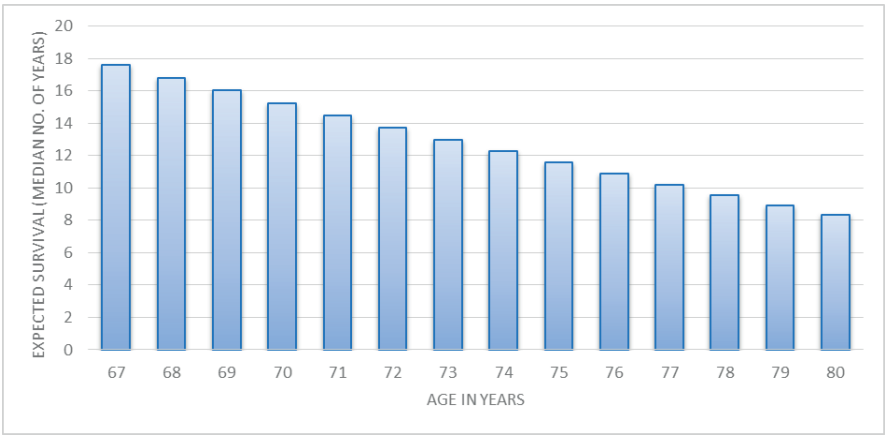

Figure 21. Life expectancy of men in Sweden. Data from the National Board of Health and Welfare (www.socialstyrelsen.se).

Data from the Nordic Cancer Registries (32) shows that PC-specific mortality is declining in men aged 75–84 years but increasing in men older than 85 years. Considering that the ageing population is getting healthier due to

improved diagnostics and better treatments (also for many diseases other than PC), it is plausible that PC mortality in the oldest men will continue to rise in the future.

Even if it is unlikely that all PC deaths can be prevented, it is reasonable to suggest that some of the PC deaths in our study could have been avoided with a different stop age. How long such a continued follow-up should be is unknown, but our data show that median age for diagnosis was 74 years. Advocating that healthy 70-year old men with PSA levels of >1.5 ng/mL should be screened until the age of 74–75 might be a vigorous approach (in terms of overdetection), although this could also increase the chance of detecting aggressive cancers in those who would have a survival benefit from diagnosis.

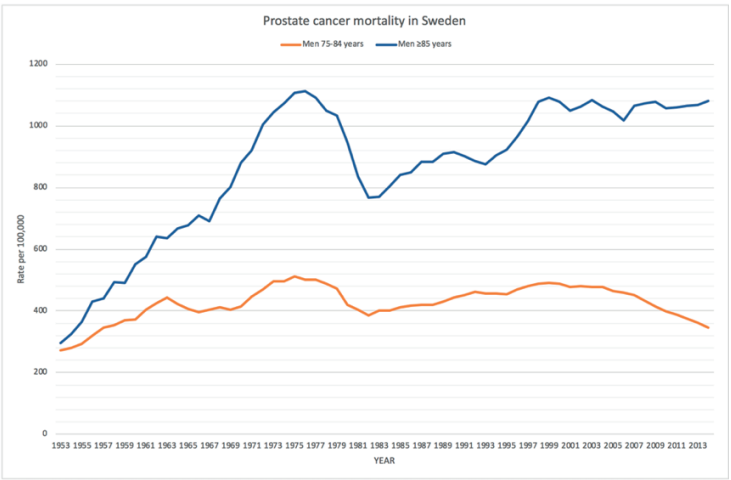

Figure 22. PC specific mortality in elderly Swedish men during the last six decades (32). Men older than 85 years have had no decline despite PSA testing becoming more accessible (opportunistic testing) in the late 1990s.

The findings of this investigation further indicate that men with an end-of-trial biopsy and a PSA level in the range 3–10 ng/mL were at lower risk of GS ≥ 7 PC than men with PSA levels of 2–3 ng/mL. Men with higher PSA levels most likely had BPH (or an anteriorly located PC) since these subjects had been thoroughly screened and biopsied before leaving the screening trial. Such men are usually monitored (often by their general practitioner) after terminating the screening trial. Men with PSA levels in the range 2–3 ng/ml, on the other hand, do not receive any follow-up, which in this cohort (with still limited follow-up) resulted in 6 PC deaths.

6 FUTURE PERSPECTIVES

The discussion in this thesis has considered the outcomes of PC screening and risk prediction. It is clear that PSA screening reduces PC-specific mortality and that PSA is a strong predictor of future PC diagnosis. However, overdiagnosis is a major problem that needs to be tackled by improving screening strategies, such as better risk stratification and individualized protocols. A "one-size-fits-all" solution is not optimal. For example, a 53-year-old man with a PSA of 2.8 ng/mL is at much higher risk of an aggressive PC than a man who is of the same age but has a PSA level of 1.1 ng/mL. The 53-year-old man with a PSA of 2.8 ng/mL is among the top 10% at that age, and it has been shown that such a PSA value corresponds to a 10-fold higher risk of later aggressive PC (203). Despite that knowledge, most urologists (and guidelines) would give the same advice to both these men: DRE and a new PSA in 2–4 years.

Screening is **no longer a question of "yes or no"**. Many men are already having PSA tests on a regular basis, albeit on their own initiative and in a suboptimal age rang (i.e., 75–85 years). The question is whether testing should be "organized or unorganized", which is presently a matter of political concern. From the perspective of quality assurance, it would be possible to improve outcome and collect information by systematizing PSA testing in voluntary nationwide programmes. Some counties in Sweden are now investigating whether this can actually be achieved, and, if so, how it can be done. A project of this type can address specific research objectives, including several essential questions that need to be considered and further analysed:

- When should screening start and stop?
- Should screening intervals be fixed or based on risk-stratification?
- What PSA cut-off is the optimal, and should the cut-off be fixed or based on values related to age?
- Should complementary biomarkers be applied, and, if so, what test should be used?
- Should complementary imaging (MRI) be used, if so, should it be done before or after other additional tests?
- How should men with benign biopsies be monitored?
- How should invitations and information regarding screening be designed, and is there a better way to reach non-attenders?

Some of these issues have been thoroughly penetrated by other researchers. Colleagues at the Memorial Sloan Kettering Cancer Center in New York, have addressed some of these questions and have developed recommendations on PC screening (184), that are worth considering. Our view is that men in Sweden should be offered information on "organized PC testing", which can involve more than just the PSA test. They should therefore be given the option to participate in organized programmes, where further assessments of PSA, additional biomarkers, and imaging techniques can be made. Such programmes would have to be coordinated within and between the different counties. Some Swedish hospitals have recently begun performing sequential diagnostic procedures (264), and it will be very interesting to see what the future holds in this context.

Future considerations

A nationwide population-based screening programme for PC will increase the number of men being diagnosed and treated, which in turn will require well-established **organizations and qualified personnel**. It is therefore of the outermost importance to have a meticulous plan for how a screening programme should be arranged and financed. It is essential that hospitals are not burdened with an impossible mission in which the expectations are high and patients with other diseases are caught in the middle. It is also of **ethical concern** to ensure that men who are invited to and diagnosed within a screening programme are offered consultations and treatments within the time periods stipulated in standardized care processes. Despite huge efforts in recent years, the waiting times for cancer patients in Sweden are still much too long, 117–280 days for PC (265).

In summary, the main obstacle in PC screening is the large risk of overdiagnosis, related to PSA testing and systematic biopsies. To decrease the harm-benefit ratio, we need to focus on developing the screening algorithm of tomorrow. Several promising strategies are currently being evaluated:

- Better and more selective biomarkers such as the 4Kscore, the STHLM3 model and the PHI test.
- Use of imaging to optimize the biopsy procedure (in selected men).
- Tissue markers, to select men who have "high-risk features" and hence not suitable for surveillance.
- Optimization of the age span for testing, to enable a more individualized approach.

Two large research projects are ongoing in Sweden: the STHLM3 MRI project (266) and the Göteborg-2 trial.

The Göteborg-2 trial (G2)

The G2 trial was initiated in 2015, based on the findings from a pilot study (236). The aim of this trial is to investigate whether mp-MRI can improve PC screening creating a better balance between harms and benefits, primarily by reducing the rate of overdiagnosis. In this RCT, 60,000 men in the Western Region (Västra Götalands Regionen) are randomized in a 2:1 ratio to a screening- or a control group. The 20,000 men in the screening group are offered a PSA test and are subsequently randomized to one of the study arms (designated I–III):

I. The reference group, whit the PSA cut-off level set at 3 ng/mL. Men with values above this threshold are invited to undergo mpMRI followed by a 10-core TRUS guided biopsy. For those who have a suspicious lesion on MRI, four additional cores, targeted to that area are to be taken.
II. Arm II is the first experimental arm, whit the PSA cut-off level set at 3 ng/mL. Men with elevated PSA are invited to mpMRI, but only have targeted (no systematic) biopsies, thus men with no lesion on MRI will not be biopsied.
III. Arm III is the other experimental arm, whit the PSA cut-off level set at 1.8 ng/mL. Otherwise uses the same schema as in the experimental arm II.

The pilot study was published in 2016 and showed promising results with the approach in arm III (236). The endpoint in the G2 trial is the rate of GS $\geq$ 7 cancers detected in various arms. Results from the first round are expected to be published in 2020.

Before introduction of a national screening program for PC, further evaluation of men's experience from screening would be valuable. We need to assess how men interpret recommendations given in a screening setting. Information and individualized decision making are central. It is therefore more appropriate to strive to reach non-informed men rather than to reach non-attenders, since not attending may be a rational choice after information.

7 CONCLUSIONS

Voiding symptoms are generally not a sign of PC in men with elevated PSA.

The initial PSA level can be used to predict men's long-term risk of PC.

The free-to-total PSA ratio has no additive value to PSA and should not be used as a reflex test in men with a PSA level below 3 ng/mL.

Men die despite screening, which shows that the current screening algorithm has pitfalls.

Screening saves lives by reducing PC-specific mortality by ~30%.

The outcomes of PC screening can be improved by starting PSA screening before the age of 60 years, improving attendance rates, and offering men with long life expectancy additional testing after the age of 70.

Men who participate in a screening programme and are free of PC at the time they terminate screening due to the stipulated stop age, are still at risk of high-grade disease and PC death.

PC deaths can be prevented by offering healthy men additional testing after the age of 70 years.

SVENSK SAMMANFATTNING

Den vanligaste cancerformen i Sverige är prostatacancer (PC). Sjukdomen drabbar främst äldre män. Mer än 70 % av de ca 10 500 män, som årligen diagnosticeras är över 65 år. Denna cancerform är vanligen långsamt växande och har en lång och symptomfri fas, då sjukdomen (om den upptäcks) kan behandlas och botas. Genom tidig upptäckt kan man undvika spridning till andra delar av kroppen. Män som får symptom av sin tumör har ofta en långt gången och icke botbar sjukdom. Sedan mitten på 90-talet kan män, genom ett enkelt blodprov, testa sig med så kallat prostataspecifikt antigen (PSA). PSA-provtagning syftar till att upptäcka PC. Den största nackdelen med PSA-testning är att många av de män som diagnosticeras aldrig skulle ha utvecklat en farlig PC. Detta kan medföra oro, onödiga behandlingar och behandlingsrelaterade biverkningar, som till exempel nedsatt sexualfunktion, urinläckage och tarmbesvär.

Under våren 2018 bedömde Socialstyrelsen att fördelarna med allmän PSA-provtagning (screening) inte uppväger nackdelarna. Denna rekommendation startade en omfattande debatt i Sverige, och många män väljer ändå att testa sig. Dessvärre leder denna typ av okontrollerad PSA-provtagning till att många män testar sig i fel ålder, ofta med bristande information och fel uppföljning. På vilket sätt ett framtida screeningprogram bör utformas är inte fastställt, och det pågår även en världsomspännande diskussion beträffande huruvida PSA-screening verkligen minskar dödligheten i PC.

Denna avhandling fokuserar på utfall och prediktion inom en screeningstudie, med syfte att öka förståelsen för PSA-screening. Delarbetena i avhandlingen härrör från en stor randomiserad, populationsbaserad screeningstudie, som startades 1995 i Göteborg, med 10 000 män i respektive grupp (en screening-respektive en kontrollgrupp). De screenade männen har erbjudits PSA-prov vartannat år och prostatabiopsi (cellprov) om värdet varit > 3.0 ng/ml. Männen som lottades till kontrollgruppen har inte fått någon inbjudan till PSA-provtagning, men de har haft tillgång till PSA-testning utanför studien (på eget initiativ). Under våren 2014 avslutades den 10:e och sista screeningomgången. Göteborgsstudien är unik på många sätt och har idag den längsta uppföljningstiden av alla screeningstudier i världen (22 år).

I **arbete I** har vi undersökt om män med förhöjt PSA och vattenkastnings-besvär hade större risk för PC än män utan symptom. I studien användes ett frågeformulär avseende vattenkastningssvårigheter. Männen genomgick

ultraljud och cellprovtagning i samband med läkarbesöket. Totalt analyserades svar från 2 353 män som genomgick undersökning i samband med sin första screeningomgång. Logistisk regression med variablerna: ålder, kvot PSA, prostatavolym och symptom användes med utfallsmåttet PC. Vi fann att ålder (odds ratio (OR) = 1.1), prostatavolym (OR 0.96), kvot PSA (OR 0.97), PSA (OR = 1.09) och vattenkastningssymptom (OR = 0.78) var statistiskt signifikant relaterade till risken för PC hos män med PSA över 3.0 ng/ml. Ett OR på 0.78 talar för att män med symptom har lägre sannolikhet för PC än män utan symptom. Från studien kan man dra slutsatsen att vattenkastningsbesvär vanligen inte är tecken på PC hos män med förhöjt PSA. Män med dessa besvär utgör inte en högriskgrupp och bör således utvärderas på samma sätt som övriga män med PSA över 3.0 ng/ml.

I **arbete II** har vi utvärderat hur den initiala PSA-nivån påverkar senare cancerutfall och död i PC. Analysen utgick från de 5 174 män som hade PSA < 3 ng/ml vid första screeningomgången. För att utvärdera det prediktiva värdet (möjligheten att förutsäga PC) av ålder, PSA och kvot-PSA för framtida insjuknande i PC användes Harrell's c-index baserat på Cox regression. Vi fann att ålder och PSA, men ej kvot-PSA var associerat med risken för framtida PC. Män med PSA < 1.0 ng/mL hade efter 19 års uppföljning en kumulativ PC risk på 8 % medan män med PSA i intervallet 2 till 2.99 ng/ml hade 40 % risk för PC. Risken för död i PC ökade från 0.3 % till 1.5 % beroende på männens ursprungliga PSA-nivå. Totalt dog 28 män av sin cancer (trots att de deltog och hade "normala PSA-värden" vid screeningens början). Slutsatsen blev att det initiala PSA-värdet var starkt kopplat till framtida utfall. Dessvärre tillförde inte ett tilläggsprov med så kallat kvot-PSA (kvoten mellan fritt och totalt PSA) något till PSA och är således inget användbart tilläggstest vid PSA under 3.0 ng/ml.

I **arbete III** analyserades effekten av organiserad PSA-screening med lång uppföljning (22 år). Studien analyserade även vilka subgrupper av män som trots screening hade en ökad risk för död i PC. Vi jämförde screening- och kontrollgruppen med en fiktiv jämförelsegrupp, där insjuknande och död i PC baserade sig på data från 1990–1994, då PSA-provtagning i Sverige var mycket sällsynt. På så sätt kunde vi uppskatta hur dödligheten skulle ha sett ut om ingen PSA-provtagning fanns att tillgå. Männen i kontrollgruppen har inte inbjudits till PSA-screening i studien, men många har testat sig på eget initiativ (utanför screeningstudien). Resultaten visade på att organiserad PSA-screening minskar PC dödligheten med ca 30 % och med nästan 50 % om man jämför med den fiktiva gruppen (baserat på att ingen PSA testning skett). För att förhindra en man från att dö av PC behövde vi bjuda in 217 män och diagnosticera 9. De män som hade högst risk för att dö av PC var de

män där screening initierades efter 60 års ålder, de som bjöds in men inte deltog samt de män med förmodad lång överlevnad, där screeningen avslutades för tidigt. För att ytterligare minska dödligheten behöver åtgärder fokuseras på att optimera start- och stoppålder samt minska antalet icke-deltagare.

Studien som rapporteras i **arbete IV** undersökte om en övre åldersgräns på 70 år (för ett screeningprogram) är tillräckligt hög eller om vissa grupper skulle vara betjänta av screening även i högre åldrar. Analysen inkluderar 4 464 män som deltog vid sitt sista screeningtillfälle. De var då mellan 67–71 år och hade ej diagnosticerats med PC under screeningperioden. Männen följdes upp i ca 9 år efter att de avslutad screening. Genom så kallad Cox regressionsanalys och Harell's c-index kunde olika faktorer (prediktiva variabler) med eventuell inverkan på framtida utfall utvärderas. Totalt hittades 186 PC-fall (varav 65 % var höggradiga) och 21 män dog av PC under uppföljningstiden. Män som avslutade studien med ett godartat cellprov och PSA 3–10 ng/ml hade en lägre risk för aggressiv PC, jämfört med de män som ej lämnat cellprov och hade PSA 2–3 ng/ml. PSA-nivån vid sista screeningtillfället var annars en viktig faktor och majoriteten av männen som dog hade ett PSA > 1.5 ng/ml vid sista provtagning i screeningstudien. Genom att fortsätta screena ca 40 % av männen (de med PSA > 1.5 ng/ml) även efter 70 års ålder, skulle vi kunna hitta 80 % av de som utvecklar farlig PC och dör av sin sjukdom.

Slutsatser

Män med ett förhöjt PSA och vattenkastningsbesvär hade en lägre risk för PC jämfört med män utan symptom.

PSA-värdet vid första screening tillfället har stor betydelse för framtida cancerutfall, då PSA-nivån ligger under åtgärdsgränsen då mannen börjar screena sig i studien. Att en del män dör av PC trots att de deltar aktivt i programmet, talar för brister med nuvarande screeningupplägg. Kvot-PSA är inget användbart tilläggstest i PSA-nivåer under 3.0 ng/ml.

Organiserad PSA-screening minskar risken att dö av PC (med ca 30 %) jämfört med de män som inte bjuds in. De som har störst risk för PC död är: män som börjar screena sig för sent (efter 60 års ålder), de som bjuds in men inte deltar och de män som har lång förväntad överlevnad och slutar PSA-screening för tidigt (vid ca 70 års ålder).

Selekterade män bör erbjudas screening även efter 70 års ålder.

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