

Thesis for doctoral degree (Ph.D.)
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Technology
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PROSTATE CANCER – ASPECTS OF SCREENING AND PROGNOSTIC FACTORS

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*“Call me morbid, call me pale
I’ve spent six years on your trail
Six long years
On your trail”*

(Morrissey, Marr)

To my family

ABSTRACT

Prostate cancer is the most common malignancy among Swedish men. Each year approx. 2 500 men die from the disease constituting almost 1/5 of all cancer death among men. Screening for prostate cancer has not been recommended in Sweden but PSA-testing is despite that commonly used. With high diagnostic activity more men are diagnosed with early stage prostate cancer, increasing the risk of over treatment. There is a strong need for better prognostic markers of prostate cancer. The aim of this thesis was to evaluate an early prostate cancer screening study and to assess different possible prognostic factors of prostate cancer.

In 1988, all men aged between 55-70 years living in the catchment area of Stockholm South Hospital were identified (26 602). 2 400 of them were randomly selected and invited to participate in a single intervention prostate cancer screening study. 1 782 men accepted, and were examined with DRE, TRUS and PSA analysis. 65 cases of prostate cancer were detected. **Study I** is based on a 15-year follow up of the screening study. There was no significant difference in the risk of dying from prostate cancer between the source population and the invited group. The risk of dying from other diseases was however significantly increased among the non-attendees, with an IRR of 1.89 (95% CI 1.65-2.16), compared to the attendees.

In **study II** we evaluated the pre-diagnostic serum values of testosterone, dihydrotestosterone and SHBG influence on prostate cancer survival. Of the 65 men with screening detected prostate cancer, 41 died during follow up. 17 died of prostate cancer. Having a DHT value above median were associated with lower risk of dying from prostate cancer with a HR of = 0.24 (95% CI 0.08-0.75). We speculate that high DHT values lead to increased stimulation of the ER β -receptor which has antiproliferative properties.

Study III assesses suPAR as a prognostic marker of prostate cancer and longevity. We measured the serum level of two different forms of suPAR among 375 men of the screening cohort. Among these we included 63 of the screening detected prostate cancer cases. Neither suPAR (I-III) nor suPAR (II-III) were associated with prostate cancer mortality after adjustment for other prognostic factors. Both suPAR-forms were however associated with decreased overall survival with HR 2.26 (95% CI 1.17-4.35) for one unit increase of suPAR (I-III). The increase of overall survival was especially due to an increase in the risk of dying from cardiovascular disease. One unit increase of suPAR (I-III) had a HR of 6.44 (95% CI 2.16-19.18).

Study IV Since low DHT level was associated with higher risk of prostate cancer death in study II, we wanted to explore if treatment with drugs lowering the DHT level (5- α -reductase inhibitors) would influence the risk of dying from PC. We used a prescription database to identify men prescribed 5- α -reductase inhibitors before diagnosis of prostate cancer. To compensate for lead time bias we also included men prescribed α -adrenoceptor antagonist for comparison. Treatment with 5- α -reductase inhibitors did not increase the risk of prostate cancer death (HR 0.94 (95% CI 0.77-1.16)). However, treatment with α -adrenoceptor antagonist did significantly reduce the risk with HR 0.82 (95% CI 0.70-0.96). We also analyzed the risk of being diagnosed with metastasized prostate cancer with DDD of medicine as exposure variable. The OR for having metastasized disease at diagnosis was 1.14 (95% 1.01-1.29) per 100 DDD of finasteride treatment.

LIST OF PUBLICATIONS

- I. **Kjellman A**, Akre O, Norming U, Törnblom M, Gustafsson O. 15-Year followup of a population based prostate cancer screening study.
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- II. **Kjellman A**, Akre O, Norming U, Törnblom M, Gustafsson O. Dihydrotestosterone levels and survival in screening-detected prostate cancer: a 15-yr follow-up study.
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- III. **Kjellman A**, Akre O, Gustafsson O, Høyer-Hansen G, Lilja H, Norming U, Piironen T, Törnblom M. Soluble urokinase-type plasminogen activator receptor as a prognostic marker in men participating in prostate cancer screening.
Submitted
- IV. **Kjellman A**, Friis S, Granath F, Gustafsson O, Toft Sørensen H, Akre O. Treatment with 5-alpha reductase inhibitors and prostate cancer survival.
Submitted

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LIST OF ABBREVIATIONS

AR	Androgen receptor
ATC	Anatomic therapeutic chemical classification system
BRCA-2	Breast cancer type 2 susceptibility protein
CI	Confidence interval
DDD	Defined daily doses
DHT	Dihydrotestosterone
DRE	Digital rectal examination
ER	Estrogen receptor
ERSPC	European randomised study of screening for prostate cancer
FSH	Follicle stimulating hormone
GPI	Glycosyl phosphatidyl inositol
Gy	Gray
HR	Hazard ratio
ICD	International classification of diseases
IRR	Incidence rate ratio
LH	Lutenizing hormone
LHRH	Lutenizing hormone releasing hormone
LUTS	Lower urinary tract symptoms
Nd-YAG	Neodymium-doped yttrium aluminium garnet
OR	Odds ratio
PA	Plasminogen activator
PAI	Plasminogen activator inhibitor
PCPT	Prostate cancer prevention trial
PLCO	Prostate lung colorectal ovarian screening trial
PPV	Positive predictive value
PSA	Prostate specific antigen
REDUCE	Reduction by dutasteride of prostate cancer events
SELECT	Selenium and vitamin E cancer prevention trial
SHBG	Sexual hormone binding globulin
suPAR	Soluble urokinase-type plasminogen activator receptor
T	Testosterone
TNM	Tumour node metastasis
TR-FIA	Time-resolved fluorescence immunoassay
TRUS	Transrectal ultrasonography
uPA	Urokinase plasminogen activator
uPAR	Urokinase plasminogen activator receptor
3 α -Adiol	5 α -Androstane-3 α ,17 β -diol
3 β -Adiol	5 α -Androstane-3 β ,17 β -diol

1 INTRODUCTION

Epidemiology

Prostate cancer is the most common malignancy among Swedish men. Each year almost 9 000 men are diagnosed¹. The age standardized incidence is shown in Figure 1. The incidence rose sharply from the late 1990's to 2006 but has since then abated. The rise was probably caused by increased PSA-testing and an increase of the mean age of men.

The median age at diagnosis is 69 years. The prevalence of prostate cancer is high in Sweden with 70 459 men living with prostate cancer (31/12 2008)².

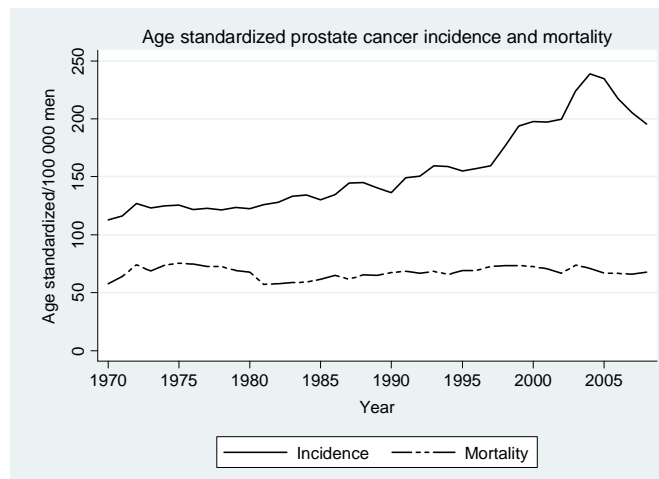


Figure 1: The trends in prostate cancer incidence and mortality in Sweden 1970-2007. Adapted from the National board of health and welfare

In 2007, 2 470 men died from prostate cancer constituting 21.5 % of all cancer deaths among men³. Prostate cancer in its terminal state causes a lot of suffering to the patient. Since other causes of mortality have decreased, especially mortality from cardiovascular disease, the relative importance of prostate cancer mortality is increasing steadily (Figure 2)^{3 4}.

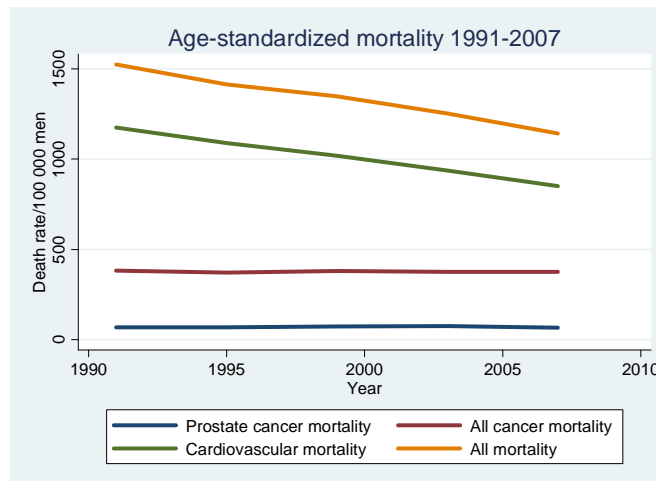


Figure 2: Age standardized mortality per 100 000 men by cause of death 1991-2007. Adapted from the National board of Health and Welfare and NORDCAN

Aetiology

There are only three known risk factors of prostate cancer: age, heredity and ethnic origin. The incidence rises sharply from the sixties and onwards. Autopsy studies have however shown an age dependent risk of finding microscopic foci of what is considered to be prostate cancer, from the age of 30 and up^{5,6}. Frequent PSA-testing leading to prostate biopsies have lowered the mean age at diagnosis.

There is a heredity form of prostate cancer constituting approximately 5 % of the cases⁷. The exact genetic location has not yet been identified and no genetic test is available. Hereditary prostate cancer is instead diagnosed on the pedigree with 3 relatives in different generations with the disease or 3 first degree relatives or early onset (before 55 years of age) among two relatives. The risk of dying from the disease increases if the relatives have been young at diagnosis⁸. It has also been shown that men in families with the heredity form of breast cancer with BRCA2- mutation have an increased risk for early onset of prostate cancer^{9,10}.

There is large variation of the incidence of prostate cancer in the world. The highest incidence is found among afro-American and the lowest among Chinese men¹¹. The difference is about 40-fold. The wide variation in screening activity and the completeness of cancer registries explains some of this disparity. Several attempts have

been made to identify risk factors but it is probably a complex interplay of genetic and lifestyle factors explaining the difference¹²⁻¹⁵.

Several other risk factors of prostate cancer have been investigated. Overweight and obesity have been proposed as risk factors but no strong association has been shown¹⁶⁻¹⁸. High dietary fat intake has been studied and there might be a weak connection to an increased prostate cancer risk¹⁹. Smoking which is a strong risk factor for many other cancers has not been established as a risk factor²⁰.

Hormones

Testosterone is essential for the normal development of the prostate both during foetal life and during puberty^{21 22}. The testosterone is also necessary for normal function of the prostate during adulthood. The Leydig cells of the testes produces 90% of the circulating testosterone, the rest is produced in the adrenal cortex. The testes are under influence of LH (luteinising hormone) released from the pituitary which in turn is stimulated by LHRH (luteinising hormone releasing hormone) from the hypothalamus. A negative feedback mechanism regulates the production of testosterone through the gonadal-hypothalamic-pituitary axis (Figure 3).

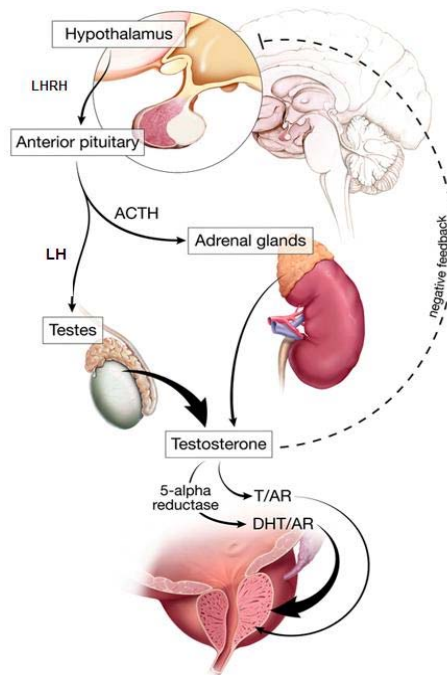


Figure 3: The gonadal-hypothalamic-pituitary axis.
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September 1, 2007, with kind permission of the publisher

Testosterone is converted in the prostate to the more potent androgen dihydrotestosterone (DHT) by the enzyme 5 α -reductase. DHT bind to the androgen receptor and the activated receptor stimulates cellular growth²³. The metabolites of testosterone and DHT can bind to other receptors causing different effects but the complete intraprostatic androgen metabolism is not known.

The importance of testosterone for prostate cancer was elegantly shown by Huggins and Hodges in 1941²⁴. They surgically castrated men with metastasised prostate cancer and were able to demonstrate pain reduction and shrinkage of metastases. It is also known that men lacking the gene for the enzyme 5 α -reductase do not develop prostate cancer²⁵.

Even if testosterone is necessary for a normal function of the prostate and for the development of prostate cancer, no direct link between the actual serum value and the risk of prostate cancer has been established²⁶.

Diagnosis

PSA

Prostate specific antigen (PSA) is a glycoprotein produced in the glandular epithelium of the prostate. The known function is to liquefy the semen after ejaculation. Blood level of PSA has been shown to correlate to the risk of prostate cancer but it is not by any means prostate cancer specific²⁷. The level can rise from various reasons i.e. benign prostatic hyperplasia, urinary tract infection and prostatitis. The test has been commercially available for 20 years. In Sweden an arbitrary upper limit of 4 ng/mL has been adapted for diagnostic purposes. For men 50-70 years old with a PSA > 4ng/ml the risk of having prostate cancer is 25%. If the cut off level is set to 3 ng/ml the risk is still as high as 20%²⁸⁻³⁰. Except for zero values there is no PSA-level low enough to exclude prostate cancer. Demonstrated in a prevention trial, even with PSA < 0.5 ng/ml, 7% were shown to harbour prostate cancer³¹.

Since benign prostatic hyperplasia which increases the PSA-level is more common among older men the PSA-level should be correlated to age³². It is also possible to assess the PSA ratio. This is the ratio between the free PSA and the total PSA. Benign prostatic hyperplasia leads to more free PSA in the blood and a high ratio decreases the risk of having prostate cancer. The free total PSA-ratio can be used to increase the specificity of PSA for a man with a moderately increased PSA value (3-10 ng/ml)³³⁻³⁵.

Digital rectal examination

Digital rectal examination is the classic way to evaluate the prostate. It is easily performed, inexpensive but subjective. However, it is not possible to palpate small cancer foci. For screening purposes the positive predictive value (PPV) could at best be 30% for patients with PSA 3-9.9 ng/mL^{36 37}. It is also used to assess the clinical T-stage for a man with cancer (Table 1). Mostly it is combined with transrectal ultrasonography which improves the PPV.

Transrectal ultrasonography (TRUS)

By using a rectal probe it is possible to get a detailed imaging of the prostate and its structure. It can be used to calculate its size, look for suspiciously malignant areas (hypoechoic), and evaluate the adjacent structures as the seminal vesicles and bladder. Unfortunately the findings of possibly malignant areas are rather unspecific and a great part of malignancies of the prostate can not be visualized with ultrasonography^{38,39}. TRUS is of immense use when biopsies are taken. It has been shown, not surprisingly, that the more biopsies you take the more cancer you will find. In the beginning of the ultrasound era 4, later 6 biopsies were taken⁴⁰. Now usually 8-12 cores are sampled with increased detection rate⁴¹. Using an injection of local anaesthetic this can be done without causing much discomfort for the patient⁴².

Pathology

95 % of the malignant tumours in the prostate are adenocarcinomas. Fine needle aspiration cytology was earlier the main diagnostic procedure for prostate cancer. For cytology the WHO grading system is used, grading the tumour into 3 grades (G1-3) of well, moderately or poorly differentiated type⁴³. With the introduction of ultrasonography and core biopsies the grading of the histology is nowadays mostly done by the Gleason system giving a grade from 1 to 5 where 1 is the more differentiated form and 5 the more malignant form of cancer⁴⁴. The Gleason score summarises the most prominent grade as the first number and the worst grade as the second⁴⁵. The score ranges from 2 to 10. Immunohistochemistry can be used to further help with diagnostic difficulties.

Evaluation before treatment

The TNM system is used to describe the tumour⁴⁶(Table 1). The T-category is decided by DRE, where the distinction between intracapsular (T1-T2) and extracapsular (T3-T4) disease has most influence on treatment decision. Assessment of the N-stage is only performed when the findings will directly influence the treatment decision, i.e. before radiotherapy. The regional lymph nodes are surgically removed and evaluated. The risk of lymph node metastases can be predicted by using Partin tables⁴⁷. The lymph nodes can also be removed during radical prostatectomy. Even if lymph node metastases are associated with worse prognosis a large proportion of these men is disease free after 10 years of follow up⁴⁸. Whether or not this is due to the excision of the nodes remains unknown^{49,50}.

Prostate cancer metastasises mainly to the skeleton. This is evaluated with a radionuclide bone scan. The recommendation is to perform this investigation if PSA \geq 20, the Gleason score \geq 8 or the T-stage \geq T3⁵¹. If metastases are found treatment with curative intent is not to be considered. Besides the TNM-evaluation, the Gleason score of the tumour and the PSA-value are important predictors for risk classification.

Table 1. Tumour Node Metastasis (TNM) classification of prostate cancer. (UICC 2009)

T	Primary tumour
TX	Primary tumour cannot be assessed
T0	No evidence of primary tumour
T1	Clinically unapparent tumour not palpable or visible by imaging
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 PSA level)
T2	Tumour confined within the prostate
T2a	Tumour involves one lobe
T2b	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: bladder neck, 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 metastasis
M0	No distant metastasis
M1	Distant metastasis

Treatment

For patients with localised disease with a life expectancy of more than 10 years, surgery or radiotherapy should be offered^{52,53}. Surgery has in a randomised trial been shown to reduce the prostate cancer specific risk of death with 5 % compared to watchful waiting^{54,55}. There are no randomised trials evaluating radiotherapy but most studies suggest an effect similar to surgery⁵⁶. Treatment of prostate cancer can have serious side effects and PSA-testing is leading to diagnosis earlier in the disease process. Therefore active surveillance has been introduced. By following the patients closely with repeated PSA testing and re-biopsies, progression of the cancer can be recognised and treatment deferred to this point⁵⁷. In this way a proportion of men will avoid the side effects of treatment because they will die from other causes before cancer progression.

Surgery

Radical prostatectomy can be performed as open surgery or laparoscopically⁵⁸, with or without robotic assistance. The prostate and often seminal vesicles are removed and the urethra re-anastomosed to the bladder neck. The overall progression free survival for men with T1-T2 tumours is 75% at 10 years postoperative follow up⁵⁹. The result for patients with Gleason score ≤ 6 , is a 15 year PSA free-survival of almost 99%⁶⁰. The two main complications of the procedure are erectile dysfunction and incontinence. The dissection can in selected cases be done with nerve-sparing technique which gives better chances of keeping potency and avoiding incontinence^{61 62}. Approximately 1-2 % of patients suffer from severe incontinence, but more common is mild stress incontinence (10-20 %). 25-75 % of patients will have erectile dysfunction⁶³.

Radiotherapy

Radiotherapy can be given as external beam radiotherapy, brachytherapy or a combination of both. There has been a rapid development of treatment modalities in recent years. External radiotherapy is delivered with 3-dimensional technique following the contour of the prostate which makes it possible to give sufficiently high doses (78 Gy), without increasing side effects⁶⁴. Brachytherapy is given by implantation of radioactive seeds in the prostate or by radio nuclides distributed through implant needles⁶⁵. The side effects of radiation can be divided in the acute and late side effects. Acute side effects include radiation induced cystitis and proctitis causing increased urinary frequency, dysuria and diarrhoea. Late side effects are chronic cystitis, rectal bleeding/secretion and erectile dysfunction^{66 67}.

Endocrine treatment

Most prostate tumours are dependent of androgens. The available endocrine treatments work by changing the hormonal milieu in the body. The aim is to lower the testosterone level in the circulation or block its effect. This can be accomplished by surgical castration, by stopping the gonadotropin release from the pituitary with LHRH agonists or LHRH blockers, by blocking the androgen receptor with antiandrogens or by giving parenteral oestrogen.

The main use of endocrine treatment is for palliative treatment of patients with metastasised disease. It has been shown to reduce the symptoms. It is not clear if giving medication before development of symptoms changes the effect on total mortality in a substantial way^{68 69}. Treatment has been shown to prolong survival if given to patients with node-positive disease after radical prostatectomy⁷⁰. It is also effective for patients undergoing radiotherapy for locally advanced disease⁷¹. The side effects of endocrine treatment are loss of libido, impotence, vasomotor (hot flushes, sweating) and in the long run osteoporosis, anaemia and cardiovascular morbidity.

Unfortunately the androgen sensitive cancer eventually develops hormone-refractory properties and the endocrine treatment loses its effect. In this case changing between different endocrine treatments can have some effect⁷². Novel chemotherapeutic agents such as doxorubicin has also shown efficacy⁷³. Hormone therapy is used before and sometimes after radiotherapy.

Androgens

The testosterone derivative 5 α -dihydrotestosterone (DHT) influence the male reproductive system both during embryogenesis and after birth^{22 74}. Testosterone is the major circulating androgen with a serum T/DHT-ratio of 10:1⁷⁵. Testosterone is converted to DHT by the enzyme 5 α -reductase which is present in 2 isoforms, type 1 mainly situated in the liver and the non-genital skin and type 2 mainly in the urogenital tract. The intraprostatic DHT/T ratio is 6:1⁷⁶.

Both T and DHT bind to the intracellular androgen receptor (AR) forming an AR-ligand complex regulating transcription of androgen-regulated genes in the DNA. DHT has a higher affinity to the AR than T^{77 78}. DHT is metabolised to androsterone, 5 α -androsterane-3 α 17 β -diol (3 α Adiol) or 5 α -androsterane-3 β 17 β -diol (3 β Adiol)⁷⁹. 3 β Adiol may have anti-androgenic activity via the estrogen receptor β ⁸⁰ (Figure 4).

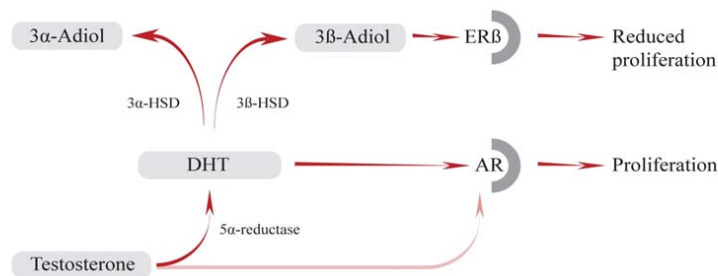


Figure 4: Schematic drawing of the intraprostatic androgen pathway. Testosterone is in the prostate rapidly converted to dihydrotestosterone. DHT forms a ligand complex with the androgen receptor (AR) stimulating transcription of androgen-regulated genes. DHT is further metabolised and the metabolites have both androgenic as anti-androgenic activity

There are two different estrogen receptors in the prostate α and β . Estrogen receptor α (ER α) is thought to be up regulated in prostate cancer. Treatment with toremifene, an ER α antagonist has been shown to reduce the risk of prostate cancer diagnosis⁸¹. Estrogen receptor β (ER β) is thought to have pro-differentiative and anti-proliferative properties in the prostate, counteracting the stimulatory effect of the androgen receptor^{82 83}. Most studies suggest a down regulation of ER β in cancer tissues compared to normal tissue but there are conflicting data⁸³. Further studies are needed to establish the role of estrogen receptors in prostate cancer development.

Even if surgical or medical castration is the first line treatment in patients with metastatic prostate cancer no strong correlation between the serum level of sex hormones and the risk of prostate cancer has been established²⁶. One reason for this

could be the relatively poor correlation between intraprostatic hormone concentrations and serum levels⁸⁴⁻⁸⁷. Another reason could be that the androgen metabolism of the prostate is insufficiently known.

Primary prevention

The strong risk factors as age, heredity and ethnicity are well known. Prostate cancer is basically an ideal candidate for exogenous preventive measures, such as dietary and pharmacological prevention, due to some specific features: high prevalence, long latency, endocrine dependency, availability of serum markers (PSA) and histological precursor lesions (prostatic intraepithelial neoplasia). Much effort has been put in to finding environmental factors explaining why some men get the disease but not others. If these factors can be identified an effective primary prevention plan can be established with elimination of carcinogenic factors and suggestions of substances with a preventive effect (chemoprevention).

Several substances have been suggested to have a preventive effect as selenium, vitamin E, vitamin D, lycopens, phytoestrogens and 5 α -reductase inhibitors.

The **SELECT** (the Selenium and Vitamin E Cancer Prevention Trial) was initiated since both treatment with selenium and Vitamin E had been shown to reduce the risk of prostate cancer diagnosis compared to placebo⁸⁸⁻⁸⁹. SELECT was the largest ever prostate cancer prevention trial. It included more than 32 000 men, randomised to selenium, vitamin E, combination treatment or treatment with placebo⁹⁰. The SELECT study was stopped ahead of time since 5 years treatment did not show any effect on the risk of prostate cancer, on the contrary men on Vitamin E actually had a somewhat increased risk of prostate cancer⁹¹.

Lycopens which are abundant in tomatoes have strong anti-oxidative effect. A meta-analysis of several studies showed a slightly lower relative risk for developing prostate cancer in men with the highest intake of tomatoes, compared to men with the lowest intake. The risk reduction was significant for cooked tomatoes but not for raw⁹².

There are several kinds of different phytoestrogens as isoflavonoids, flavonoids and lignans. Soya bean products are a rich source of isoflavonoids and some studies in south east Asia have shown a correlation between high intake of Soya bean and reduced prostate cancer risk. Other studies have failed to show such a correlation⁹³. It has been shown that the effect of phytoestrogens could be dependent on the genes of the subject since high intake of phytoestrogens substantially reduces prostate cancer risk among men with specific polymorphic variation in the promoter region of the estrogen receptor-beta gene⁹⁴.

5 α -reductase inhibitors as finasteride and dutasteride function by inhibiting the enzyme converting testosterone to the more active metabolite dihydrotestosterone (DHT). Low DHT means less stimulation of the androgen receptor. Long time use leads to shrinkage of the prostate by approximately 30%. The main indication for treatment is benign prostatic hyperplasia where relief of symptoms and better urinary flow have been shown in randomised trials⁹⁵. Treatment also lowers PSA by 50% and this should be

kept in mind when evaluating the PSA value for a man on 5 α -reductase inhibitor medication.

In the Prostate Cancer Prevention Trial (**PCPT**), the preventive effect of treatment with finasteride was investigated³¹. 18 882 men were randomised to placebo or finasteride treatment for seven years. The men were 55 years of age or older, had a normal digital rectal examination and a PSA \leq 3.0 ng/mL. They had annual check ups and if the PSA exceeded 4.0 ng/mL or if the DRE was abnormal biopsies were recommended. If they had not had biopsies taken earlier all men were offered biopsies at the end of the study. 9 060 men had biopsies taken at some point during the 7 year period and were included in the final analysis. The main result was a reduction of prostate cancer detection. Prostate cancer was found in 18.4% of men on finasteride and 24.4% of men on placebo. If only looking at the men having biopsies before the end of study the difference was smaller (26.5% vs. 29.5%) and not statistically significant. The life time risk of being diagnosed with prostate cancer is 17%. This study found prostate cancer in 24.4% of men on placebo during 7 years. This raises the question of over diagnosis. What kind of cancers were diagnosed and would they ever have been diagnosed if not biopsies had been taken? Paradoxically 37% of cancers diagnosed in the finasteride arm had a Gleason score of 7-10 compared to 22.2% in the placebo arm. This implies that treatment with finasteride actually could increase the risk of being diagnosed with high grade, potentially more malignant prostate cancer. These findings have caused hesitation in the urological community to implement finasteride as chemoprevention. Much effort has been put in to trying to explain the difference in high grade cancer incidence. It has been attributed to several potential biases. First treatment with finasteride shrinks the prostate and when adjusting for the prostate volume the risk of high-grade diagnosis was the same⁹⁶. Secondly finasteride is known to alter the histological appearance of the prostate and could bias the histological evaluation⁹⁷⁻⁹⁹. Thirdly the PSA sensitivity to detect prostate cancer was increased in the finasteride arm^{100 101}. The study has been re-analysed with different statistical methodology but these results require cautious interpretation^{102 103}. The landmark finding in the PCPT is the knowledge that prostate cancer can be found even in men with low PSA-levels, actually at any PSA level.

Dutasteride is a 5 α - reductase inhibitor which inhibits both isoenzymes of 5 α -reductase, type I and type II. In an ongoing randomised trial called **REDUCE** a total of 8 229 men have been randomized to receive dutasteride or placebo for 4 years¹⁰⁴. Eligible men were 50 to 75 years old, had a serum prostate specific antigen of 2.5 to 10 ng/mL (men aged 50-60) 3.5-10ng/mL (>60 years), and had a negative 6 to 12 core biopsy within 6 months prior to enrolment. Repeated biopsies were taken at 2 and 4 years. The results have not yet been published but preliminary results were presented at 2009 years meeting of the American Urological Association. The results for the primary end point biopsy-detectable prostate cancer at 4 years showed a 23% relative risk reduction for dutasteride compared with placebo ($p < 0.001$). There was no difference between the risks for high-grade (Gleason score 7-10) tumours between the two groups.

In conclusion no effective primary prevention for clinically significant prostate cancer has been established.

Screening

If screening for a disease should be considered some requirements should be met, these were summarized in 10 points by Wilson and Jungner in 1968¹⁰⁵:

Wilson and Jungner classic screening criteria

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

These criteria can be summarized into four main conditions: The disease must be an important health problem, there must be an effective diagnostic test, there should be an effective treatment and all this should be done to an acceptable cost.

Prostate cancer is indeed an important health problem. PSA testing is an inexpensive and easy diagnostic procedure. However the sensitivity and specificity for finding potentially life threatening cancers are not known. The cut-off level of the PSA-level is under much debate since there is no clear cut point when the PPV changes dramatically. Prostate cancer has most often a latent phase and screening with PSA leads to an earlier diagnosis of the disease. This stage migration is evident in countries where PSA-testing has become more common and it increases the risk of over treatment²⁸. Treatment with radical prostatectomy has in a randomized trial been shown to reduce the disease specific mortality⁵⁵. The cost for PSA-testing and the following treatment and follow-up of patients is immense and must be weighed against the benefit in survival.

The first results from two large scale randomized prostate cancer screening trials were presented in 2009:

The European Randomised Study of Screening for Prostate Cancer (**ERSPC**) randomised 72 952 men aged 55-69 years to screening and 89 435 men in the same age group to a control group¹⁰⁶. The men in the screening group had PSA analysed and if PSA>3 ng/mL biopsies were taken. The PSA-testing was repeated every fourth year (some variations between centres). The first results presented were on a median follow-up of nearly 9 years. In the men randomised to screening, 82.2% were screened at least once, and 214 men died of prostate cancer. In the control group 326 men died of prostate cancer. The cumulative incidence of prostate cancer was 4.8% in the control

group and 8.2% in the men allocated to screening. The relative risk of dying from prostate cancer was 0.80 (95% CI 0.68-0.96). To save one man from dying of prostate cancer 1410 men needed to be screened and of these 48 men needed to be treated for prostate cancer.

The Prostate Lung Colorectal Ovarian Cancer Screening Trial (**PLCO**) is an American study on 38 343 men aged 55-74 years randomised to screening and 38 350 men randomised to a control group. The screening procedure was annual PSA testing for 6 years and annual DRE for 4 years. The PSA cut off for biopsies was 4 ng/mL. The compliance to screening was 85% but the control group had a high proportion having PSA-testing outside the study, reaching 52% at six years. After 7 years of follow-up there were 50 deaths from prostate cancer in the screening group compared with 44 in the control group giving a non statistically significant relative risk ratio of 1.13.

The mortality from prostate cancer was much lower in the PLCO compared to ERSPC, probably because PSA-testing and active treatment have been implemented earlier in the US. Many men in the PLCO study were pre-screened and prostate cancer patients got more aggressive treatment during the study period lowering the risk of prostate cancer death even more. The very high contamination rate (screening in the non-screening control group) also diluted any differences between study groups. The ERSPC had less of these problems. Longer follow up in the ERSPC study will probably decrease the numbers needed to screen to save patients from prostate cancer death.

Prognosis

The prognosis after prostate cancer diagnosis is hugely variable. The most important clinical parameters used to assess the patient with regard to prognosis are PSA, Gleason score and tumour stage¹⁰⁷⁻¹¹². By using nomograms the individual patients' risk of dying from prostate cancer following radical treatment can be calculated and management planned accordingly^{113 114}. Besides the PSA-value also the PSA velocity (rate of PSA change over time), the PSA ratio (free PSA/total PSA) and PSA density (PSA/prostate volume) could add some information on prognosis¹¹⁵⁻¹¹⁷. For the pathological evaluation not only the Gleason score but also the number of biopsy cores positive, the total length of cancer in the biopsies and the tertiary grade of Gleason grade in the specimen, can add information¹¹⁸.

Even with all this prognostic information there is still a great need for new prognostic biomarkers to enhance the clinical management and help to distinguish between indolent and aggressive disease. A lot of research is ongoing. Table 2 gives an overview of different new biomarkers for prostate cancer prognosis.

Table 2: Overview of blood and tissue biomarkers for prostate cancer prognosis

Abbreviation	Full name	Measured in	Assessed use
KLK2	Human kallikrein-related peptidase 2	Serum	Prediction of extracapsular extension, tumour volume and biochemical recurrence ^{119 120}
IGFBP-3	Insulin like growth factor binding protein 3	Serum	Inversly correlated to the risk of bone metastasis ¹²¹
IL-6	Interleukin-6	Serum	Increased in metastatic and androgen-independent PC ^{122 123}
EPCA-2	Early prostate cancer antigen-2	Serum	Differentiate local from metastatic PC ¹²⁴
uPA/uPAR	Urokinase plasminogen activator and receptor	Serum	Increased serum levels associated with bone metastasis ¹²⁵
AMACR	α -Methylacyl-CoA racemase	Tissue	Decreased expression associated with biochemical recurrence and PC-death ¹²⁶
EZH2	Enhancer of zeste homolog 2	Tissue	Increased expression marker of PC progression ¹²⁷
CRISP-3	Cysteine-rich secretory protein 3	Tissue	Predictor of PC recurrence after surgery ¹²⁸
E-cadherin	E-cadherin	Tissue	Low production associated with shorter survival ¹²⁹
ANXA3	Annexin A3	Tissue	Decreased level marker of worse PC prognosis ¹³⁰
PSCA	Prostate stem cell antigen	Tissue	High levels correlated to higher stage and risk of metastasis ¹³¹
TGF- β 1	Transforming growth factor β 1	Plasma and tissue	Tissue levels correlated to risk of lymph node metastasis, plasma levels to metastasis and biochemical recurrence ^{132 133}

When advising on therapy it is also important to have information about the patients' general health since surgery or radiation therapy should only be considered if the patients have an expected survival of at least 10 years. Predicting the life expectancy is difficult and clinicians are not always perfectly accurate^{134 135}. Life tables give

prediction of the average remaining life years of a group of individuals and reflect population-specific characteristics. For example, a 75 year old man in Sweden 2008, had a median predicted survival of 10.7 years¹³⁶. Using life tables can help the clinician but their use in prostate cancer patients has been shown to have low accuracy¹³⁷. A clinical morbidity index as the Charlson co morbidity index¹³⁸ is the most useful tool. Its use on prostate cancer patients has been shown to predict the risk of non-prostate cancer related death within 10 years of definitive treatment with 84.3 % accuracy¹³⁹.

Soluble urokinase-type plasminogen activator receptor (suPAR)

The ability for a cancer to spread locally and to metastasise is dependent on increased degradation of extra cellular matrix and of the basement membrane. The urokinase plasminogen activator (uPA) system is believed to play a key role in this process¹⁴⁰. uPA binds to the urokinase plasminogen activator receptor (uPAR) on the cell surface (Figure 5). This leads to cleavage of plasminogen to the active serine protease plasmin. Plasmin degrades extra cellular matrix proteins and activate different other proteolytic enzymes. uPA has also been shown to be associated with non-proteolytic processes as cellular adhesion and chemo taxis¹⁴¹. The action of the activated uPA is balanced by different plasminogen activator inhibitors (PAI1, PAI2).

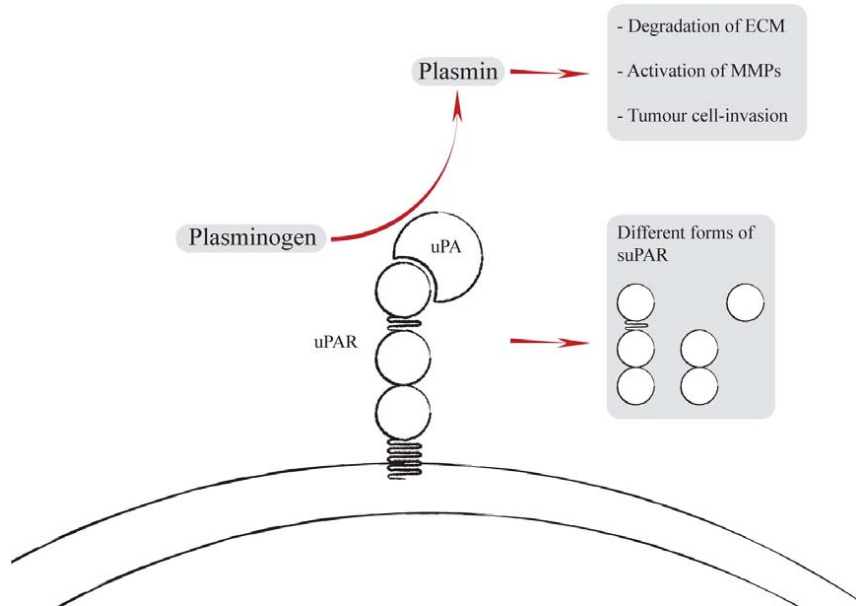


Figure 5: The urokinase plasminogen activator system. The uPAR on the cell surface binds the urokinase plasminogen activator (uPA), leading to conversion of plasminogen to plasmin. The uPAR can be cleaved from the cell surface and found in body liquids as soluble uPAR (suPAR)

The uPAR is a three domain, highly glycosylated protein attached to the cell surface by a glycolipid anchor (GPI)¹⁴². In immunohistochemical studies of prostate cancer, uPAR is found to be expressed in macrophages and neutrophils at the invasive front¹⁴³. The GPI anchored uPAR can be shed from the cell surface and increased levels of soluble uPAR (suPAR) are found in blood from cancer patients. High uPAR levels in both tumour tissue and blood have been shown to correlate with poor prognosis in several types of cancer¹⁴⁴⁻¹⁴⁷. In prostate cancer high levels of suPAR have been found in more aggressive tumours and metastasis¹²⁵. The suPAR level has also been shown to correlate to the risk of finding prostate cancer in biopsies¹⁴⁸.

uPAR can be cleaved in the linker region between domain I and II by uPA, resulting in different molecular variants of suPAR termed suPAR (II-III) and the liberated domain I, suPAR (I)¹⁴⁹. Experiments using transgenic mice with combined over-expression of uPA and uPAR indicate that uPA is the only protease able to cleave uPAR in vivo¹⁵⁰. Thus, the amount of the cleaved suPAR forms could be an indicator of the activity of the PA system, and the cleavage products are strong prognostic markers when measured in blood from patients with non-small lung cancer and ovarian cancer^{144 151}.

SuPAR also reflects the level of activity of the immune system and is involved in several immune regulating mechanisms. Several studies have focused on suPAR levels during infectious diseases and among patients treated in intensive care units¹⁵²⁻¹⁵⁴. SuPAR is also believed to be a marker of low-grade inflammation and could as such be of prognostic value to predict the risk of diabetes, hypertension and cardiovascular disease.

2 THE PRESENT STUDY

The aims of this thesis were

- To evaluate the effect of a randomized single intervention prostate cancer screening study on prostate cancer survival
- To compare overall mortality between attendees and non attendees in this study
- To evaluate the level of DHT in serum at diagnosis as a prognostic factor in prostate cancer
- To assess different forms of suPAR as prognostic factors for prostate cancer survival
- To assess different forms of suPAR as prognostic factors for overall survival
- To evaluate the influence of treatment with 5- α -reductase inhibitors on prostate cancer survival and the risk of developing metastasised disease

3 MATERIAL AND METHODS

Study population

Study I, II and III are all derived from the same cohort of patients. Using the Swedish census records comprising the entire population with current addresses, all men born between 1918 and 1933 living in the catchment area of Stockholm South Hospital were identified in 1988. After excluding men with an earlier prostate cancer diagnosis 26 602 men remained. 2 400 of these were randomly selected and invited to participate in a prostate cancer screening study. Some men were not reachable by mail ($n = 17$) or had previously diagnosed prostate cancer ($n = 21$) not detected before randomisation and were excluded. Of the remaining men 1782 (74%) accepted the invitation, thus 580 (26%) did not attend. The participants were examined with DRE, TRUS and PSA. If they had abnormal findings on DRE and/or TRUS they underwent TRUS guided biopsies. If PSA was more than 7 ng/mL, repeated TRUS was performed. If PSA was more than 10 ng/mL, randomized quadrant biopsies were taken. With this protocol 65 patients (3.6% of attendees) with prostate cancer were diagnosed³⁶.

Study I is based on a 15 year follow up of the whole cohort of men. Unfortunately the file containing the registration number of the original 26 602 men could not be retrieved due to a change of record holders. We reconstructed the cohort with the help of Statistics Sweden. This reconstructed cohort comprised 27 204 men, that is, 602 (2%) more than the original source population. We had the registration numbers of the 2 400 men invited to the screening procedure and all of them were included in the reconstructed cohort.

Study II is a 15 year follow-up of the 65 men diagnosed with prostate cancer evaluating the serum DHT level in relation to survival.

Study III is a 15 year follow-up of 375 men participating in the screening study evaluating suPAR in relation to survival. It includes 63 men with screening detected prostate cancer. The other 312 men belonged to one group with low PSA and high fPSA/PSA ratio ($n = 194$), one group of men with large prostates, high PSA but benign biopsies ($n = 79$) and one group with clinically detected PC during the first 10 years of follow up ($n = 39$).

Study IV

Study IV is conducted within the population of North Jutland, Aarhus and Viborg counties, Denmark (approximately 1 400 000 inhabitants) from January 1 1991 to December 31 2001.

All men were linked to the Danish Cancer registry to identify cases of prostate cancer. From the Danish registry of causes of death we identified the men who died from prostate cancer. Using the Prescription Database, we identified all individuals in the study population who received prescriptions for 5- α -reductase inhibitors or α -adrenoceptor antagonists and then assessed the risk of dying from prostate cancer after treatment with 5- α -reductase inhibitors and/or α -adrenoceptor antagonists.

Material

Registries

The Swedish registry of population (Study I, II and III)

Data is collected and updated by the local tax offices. The register contains official Swedish census data of all residents in Sweden with information on name, national registration number, current address and date of death if the subject is recently diseased. The registry was used to assess vital status of the study objects.

The Swedish cancer registry (Study I and III)

It is since 1958 mandatory for hospital departments and histopathological laboratories in Sweden to report all malignancies to the Swedish Cancer Register. Reports are sent to one of six regional oncology centre for coding and quality checks. Each year approximately 50 000 malignancies are registered. The completeness of the registry is excellent. We collected information on cancer diagnosis and the date of diagnosis from the registry.

The Swedish cause of death registry (Study I and III)

The registry includes information on all deaths among Swedish residents. The causes of death are classified according to the English version of the International Statistical Classification of Diseases and Related Health Problems, Tenth Revision (ICD-10), including the official updates published on the WHO website. The information is based on the death certificate issued by a physician. We collected information on date of death and the underlying cause of death from the registry.

The Danish central population registry (Study IV)

The registry was established in 1968. This registry assigns all Danish citizens a unique personal identification number that encodes gender and date of birth, and keeps information on residence, emigration and vital status. We used the registry to identify all men living in the North Jutland, Aarhus and Viborg area between 1989 and 2001.

The Danish Cancer registry (Study IV)

The registry was initiated in 1943 as a population-based countrywide registry. Newly diagnosed cancers are routinely reported to the registry through hospitals, outpatient clinics and general practitioners. Records for each patient include name, central population identification number, date of birth, residence at date of cancer diagnosis, method of verification of the cancer, extent of disease at diagnosis and treatment. From the registry we identified patients with prostate cancer and if the tumour has metastasised.

The National registry of causes of death (Study IV)

This registry includes individual based data of all deaths occurring among residents in Denmark¹⁵⁵. It is based on the death certificates from physicians. We identified all men who died from prostate cancer.

The Pharmacoepidemiological Prescription Database of North Jutland (Study IV)

The Regional Prescription Database includes information on prescription medications dispensed in Denmark in North Jutland County since 1991, Aarhus County since 1996,

and in Viborg County since 1998. It contains data on more than 70 million dispensed prescriptions, includes a personal identification system, and can be linked to other Danish registries. Using the Prescription Database, we identified all individuals in the study population who received prescriptions for 5- α -reductase inhibitors (ATC code G04CB) or α -adrenoceptor antagonists (ATC code G04CA).

End-point committee (Study II)

In study II the medical journals of the screening detected prostate cancer patients that had died before 15 years follow up were retrieved. Three independent senior urologists reviewed the medical records separately and designated the cause of death. In case of disagreement (one case), the majority ruled.

Serum analysis (Study II and III)

At the screening procedure in 1988-1989 blood samples were drawn from all 1 782 participating men. The samples were drawn between 08:00 and 11:00 to reduce the distortion by diurnal variation. Two venous blood samples were drawn. One for PSA-measurement and the other was immediately stored at -70. In 1993 the blood samples were analyzed for DHT, testosterone and sexual-hormone binding globulin (SHBG)¹⁵⁶. DHT, testosterone and SHBG were measured through different forms of radioimmunoassay further described in Study II¹⁵⁷.

In 2001 the serum was used for analysis of suPAR. We measured serum levels of suPAR using time-resolved fluorescence immunoassay (TR-FIA). Intact suPAR (suPAR (I-III)) was measured with TR-FIA I and intact + cleaved suPAR using TR-FIA 2. The methodology is fully described in earlier paper¹⁵⁸. The assays were validated for their use in serum from this collection using a pool of 50 different samples. The amount of suPAR domain II+III (suPAR (II-III)) was calculated. The results are presented in Study III.

Statistical analyses

Survival analysis is used to assess the probability of remaining free of a specific outcome after a certain time. It also implies that the incidence of the outcome is not constant over time. In all studies we evaluated the event death related to different exposures. The Kaplan-Meier method calculates a new survival proportion for each event that occurs and the result is usually presented as a curve. To test if there is a difference between two groups in survival the log-rank test is used. By using the Cox proportional hazards model it is possible to compare effects of several variables on hazard¹⁵⁹. The hazard is the risk of an outcome in a certain time interval, assuming survival to that time. The model generates a hazard ratio (HR) for each covariate included in the model and the HR is the relative hazard when two groups are compared. Poisson regression is a variant of multiple regression where the outcome is a count. It calculates incidence rates and incidence rate ratios.

4 RESULTS AND DISCUSSION

Study I: 15-Year followup of a population based prostate cancer screening study

Results

The aim of the study was to evaluate the one time screening procedures impact on prostate cancer survival and to compare the long time overall survival in attendees and non-attendees. In total 27 146 men were followed, including 1769 attendees, 605 non-attendees and 24 772 men in the source population. With median follow-up of 12.9 years the risk of being diagnosed with prostate cancer during follow-up was not significantly different among the groups. Nor was the risk of death from prostate cancer. The invited population did not differ from the source population with respect to overall survival, Incidence rate ratio (IRR) 1.10 (95% CI 0.83-1.46). When comparing the source population with the screening attendees and non-attendees the IRR for death from another cause was 0.82 (95% CI 0.76-0.90) and 1.53 (95% CI 1.37-1.71), respectively. With the attendees as the referent group the IRR of death from other causes in non-attendees was 1.89 (95% CI 1.65-2.16). The difference in overall survival was constant over time except for year 1 of follow-up, during which excess mortality in non-attendees was strongly increased with IRR 4.58 (95% CI 2.58-8.11) compared to the source population.

Discussion

There are several limitations to evaluate prostate cancer screening from the results from this study. First the initial study was designed to evaluate different diagnostic procedures to find prostate cancer and it was in the beginning of the PSA-era. Only 3 cases were detected on PSA-evaluation alone without DRE or TRUS findings. Today most patients are referred to biopsy after PSA elevation without any suspicious DRE finding. The biopsy protocol with 2-4 histological cores and 3 fine needle aspirates was probably less efficient than more contemporary biopsy strategies. Even considering these differences the diagnostic yield with a detection rate of 2.7% in a first screening round is comparable with the results of ERSPC which had a detection rate between 1.1 and 4.2% in the first round¹⁶⁰. However, the current study was just a single intervention and repeated screening procedures add cases and the end rate of screening detected cases in the ERSCP study was 5.8%¹⁰⁶.

Secondly the treatment offered to the 65 cancer patients may not have been effective enough. 41 men were offered treatment with curative intent with radical prostatectomy, low-dose external radiation and Nd-YAG laser therapy. The latter two procedures are today considered obsolete. 11 (16.9%) men received radical prostatectomy and it can be noted that none of them died from prostate cancer during follow up. In the ERSCP study 40.3% of men in the screening arm had a radical prostatectomy.

We did not find any difference in disease-specific mortality, the most widely accepted end point in randomized cancer screening trials. The analysis of disease specific mortality is vulnerable since bias in the registration of cause of death could influence the results. All cause mortality on the other hand does not require an opinion on the

cause of death, since it only measures if the patient is alive or not. It can also measure any lethal side effects of treatment. When assessing all-cause mortality we could not see any difference in overall survival between the invited population and the source population. When dividing the invited men into attendees and non-attendees there was a huge difference in overall survival with the screening non-attendees having an almost two-fold increased risk of dying. This has not been described earlier in randomised prostate cancer screening studies but it has been seen in other screening studies. In a nine year follow-up of participants and non-participants of a sigmoidoscopy screening study the non-participants had an increased risk of overall mortality with mortality rate ratio of 2.4 (95% CI 1.7-3.4)¹⁶¹. They also compared the mortality of the invited cohort with the standard mortality rate (SMR) of the population. The invited participants had a SMR of 0.5 of the expected. The reason for this healthy volunteer effect is probably multiple. Several earlier cancer screening studies have indicated that socioeconomically underprivileged people are less motivated to participate in screening¹⁶²⁻¹⁶⁴.

All cause mortality also helps when the benefit from a screening program should be put in perspective when making decisions on a population basis. Even if prostate cancer screening could be shown to lower the disease specific mortality it might not influence all cause mortality. In the ERSPC prostate cancer screening was shown to lower disease specific mortality but the overall mortality was unchanged with a Rate Ratio of 0.99 (95% CI 0.97-1.02). As described earlier the healthy volunteer effect must also always be considered when interpreting screening studies. Among the 7 centres recruiting patients in the ERSPC, 4 of them, with 34% of study patients, had informed consent from the patients before randomisation. This will reduce the problem but even with consent before randomization not all men will participate (in the ERSCP 5.7%). The problem of generalising the results to the whole population remains.

Study II: Dihydrotestosterone levels and survival in screening detected prostate cancer: a 15 year follow-up study

Results

The aim was to evaluate the pre-diagnostic values of testosterone, dihydrotestosterone and SHBG influence on prostate cancer survival. Among the 65 men with screening detected prostate cancer 41 died during follow-up. 17 died from prostate cancer designated by our end-point committee. Testosterone and SHBG values were not associated with prostate cancer survival. Having a DHT value above the median was associated with a HR of 0.24 (95% CI 0.08-0.75) of dying from prostate cancer. When adjusting for PSA, tumour stage and age, a high DHT value remained protective against prostate cancer death (HR 0.23 95% CI 0.06-0.89). DHT did not influence the risk of dying from other causes.

Discussion

How can high DHT protect from prostate cancer death? First there may be an association between low DHT values and more aggressive tumours. One hypothesis for this could be that DHT is degraded in the prostate to β Adiol which in turn stimulates

the ER β -receptor leading to inhibition of epithelial growth⁸⁰. Less DHT intraprostatically means less 3 β Adiol and reduced stimulation of the ER β -receptor. Secondly there is evidence that manipulation of the DHT level in adults may affect tumour grade. In the PCPT study described earlier the treatment arm receiving finasteride had a higher proportion of high-grade tumours³¹. This has also been seen in an analysis of the prostate cancer incidence among finasteride users in the Finnish Prostate Cancer Screening Trial where long-time use of finasteride was associated with an increased risk of high-grade cancer¹⁶⁵.

This is the first study investigating androgen levels in a long time follow-up of screening detected prostate cancer patients. As mentioned earlier the prognostic significance of blood levels of androgens are very uncertain²⁶.

Ideally the androgens levels should have been measured intraprostatically since the correlation between serum and intraprostatic concentrations are not obvious^{86 87}. Any misclassification would however be non-differential between the comparison groups and lead to a dilution of the differences. The facts that DHT was specifically associated with death from prostate cancer and did not have any impact on other causes of mortality do however in our opinion point to a true association.

In the analysis of DHT as a continuous variable, adjusting for PSA lead to a diminished effect of DHT. You could say that DHT had little prognostic value besides PSA. On the other hand, DHT and PSA could be components in a casual chain were DHT comes first. Since our study was small we were not able to stratify the analysis by PSA levels.

Study III: Soluble urokinase-type plasminogen activator receptor as a prognostic marker in men participating in prostate cancer screening

Results

The urokinase plasminogen activator system is involved in tissue remodelling processes and is up regulated in many types of malignancies. It has also been seen to reflect the level of activity of the immune system and is probably also a marker of low-grade inflammation. We examined the serum levels of different forms of soluble urokinase-type plasminogen activator receptor (suPAR (I-III) and suPAR (II-III)) as prognostic markers in a prostate cancer screening cohort. Of the 375 men investigated 152 died during follow-up. We analyzed the levels of different forms of suPAR by their quartiles and as a continuous variable. The 63 men, in the cohort with screening detected prostate cancer who had high values of either suPAR form had an increased risk of prostate cancer mortality. This difference was however not significant and the association was lost in Cox regression adjusting for age, tumour stage, tumour grade and PSA.

When analyzing overall mortality we found that increase of both suPAR (I-III) and suPAR (II-III) values were associated with significant decreased survival (HR 2.26 (95% CI 1.17-4.35) and HR 2.53 (95% CI 1.56-4.10) respectively.

We analysed the risk of death from cardiovascular disease during follow-up. Belonging to the highest quartile of any of the suPAR forms was associated with a highly significant increased risk of cardiovascular death with HR 3.27 (95% CI 1.38-7.73) for suPAR (I-III) quartile 4 vs. quartile 1, adjusted for age.

Discussion

We were not able to demonstrate any prognostic value of suPAR for prostate cancer in this cohort. Maybe this was due to lack of statistic power (not enough patients and events). This is to our knowledge the first study evaluating suPAR levels and prostate cancer mortality in a long time follow-up. High suPAR levels have been shown to have some correlation to the risk of biochemical progression after radical prostatectomy. 429 patients who underwent radical prostatectomy had their uPA and suPAR levels analysed before and 6 weeks after surgery. Preoperative suPAR level was associated with biochemical progression in univariate but not in multivariate analyses. The circulating levels of suPAR were higher in prostate cancer patients compared to healthy men but there was a large overlap between groups. Interestingly the level of suPAR went down significantly after prostate removal¹²⁵. This leads us to the question whether the blood level of suPAR correlates to the tissue level. In breast cancer there seems to be a constant and direct correlation between cancer cell numbers and amount of suPAR released and in the same study the plasma suPAR level was highly correlated to the tumour volume¹⁶⁶. On the other hand other researches have failed to show any correlation between tumour tissue suPAR level and serum level¹⁴⁵.

SuPAR is present in blood in both intact and cleaved forms. In the time-resolved fluorescence immunoassays (TR-FIA) used in our study we were able to analyse suPAR (I-III), suPAR (I-III) + (II-III) and the calculated value of suPAR (II-III). After our analyses were performed the opportunity to analyse suPAR (I) has emerged¹⁵⁸.

SuPAR (I) has been shown to be increased in men with prostate cancer and also improve prediction of positive biopsies in men with elevated PSA values¹⁶⁷. It had of course been valuable if we would have been able to analyse suPAR (I) as well.

During the 15 years of follow up 49 cases of prostate cancer were clinically diagnosed in the cohort. We made a separate analysis for all prostate cancer patients disregarding the date of diagnosis. The prognostic effect of suPAR did not change considerably but it is hard to interpret since we had to use the new prostate cancer cases PSA values from diagnosis but had their suPAR analysed in blood collected at the initial screening.

It is not known if suPAR has any biological role in the body or merely reflects the uPAR levels and the activity in the uPA/uPAR signalling. It has been shown that suPAR actually can inhibit proliferation of prostate cancer cell lines in vitro. By giving recombinant suPAR to the human prostate cancer cell line DU145, Piccolella et al showed that this could induce apoptosis and decrease the migration properties of the cells¹⁶⁸. These cell lines had an active uPA/uPAR system. When testing the administration of suPAR on LNCaP cells, in which uPA/uPAR signalling is inactive, no effect on cell proliferation was seen. This means that the level of uPA/uPAR signalling determines if suPAR has any influence on proliferation and migration.

Why was overall survival diminished with higher suPAR levels? Plasma levels of uPA and suPAR have been showed to be positively associated with the presence of cardiovascular disease in patients on dialyses¹⁶⁹. The uPA expression is elevated in endothelial cells, smooth muscle cells and macrophages in atherosclerotic aortas and coronary arteries¹⁷⁰. This leads to the hypothesis that it could be atherogenesis leading to increased mortality. Either promoted by increased activity in the uPA/uPAR axis leading to increased cleavage of suPAR, and/or by a direct effect of the liberated

suPAR. There are no earlier studies on the relationship between suPAR levels and the risk of cardiovascular death.

Our results are however not completely new. In two studies on patients with colorectal malignancies, increased total suPAR values predicted shorter survival independent of other known prognostic markers of colorectal cancer^{171 172}. The risk for overall death was approximately twofold for one unit increase of suPAR on the log-scale.

Our study differs in investigating the different forms of suPAR and also has information on cause of death. By making a sub analysis on the men that died of cardiovascular disease we showed that this was the main reason for the overall increased mortality for men with high suPAR levels. One big drawback in doing this analysis was that we did not have any information on other common risk factors for cardiovascular death besides age and gender. We lack information on smoking status, blood lipids, co morbidity etc. In one recently published study on 255 patients operated on for symptomatic carotid atherosclerosis the suPAR (I-III) and suPAR (II-III) levels were not increased by smoking or by elevated blood lipids (HDL, LDL, cholesterol, triglycerides)¹⁷³. In fact the mean suPAR (I-II) level was actually lower in the patients smoking.

Study IV: Treatment with 5-alpha reductase inhibitors and prostate cancer survival

Results

There is at present no data on long-term survival in prostate cancer among men treated with 5- α -reductase inhibitors. We compared survival after diagnosis of prostate cancer in a cohort of users of 5- α -reductase inhibitors with that of the background population. To compensate for lead time bias we also compared survival of users of α -adrenoceptor antagonists. We found 3 791 men diagnosed with prostate cancer during the study period. 199 had been treated with 5- α -reductase inhibitors, 613 with α -adrenoceptor antagonists and 173 with combination therapy. The mean age at diagnosis was 73.6 years and the mean follow up time was 3.7 years. In total 3 075 men died during follow up, and among these 2 284 of prostate cancer.

In the Cox regression stratifying for age and year of diagnosis the HR for prostate cancer death after treatment with 5- α - reductase inhibitors was 0.93 (95% CI 0.76-1.14). Treatment with α -adrenoceptor antagonists significantly reduced the risk of prostate cancer death (HR 0.78 (95% CI 0.67-0.90)). After adjusting for local tumour status, none of the treatments were significantly associated with survival after PC diagnosis. We also analyzed the risk of being diagnosed with metastasised prostate cancer with DDD of medicine as exposure variable. The odds ratio (OR) for having metastasised cancer at diagnosis compared to untreated men were 1.14 (95% CI 1.01-1.29) per 100 DDD of finasteride treatment and 0.89 (95% CI 0.81-0.98) per 100 DDD of treatment of α -adrenoceptor antagonists.

Discussion

We were not able to demonstrate any difference in disease specific survival after diagnosis of prostate cancer among men treated with 5- α -reductase inhibitors compared to men without treatment. There is an apparent risk of lead time bias in a study like this. Since lower urinary tract symptoms (LUTS) leads to further evaluation of the prostate with PSA-testing and DRE, biopsies will be taken and prostate cancer revealed earlier than without LUTS. The diagnostic activity with PSA-testing was however low in Denmark during the study period¹⁷⁴. The diagnosis of prostate cancer also leads to a treatment decision and if the treatment is effective an additional prolonged survival would be expected. In this population the proportion of men treated with prostatectomy and radiation therapy was very low compared to Sweden. 6.9% had radical prostatectomy and 1.5% radiation therapy as primary treatment but the treatment data is not complete.

To assess the lead time bias we included men treated with α -adrenoceptor antagonists for comparison. These men had a statistically significant reduced mortality. We were not able to identify any bias explaining why the men treated with α -adrenoceptor antagonists could have stronger lead time bias than the men treated with 5- α -reductase inhibitors. Maybe the PSA reduction caused by treatment with 5- α -reductase inhibitors could lead to deferred diagnosis of PC, but the age at diagnosis was the same between groups. In our analysis of the risk of being diagnosed with metastasised disease, treatment with finasteride significantly increased this risk compared with untreated men. Since lead time bias should be the same for the men treated with finasteride as for the men treated with α -adrenoceptor antagonists, their ORs should be compared. The OR for finasteride treatment is then 1.28/100 DDD.

It can be speculated that the lead time bias among patients treated with 5- α -reductase inhibitors is outweighed by a biologically adverse effect of reducing DHT in the prostate. It is also possible that treatment with α -adrenoceptor antagonists has a tumour suppressive effect. Both α -adrenoceptor antagonists doxazosin and terazosin have in vitro been shown to induce apoptosis in prostate cancer cell lines^{175 176}. Treatment with these drugs has also been associated with a reduced risk of being diagnosed with prostate cancer¹⁷⁷.

We do not know how long treatment it would take to see an effect, but possibly the duration of treatment in our data is too short. The mean exposure for the 5- α -reductase inhibitor group was 314 DDDs. It must however be stressed that the exposure data is truncated to the left since the counties of Aarhus and Viborg were not included in the Prescription register until 1996 and 1998, respectively. The absolute value of DDD should therefore be interpreted with caution.

5 CONCLUSIONS

- Participating in a one time prostate cancer screening study did not influence prostate cancer survival
- Non-attendees in the screening program had an almost twofold increased risk of overall death compared to attendees
- A high DHT value at diagnosis of prostate cancer lowered the risk of prostate cancer death in long time follow-up
- High levels of suPAR (I-III) and (II-III) were associated with decreased prostate cancer survival, but the association was not statistically significant and lost in multivariate analyses
- High levels of both subtypes of suPAR were associated with decreased overall survival, especially attributable to cardiovascular mortality
- Treatment with 5- α -reductase inhibitors did not increase the risk of prostate cancer death
- Treatment with α -adrenoceptor antagonists protected from prostate cancer death
- Treatment with 5- α -reductase inhibitors significantly increased the risk of being diagnosed with metastasised prostate cancer

6 FUTURE RESEARCH

The cohort of 27 000 men constituting the source population of the screening study has now reached a mean age of 85 years. We are planning to update our outcome data and will have more outcomes i.e. adding to statistical power in survival analysis. Probably this will not change our general conclusions but it is interesting to see if our finding of a large difference in overall survival between attendees and non-attendees will continue in the same magnitude over time.

We will also benefit from longer follow-up with more outcomes adding to statistical precision in assessing DHT and long time survival.

When reading the literature on the androgens relationship to the prostate and prostate cancer it becomes evident that our knowledge is limited. We lack full understanding on their metabolism and action in the prostate. We are also in need of further knowledge of their influence on other structures in the body. We recently published a paper in which we described the changes of the serum values of androgens and precursors after radical prostatectomy¹⁷⁸. The levels of LH and FSH were significantly increased and the level of DHT decreased 90 days after surgery. The level of testosterone was unchanged after surgery. We speculate that the increase of LH and FSH is a response to the lowering of DHT. We have observed that many patients after radical prostatectomy not only are suffering from erectile dysfunction but also from loss of libido. Maybe DHT is involved in the regulation of libido and supplementation with DHT could be a way of improving libido after surgery.

The puzzling findings in the PCPT lead us to evaluate DHTs influence on long time prostate cancer survival. We believe that our findings merit additional investigation. We are planning to try to ascertain the 3β -Adiols role further. This can be achieved by giving finasteride treatment to a group of men before radical prostatectomy and then measuring the level of 3β -Adiol and other metabolites in the prostate specimen compared with untreated men. It would also be possible to measure the level of ER- α and ER- β - receptors in the prostatic tissue with or without finasteride treatment.

We were not able to show any prognostic role of suPAR levels for prostate cancer outcome in our material. The statistical precision was low and we will hopefully be able to do these analyses on blood samples from a larger cohort of prostate cancer patients. Our interesting finding of suPAR prognostic ability for predicting cardiovascular mortality also merits further investigation.

Besides the need for prognostic markers for avoiding over treatment of prostate cancer, there is also a need for improved treatment of men with advanced disease at diagnosis. There is evidence that men with lymph node metastasis benefit from radical prostatectomy in combination with endocrine treatment, in contrast to endocrine treatment alone^{179 180}. Maybe removal of the primary tumour reduces the shedding of metastatic cancer cells. The production of different growth factors from the tumour can also help the tumour to metastasise¹⁸¹. In a randomised trial, it has also been convincingly shown that adding radiation therapy to endocrine treatment improves survival in men with advanced disease¹⁸². Almost 900 patients with locally advanced

prostate cancer (T3 = 78%) were randomised to endocrine treatment or endocrine treatment combined with radiotherapy. Combination treatment halved the 10 year prostate cancer-specific mortality (RR 0.44). We need to better understand how to combine our treatment modalities in advanced cases, and probably be more active with both surgery and radiotherapy.

7 SAMMANFATTNING

Bakgrund

Prostatacancer är den vanligaste cancerformen hos män i västvärlden. I Sverige diagnostiseras ca 9 000 nya fall varje år och 2 500 män dör av sjukdomen. Sedan 20 års tid finns det möjlighet att med ett blodprov kontrollera nivån av prostataspecifikt antigen (PSA). Förhöjda värden kan indikera förekomst av prostatacancer. Screening för prostatacancer dvs. att alla män inbjuds att kontrollera sitt PSA-värde har inte införts i Sverige. Prostatacancer har ett väldigt varierande förlopp. En del patienter har en beskedlig tumör som växer långsamt. Andra drabbas av en tumör som snabbt riskerar att sprida sig i kroppen. Botande behandling ges med kirurgi eller strålbehandling. Båda dessa behandlingar kan medföra biverkningar som sänker livskvaliteten. Vid screening för prostatacancer finns ändå stor risk för överbehandling. Med det menas att man behandlar en tumör som kanske inte skulle ställa till med några besvär för patienten.

Syfte

Avhandlingens syfte var att analysera långtidsresultatet av en svensk screeningstudie. Vidare att utvärdera två olika prognostiska markörer och undersöka risken att dö av prostatacancer hos patienter som behandlats med medicin mot prostatabesvär.

Delstudie I

1988 startade den s.k. SÖS-studien. Av alla män, mellan 55-70 år, som bodde i Södersjukhusets upptagningsområde (ca 27 000) inbjöds 2 400 att delta i en screeningstudie. De 1 782 män som deltog undersöktes med palpation av prostatan, ultraljud och PSA-provtagning. Vid misstanke om prostatacancer togs vävnadsprover. 65 män med prostatacancer diagnostiserades bland de undersökta. Vi har nu utfört en uppföljning 15 år efter studiestart via uppgifter från cancerregistret och dödsorsaksregistret. Vi fann ingen skillnad i risken att dö av prostatacancer mellan de 2 400 män som inbjöds till undersökningen och de icke inbjudna männen. Vi fann emellertid en kraftigt ökad risk att dö av andra orsaker hos den grupp av män som inbjöds men inte kom till undersökningen. Risken att dö under 15-års tid var nästan dubbelt så hög hos icke-deltagarna jämfört med hos deltagarna.

Delstudie II

I delstudie två undersökte vi om nivån av dihydrotestosteron (DHT) kunde ha prognostisk betydelse för prostatacancer. Testosteron som är det viktigaste manliga könshormonet omvandlas i prostatan till DHT. DHT stimulerar tillväxt av prostatan men dess nedbrytningsprodukter tros också kunna ha en hämmande effekt. Hos de 65 männen med prostatacancer, diagnostiserade i screeningstudien, mätte vi nivån av DHT. Vi följde patienterna i 15 år och lät en expertgrupp granska deras journaler om de hade dött under uppföljningstiden. 17 av de 65 männen hade dött av prostatacancer. Risken att dö av prostatacancer minskade vid hög DHT nivå.

Delstudie III

Souble urokinase-type plasminogen activator receptor (suPAR) är ett ämne som påverkar nedbrytningen av vävnad i kroppen. SuPAR har noterats vara förhöjt i blodet vid ett flertal olika tumörformer. Även inflammation och diabetes tros kunna ge förhöjda värden. Vi mätte nivån av två olika former av suPAR hos 375 av de män som deltog i screeningstudien. SuPAR nivåerna var inte korrelerade till risken att dö av prostatacancer. Vi fann dock att höga nivåer av suPAR var förenat med en ökad risk att dö generellt. Speciellt ökade risken att dö av hjärt-kärlsjukdomar.

Delstudie IV

I delstudie II noterades att låg nivå av DHT var förenat med ökad risk att dö av prostatacancer. En grupp av läkemedel (5 α -reduktashämmare) används vid godartad prostataförstoring. 5 α -reduktashämmare verkar genom att sänka nivån av DHT i prostata. Vi studerade förskrivningsregistret i Danmark för att identifiera alla män som fått denna medicin och senare utvecklat prostatacancer. Vi jämförde dem med en grupp som fått annan medicin mot prostatabelsvär (alfa-adrenoreceptorantagonister) samt män som inte fått någon prostatamedicin alls. Behandling med 5 α -reduktashämmare ökade inte risken att dö av prostatacancer. Behandling med alfa-adrenoreceptorantagonister minskade dock risken. Risken att ha en spridd cancer redan vid diagnos ökade dock med nästan 30 % om man fått behandling med 5 α -reduktashämmare jämfört med alfa-adrenoreceptorantagonister.

Konklusion

Screening för prostatacancer, som den gjordes i SÖS-studien, minskade inte risken att dö av prostatacancer vid långtidsuppföljning. De som kommer till screeningundersökning är betydligt friskare än de som uteblir och denna skillnad finns kvar även efter 15 år. DHT mätt i samband med screeningundersökningen gav viss prognostisk information där låg nivå verkade förknippat med en ökad risk att dö av prostatacancer. SuPAR-nivån i blodet var inte korrelerad till risken att dö av prostatacancer. Däremot var höga suPAR-nivåer starkt förknippade med ökad risk att dö av hjärt-kärlsjukdom. Behandling med 5 α -hämmare, innan diagnos av prostatacancer, ökade inte risken för prostatacancerdöd men behandling med alfa-adrenoantagonister minskade risken.

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