Early Prostate Cancer

On prognostic markers and predictors of treatment outcome after radical prostatectomy

by

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The great tragedy of science:
The slaying of a beautiful hypothesis by an ugly fact

Thomas Huxley

To Annelie Sarah & William

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List of papers

The thesis is based on the following five studies:

I) Khatami A, Damber J-E, Lodding P, Pihl CG, Hugosson J.
Does initial surveillance in early prostate cancer reduce the chance of cure by radical prostatectomy? A case control study.
Scand Journal of Urology and Nephrology. 2003; 37(3):213-7.

II) Khatami A, Pihl CG, Norrby K, Hugosson J, Damber J-E.
Is tumour vascularity in prostate core biopsies a predictor of PSA recurrence after radical prostatectomy?
Acta Oncologica. 2005; 44(4):362-8.

III) Khatami A, Aus G, Damber J-E, Lilja H, Lodding P, Hugosson J.
PSA doubling time predicts the outcome after active surveillance in screening-detected prostate cancer: results from the European randomized study of screening for prostate cancer, Sweden section.
International Journal of Cancer. 2007 Jan 1;120(1):170-4.

IV) Khatami A, Hugosson J, Wang Wanzhong, Damber J-E.
Ki-67 in screen-detected, low-grade, low-stage prostate cancer; relation to PSADT, Gleason score and PSA relapse after radical prostatectomy.
Manuscript

V) Khatami A, Aus G, Damber J-E, Lilja H, Wessman C, Hugosson J.
PSA doubling time (PSADT) is influenced by prostate volume and the presence of high-grade cancer: Results from the European Randomized study of Screening for Prostate Cancer (ERSPC) Sweden section.
Manuscript

Abstract

The incidence of prostate cancer (PC) has increased by 4.6% annually in Sweden during the past ten years. Today's clinically used prognostic markers are not accurate enough to separate the potentially life-threatening tumours from the insignificant ones in 50-80% of newly diagnosed PCs. Curative treatment of all men with early PC results in substantial overtreatment and subsequently a large number of men would suffer from the side effects of this treatment. There is an urgent need for more accurate prognostic tools to distinguish the insignificant PC from the potentially lethal PC in its early stage.

We studied whether an initial period of surveillance in these patients might decrease their chance of cure by radical prostatectomy. The prognostic significance of tumour vascularity (TVC) from biopsy was evaluated. The outcome in 270 consequent screening-detected PC patients under active surveillance was studied and PSA doubling time (PSADT) as a predictor of outcome was evaluated. The proliferation marker Ki-67 was evaluated as a prognostic marker. The factors that influence the variations in PSADT were explored in the entire cohort in the screening study and in the men with PC.

The results revealed that up to two years of surveillance in patients with early PC did not reduce the chance of cure by radical prostatectomy. TVC and Ki.67 were both significantly correlated to PSA relapse after prostatectomy. However, these markers could not improve the prognostic information generated from routinely used markers.

Some 61% of patients were treatment-free after a follow-up period of 63 months in the active surveillance cohort. No patient has developed bone metastasis or died from PC. Fourteen patients died for reasons other than PC during the follow-up. PSADT was the only significant predictor of PSA relapse after radical prostatectomy in this cohort of patients. PSADT is mainly influenced by prostate volume and the presence of high-grade PC.

The active surveillance approach offers an alternative to active treatment in patients with early-detected, low-stage, low-grade PC. PSADT seems to be a useful, reliable and discriminating prognostic marker of disease progression and active treatment during the follow-up of patients with screening-detected early PC who opt for the active surveillance strategy.

Abbreviations

ACT	Alhla-1-antichymotrypsin
AR	Androgen receptor,
AS	Active surveillance
BPH	Benign prostate hyperplasia
CG	Control group
DHT	Dihydrotestosterone
DRE	Digital rectal examination
ERSPC	European randomized study of screening for prostate cancer
F/T-PSA	Free to total PSA
MVD	Microvessel density
PLCO	The prostate, lung, colorectal and ovarian cancer screening trial
PC	Prostate cancer
PSA	Prostate specific antigen
PSAD	PSA density
PSADT	PSA doubling time
PSAT	PSA density of the transitional zone
RP	Radical prostatectomy
RT	Radiation therapy
SG	Surveillance group
TRUS	Transrectal ultrasound
TUMT	Transurethral microwave thermotherapy
TURP	Transurethral resection of the prostate
TVC	Tumour vascularity
VEGF	Vascular endothelial growth factor
WW	Watchful waiting

Introduction

Early prostate cancer; curative-intent treatment or not, that is the question.

In Sweden in 2005, one man was diagnosed with prostate cancer (PC) every hour and one man died of (PC) every three hours (Swedish national board of health and welfare, 2007).

During the past twenty years the incidence of PC has increased in developed countries (Parkin, Pisani et al. 1999; Bray, Sankila et al. 2002; Parkin, Bray et al. 2005; Ferlay, Autier et al. 2007; National cancer institute 2007). One of the major reasons for such a unanimous increase is that during this period of time urologists have adapted new diagnostic tools to diagnose PC at an earlier stage. Today, the majority of PCs in developed countries are diagnosed in asymptomatic men at a very early stage and subsequently these men are much younger in comparison to men who were diagnosed with PC twenty years ago. In other words, PC in the majority of men is diagnosed 10-20 years before the clinical symptoms are developed (Galper, Chen et al. 2006).

As a physician one should be very enthusiastic over such a shift in the diagnosis. It is the ideal improvement to find the disease before it can cause any symptoms and subsequently the chance of cure through active treatment for each patient is improved. However, the dilemma in dealing with this disease is that far from all the patients with PC would be bothered by the disease during their lifetime (Albertsen, Hanley et al. 2005).

The above circumstances has also created another problem, namely that the curative treatment of all men with early-detected PC would result in substantial overtreatment (Bangma, Roemeling et al. 2007). The active treatment of PC has several potential short- and long-term, quality of life-reducing side effects such as incontinence, impotence and bowel disorders. This fact is a major consideration, which makes the accurate selection of men with early PC for active treatment so essential.

Although the diagnosis of PC has undergone a revolution over the last twenty years, the clinically used prognostic tools at the time of diagnosis are still rather inaccurate. Because of this uncertainty a substantial number of patients with early PC often prefer the safe way out at the time of diagnosis, which is active treatment, and the risk of overtreatment subsequently increases.

Paradoxically, the prostate cancer mortality rate is still very high (Swedish national board of health and welfare, 2007). This can be interpreted as such that despite the new diagnostic improvement there is ongoing undertreatment in men with aggressive lethal prostate cancer.

To prevent the overtreatment of patients with early PC and to improve undertreatment in patients with potentially lethal PC there is an urgent need for new prognostic tools which can distinguish potentially life-threatening PC from the "innocent" ones.

In this thesis different aspects of this prognostic dilemma in PC have been explored.

The prostate gland

Anatomy

The prostate gland arises from the urogenital sinus mesenchym. The development of the prostate is under the control of dihydrotestosterone (McNeal 1981). The prostate gland is located caudally to the urinary bladder and encloses the urethra all the way down to sphincter muscle. The prostate inferiorly rests on the pelvic floor and the sphincter muscle. The seminal vesicle and the vas deferens are posterior to the prostate. The two layer of Denonvilliers fascia separate the prostate and seminal vesicle from the rectum at the dorsal aspect. Neurovascular bundles that supply the corpora cavernosa are to be found posterolaterally to the prostate.

The prostate is about 15-20 ml in adult men. Based on predisposition to altered pathological processes in different parts of the gland, the prostate has been described as consisting of three zones: the peripheral zone, from which more than 75% of cancers are originated; the transitional zone, which harbours the glandular tissue where excessive growth causes benign prostate hyperplasia; and the central zone, which in comparison with other two zones is principally free from diseases (McNeal 1981; McNeal 1988; McNeal 1988; McNeal, Redwine et al. 1988).

Physiology

The prostate gland is inactive during childhood. After puberty, because of the increasing level of circulating androgens, mainly testosterone, the prostate gland becomes active and develops. Inside the prostate the testosterone and the adrenal androgens are metabolized to dihydrotestosterone (DHT) by 5-alpha reductase, an enzyme which is located mainly on nuclear membrane. DHT is 2.5 times more potent than testosterone. DHT binds to androgen receptor (AR) within the glandular cells. The complex DHT-AR activates several cell functions by targeting the DNA sequences in the nuclei and results in growth and proliferation. The function of the prostate is principally unknown. However, two possible functions have been suggested. There is a high production of immunoglobulin in the prostate and the gland seems to have a protective function against local infections. The second function is the importance of prostate secretion in the motility of the spermatozoa (Fredricsson 1994).

Prostate specific antigen (PSA) is a glycoprotein that is secreted from the epithelial cells of the prostate to the lumen. The luminal fluid of the prostate is mixed with the semen during ejaculation. The PSA lyses Seminogelin, a protein which is initially derived from seminal vesicles. This reaction facilitates spermatozoa migration within the female reproductive tract.

Under normal circumstances only a small proportion of the PSA is absorbed into the bloodstream. The conditions that disrupt the basal cell layer lead to increased absorption of the PSA and thereby an increased serum value of the PSA.

Epidemiology

The age-standardized (population in 2000 in Sweden) incidence of PC in 2005 was 233.2 cases/100,000 men in Sweden. The age-standardized incidence of PC in 1986 was 134.7, which reveals that the incidence of PC in Sweden increased on average by 2.9% annually during the past 20 years and by 4.6% annually during the past 10 years (Swedish national board of health and welfare, 2007). Prostate cancer is the most common cancer in Sweden

(19.4% of all cancer in 2005) and accounts for 36.5% of all cancers among men. There are certain regional variations in Sweden with a lowest incidence of 174.4 and a highest incidence of 267.1/100,000 men (Swedish national board of health and welfare, 2007).

The incidence and mortality rates in the United States gradually increased through to the middle of the 1980s. PSA testing was introduced in 1986 and in the US the incidence of PC doubled between 1986 and 1992. The incidence rates have declined since 1993 but remain substantially higher than before 1986 (Parkin, Bray et al. 2005). The age-adjusted incidence of prostate cancer in the USA for 2003 was 150/100,000 men. Black Americans had the highest incidence, 221.8, while white Americans had an incidence of 138.9. The lowest incidence observed was 72.2 among American Indians and Alaska Natives.

In 2006, PC was the commonest form of cancer in men in Europe and accounted for 24.1% of all cancers. The age-standardized incidence rate (European standard) shows substantial differences between the European countries. Ireland had the highest incidence with 182/100,000 men, and the Republic of Moldova had the lowest incidence with 17.7/100,000 men (Ferlay, Autier et al. 2007).

Prostate cancer is the second most common cancer in men worldwide. The number of new cases in 2002 was estimated at 679,000. PC is responsible for 11.7% of new cancers and the incidence is almost four times higher in developed countries compared to developing countries, 19% versus 5.3%. (Parkin, Bray et al. 2005).

One interesting observation is that in the late 1980s PSA was adapted as a diagnostic test for PC. This could be partly responsible for the increased incidence of early/latent PC. However, the fact that even mortality in PC had increased during the same period has been interpreted as such that there is also a genuine increased incidence of the disease (Parkin, Bray et al. 2005).

PC is the most common cause of cancer death in Swedish men. In 2004, 2,549 men died of PC in Sweden. The age-standardized mortality rate in PC has been relatively constant during the past decade (1997: 72.68/100,000 men and 2004: 71.4/100,000 men). However, the age-standardised mortality from PC in men younger than 65 years old has decreased from 4.6/100,000 in 1997 to 3.2/100,000 in 2004 (Swedish national board of health and welfare, 2007).

The number of deaths from PC in Europe increased by 16% between 1995 and 2006 (Ferlay, Autier et al. 2007).

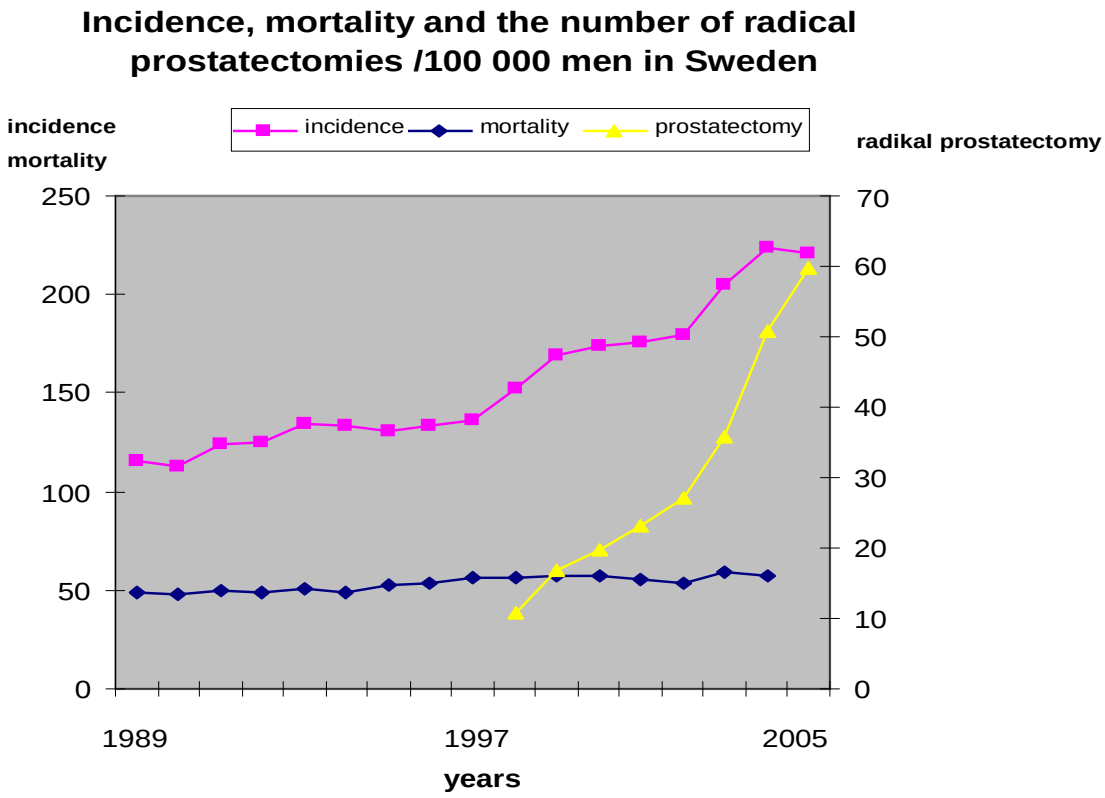
In the United States the age-adjusted (USA population 2000) mortality rate in 2003 was 26.6/100,000 men. The mortality rate among black Americans was 58/100,000. White Americans had a mortality rate of 24.5/100,000 (National cancer institute 2007).

The mortality rate has begun to decline in United States during the past decade. This observation has been discussed frequently in the literature. Some authors interpreted the decrease in mortality as a result of almost 20 years of aggressive screening and treatment of PC (Galper, Chen et al. 2006). However, almost the same trend in mortality has been seen in other countries in the absence of aggressive screening. Because of the long lead time for screen-detected PC, longer follow-up is needed before an accurate conclusion can be drawn about the rationale behind the changes in the PC mortality rate.

In conclusion, the study of the epidemiology of PC revealed the same pattern in almost all the developed countries. The incidence of PC has increased substantially during the past 20 years. In the US, after a substantial initial increase the incidence has decreased since 1993. The PC mortality rate has decreased slightly during the past 20 years. The number of men who received curative treatment has increased substantially during the past 20 years. The number of men with advanced PC and metastasis at diagnosis has decreased.

Figure 1 shows the incidence, mortality and number of radical prostatectomies/100,000 men in Sweden during the past 15 years.

Figure 1)



Diagnosis

Prostate-specific antigen (PSA)

PSA is a glycoprotein and a serine protease produced by the prostatic epithelial cells. This protein was first described in 1971 and was isolated from seminal plasma (Hara, Koyanagi et al. 1971). Kuriyama et al developed the first assay for PSA in serum in 1981, and PSA was suggested as being a useful marker for the detection of prostate cancer (Kuriyama, Wang et al. 1981; Wang, Papsidero et al. 1981).

It took a few years before PSA was adapted by urologists as a marker for PC detection. Today, PSA is the most widely used tumour marker in urology (Polascik, Oesterling et al. 1999). The cumulative seven-year risk of being diagnosed with PC in a biennial PSA-screening programme was 34%, 44% and 71% for those men with initial PSA values of 3-6 ng /mL, 6-10 ng /mL and >10ng /mL. (Aus, Damber et al. 2005).

There are several limitations on the use of PSA as a diagnostic tumour marker. The two main ones are: firstly, the PSA has high tissue specificity but is not a cancer-specific protein. The PSA value is increased in patients with BPH and inflammatory process or infections in the prostate. This substantially reduces the sensitivity of the PSA as a diagnostic tumour marker. Secondly; there is no cut-off value for PSA when the risk of having cancer is eliminated. Even in a very low interval of PSA the men are at risk of having PC at core biopsy. In the US prevention study PC was diagnosed in men with a PSA between 3.1-4ng/mL in 26.9%. Even in men with a PSA between 1.1-2 ng/mL 17% had cancer in the core biopsy.(Thompson, Pauler et al. 2004).

To improve the specificity and sensitivity of the PSA in the early detection of PC, several diagnostic tools based on PSA have been suggested.

PSA density (PSAD)

PSAD is the total PSA divided by the volume of the entire prostate measured by TRUS. The PSA density should theoretically be higher in men with PC as the leakage of PSA in the blood is ten times higher in cancer cells compared to normal prostatic tissue and therefore the PSAD was suggested as improving the sensitivity of the PSA (Babaian, Fritsche et al. 1990). PSAD has the same limitation as PSA itself because of the impact of inflammatory processes or infection. Another problem is that the calculated prostate volume could be altered substantially as the measurement method is highly dependent on the person who performed the TRUS.

PSA density of the transitional zone (PSAT)

PSAT is a modification of PSAD. The theory behind it is to eliminate the PSA increase caused by BPH by measuring the transitional zone and thereby purifying the PSA increase caused purely by cancer (Zlotta, Djavan et al. 1997). The shortcoming of PSAT is mainly based on the difficulty of assessing accurately the transitional zone by TRUS, especially in men with small prostates.

Age-specific PSA

The rationale behind age-specific PSA is the association between prostate volume, age and the PSA (Oesterling, Jacobsen et al. 1993). Strict launching of this method in clinical practice could result in excessive biopsy in younger men as well as underdiagnosis of aggressive cancers in older patients.

Free to total PSA (F/T-PSA)

PSA in serum is combined with alhla-1-antichymotrypsin (ACT) in 56-95% and in a smaller proportion combined with alpha-2 macroglobulin. Only a small fraction of PSA is in free form (Lilja, Christensson et al. 1991). The proportion of PSA complexed to ACT is higher in patients with cancer than BPH (Stenman, Leinonen et al. 1991). In 1998, Catalona et al presented the results from a multicentre study using the percentage of free PSA to enhance differentiation of PC from BPH; men with F/T-PSA <10% have cancer in the core biopsy in 56% compared to 8% in men with F/T-PSA >25% (Catalona, Partin et al. 1998). F/T-PSA allows a clearer distinction between men with prostate cancer and men with BPH rather than total PSA. However, the F/T-PSA's power of discrimination between PC and BPH is decreased to an insignificant level in men with a prostate volume above 40 cm^3 (Catalona, Smith et al. 1995; Stephan, Lein et al. 1997).

Transrectal ultrasound, TRUS

The role of traditional grey-scale TRUS in early prostate cancer as a detector of PC is very restricted. The majority of the tumours detected because of a limited increase in PSA are T1c and have a small volume and in the majority of cases are not visible on TRUS. The TRUS has two potential roles in diagnosing PC; an improvement in accuracy of prostate biopsy through better direction for systematic sampling and identification of lesions suspected of malignancy (Heidenreich H 2007). PC suspicion cannot be dismissed based on a normal TRUS.

Core biopsy

AUA Guidelines recommended that a TRUS-guided 18G core biopsy should be the standard way of obtaining material for histopathological examination. Patients with

elevated PSA who underwent a core biopsy with normal results and who have a persistently high level of PSA should be recommended to undergo a second biopsy procedure. The detection rate of a second biopsy set is about 10-35% (Djavan, Ravery et al. 2001; Applewhite, Matlaga et al. 2002). After two sets of biopsies the vast majority of clinically significant tumours are detected (Djavan, Ravery et al. 2001).

Treatment option in organ-confined prostate cancer

Curative treatments

Radical prostatectomy

The aim of the procedure is to remove the disease. This operation was applied at the beginning of the 20th century by Young (Young 1905).

The operation has undergone modifications, especially during the past three decades, which had led to minimization of the major side effects in the form of incontinence and impotence (Walsh and Donker 1982). The operation can be performed by using different approaches, perineal, retropubic, laparoscopic or robot-assisted. Radical prostatectomy is the only curative treatment for localized PC that has indicated a cancer-specific survival benefit compared to conservative management in a prospective, randomized trial (Holmberg, Bill-Axelson et al. 2002).

Radiation Therapy (RT)

Since 1960, radiation therapy has been used in the treatment of prostate cancer. There have been several modifications in RT to limit the short- and long-term side effects, especially genitourinary, gastrointestinal toxicity. The aim of these modifications was to concentrate the radiation energy to the prostate and minimize the amount of radiation to other organs, thereby decreasing the toxicity rate and increasing the efficacy (Eade, Hanlon et al. 2007). Three-dimensional, CT-guided treatment planning, dose escalation and brachytherapy are the methods which have improved the precision of RT. There are several longitudinal studies to evaluate the effect of different RT dosages as a curative treatment (Pollack, Zagars et al. 2002; Zietman, DeSilvio et al. 2005). However, there is not a single prospective, randomized trial that compares RT to radical prostatectomy or

conservative management. “RT may be effective in the treatment of patients with localized prostate cancer” (Aus, Abbou et al. 2005).

Active surveillance (AS)

The concept of AS is fundamentally different from watchful waiting (WW). In the AS approach the curative treatment is still an alternative, which is not the case in WW. This is the reason why patients in an active surveillance programme are generally younger than patients in a WW cohort.

The patients with clinically low-risk PC will be monitored closely by repeated PSA and biopsy and with signs of progression the patients would be offered active treatment.

One major reason that AS has been popularized during the last decade is the lack of accurate prognostic markers in early localized PC to identify the non-life-threatening tumours.

Two major expectations of the AS approach are to decrease the overtreatment of patients with indolent PC compared to curative treatment of all patients and to minimize the risk of undertreatment of aggressive tumours in patients in comparison to WW.

The ideal characteristics for a patient who can be offered AS are PSA <10ng/ml, a Gleason score of <7, T1c –T2a. These characteristics describe 50% of newly diagnosed PC in western countries (Klotz 2005). In a prospective study comprising 299 patients with low-risk PC, 65% of the patients were without any active treatment after eight years. The prostate cancer-specific survival rate was 99.3% at eight years (Klotz 2006).

Non-Curative Treatment

Watchful waiting (WW)

The fact that the majority of men harbouring a prostate cancer will be unaffected by the disease during their lifetime had led to the WW approach being promoted on a varying level in different countries. Traditionally, WW was adapted more in northern Europe than in the US and the rest of Europe. The patients would not receive any treatment until the debut of a symptom related to PC, whereupon symptom-relieving treatment would be started.

This is an excellent approach in men with a short life expectancy and without poorly differentiated tumours. There is a substantial number of studies which support such an

approach in these selected patients. Albertsen et al. demonstrated that cancer-specific mortality after 15 years for patients with localized PC aged 55-74 years was strongly related to the Gleason score. The cancer-specific mortality for patients with a Gleason score of 2-4, 5, 6, 7 and 8-10 was 8%, 14%, 44%, 76% and 93% (Albertsen, Hanley et al. 1998; Albertsen, Hanley et al. 1999).

Screening for prostate cancer

Introducing population-based screening for a disease requires three essential conditions:

- The disease constitutes a serious health problem in society.
- The disease can be diagnosed in an early preclinical stage.
- The treatment must be effective, prevent the disease from progressing and decreasing mortality.

PC is a serious health problem in developed countries and PC is feasible to diagnose at an early preclinical stage. The debate on PC screening is about the validity of the third demand. There is one prospective randomized trial from the city of Quebec that suggests a 62% reduction in cause-specific mortality in the screened men after a median follow-up of 7.9 years. (Labrie, Candas et al. 2004). This study have been criticized because of the low compliance rate in the invited-to-screen group (24%) (Pinsky 2004). In this study the mortality reduction was calculated in the men who were participating in the screening arm and not the entire screened arm. Nevertheless, the question that must be answered by a prospective randomized screening study is “what happens if a screening programme is applied to the general population” (Roemeling, Roobol et al. 2007). The Quebec study has failed to clarify this question.

There is a prospective, randomized study that shows a reduced risk of distant metastasis, cancer-specific mortality and overall mortality in patients with localized PC who received radical prostatectomy at diagnosis compared to patients that were managed by watchful waiting until the clinical symptoms occurred. (Bill-Axelson, Holmberg et al. 2005). This study showed a 5% reduction in cancer-specific mortality in the radical prostatectomy arm after a median follow-up of 8.2 years. The number of patients that needed to be

treated to prevent one man from PC death was 19. The impact of potential life-long side effects of the treatment should be considered in an interpretation of the study results.

There are two ongoing, prospective, randomized studies to evaluate the effect of PSA screening. The European Randomized Study for Screening of Prostate Cancer (ERSPC) (Schroder, Denis et al. 2003) and the US Prostate, Lung, Colon and Ovary trial PLCO (Andriole, Levin et al. 2005). The results from these studies are expected within a few years. At the present time there is not enough scientific evidence to legitimize general screening for prostate cancer. Another major issue that must be clarified apart from the issue of reduced mortality is the management of patients with early disease, i.e. clinically insignificant cancer, which will increase dramatically if PSA screening is introduced. It is essential to have a strategy for handling these patients before starting a screening programme to reduce the risk of overtreatment of patients with early PC (Bangma, Roemeling et al. 2007).

Finally, the men with a positive family history of PC run a high risk of developing the disease. The risk is correlated with the number and the age of the relatives who had the disease. A man with no family history of PC has an 8% lifetime risk of developing the disease. A man whose father had the disease after the age of 70 years has a 12% lifetime risk and a man with three first-degree relatives with the disease before the age of 70 years has a 40% lifetime risk (Gronberg, Wiklund et al. 1999; Bratt 2007).

In counselling the men with a family history of PC it is important to estimate an individual risk profile. These men are more likely to opt for screening because of their family history and if, after the individual risk assessment and information, they decide to participate in a PSA-based follow-up (screening), it is recommended that the follow-up starts five years before the age of the youngest relative at diagnosis or at least 10 years before the age of the relative who developed metastasis. (Bratt 2007).

Prognostic markers in early prostate cancer

The prognostic information in early PC can be originated from blood/serum or prostate tissue from a core biopsy.

PSA, ratio of free-to-total PSA, complex PSA and PSA density have been discussed before. These markers can be useful in improving the precision of PSA as a diagnostic test but offer limited additional prognostic information in a large cohort of men with early-detected PC with a homogeneous Gleason score (<7), T-stage (T1c) and PSA range (<10) (Babaian, Fritsche et al. 1990; Catalona, Partin et al. 1998; Aus, Damber et al. 2005).

PSA kinetics, PSA velocity and PSADT

The most usual cause of increased PSA in the range 4-10 ng/mL is BPH. It is not realistic, based on a single PSA measurement in this range, to make an accurate assertion that the PSA rise is due to the presence of cancer or BPH. To improve the accuracy of the PSA as a marker of PC, serial measurement of the PSA during the time period has been suggested. The rationale behind this assumption is that a rising PSA caused by cancer is more rapid than a PSA rise caused by BPH. The idea is that a series of PSA measurements over a period of time could distinguish those men with a very low risk of cancer in a biopsy. In a case control study Carter et al demonstrated that five years prior to diagnosis men who developed PC had a significantly greater increase in the PSA rate per year (PSA velocity) than men in a control group and men with BPH. (Carter, Pearson et al. 1992).

In 1992, Carter et al calculated PSADT in men with no prostatic disease, men with BPH and in men with prostate cancer. Patients with cancer had a linear increase initially but seven years before clinical detection of the PC the initially linear increase in PSA switched to an exponential phase, a switch that was absent in the other two groups in the study (Carter, Morrell et al. 1992). Schmidt et al. presented their work on PSADT in 1993 and suggested that the increase in PSA in patients with PC was exponential (log linear) and that PSADT could be used as a proxy for increasing tumour volume (Schmid, McNeal et al. 1993).

Several authors have investigated the PSA velocity and the PSADT as a prognostic marker and the results strongly support the hypothesis that a rapid rise in PSA expressed as PSA velocity or PSADT in patients with prostate cancer is strongly associated with more severe prognosis (Roberts, Blute et al. 2001; Stephenson, Aprikian et al. 2002; Ward, Zincke et al. 2004; Bates, Pickles et al. 2005; Freedland, Humphreys et al. 2005; Lee, Levy et al. 2005; Lin, Schultz et al. 2005; Freedland, Humphreys et al. 2007).

One study of 2,290 men who underwent radical prostatectomy with a median follow-up of 7.1 years revealed that the preoperative PSA velocity was a predictor of biochemical progression. In the same study preoperative PSADT was a significant predictor of clinical progression and cancer death (Sengupta, Myers et al. 2005).

There is massive support in the literature for the prognostic value of PSA kinetics but in the diagnostic area PSA kinetics have not been shown to be as useful. In a recent study, Spurgeon et al reported that neither PSADT nor PSA velocity could predict cancer detection or the presence of high-grade cancer in biopsy (Spurgeon, Mongoue-Tchokote et al. 2007). The PSADT and repeated biopsies have been suggested as being excellent prognostic tools for monitoring the patients during active surveillance (Klotz 2005; Klotz 2006)

The histopathological evaluation of prostate cancer

This evaluation is based on a core biopsy. The information that should be extracted from the histopathological assessment is the following: the number of cores with cancer, the total amount of cancer in biopsies or the percentage of cancer in biopsies, the presence of high-grade prostatic intraepithelial neoplasm and the Gleason score. The reason why these parameters are important is the prognostic value of each factor.

Gleason score

The Gleason system is the most commonly used histopathological grading for PC (Gleason 1966). The Gleason score has been shown by several authors to be a strong prognostic marker in organ-confined PC (Epstein, Carmichael et al. 1993; Epstein, Pizov et al. 1993; Partin, Yoo et al. 1993; Epstein, Walsh et al. 1994).

The amount of cancer in biopsies

McNeal et al demonstrate the strong correlation between the tumour volume and disease progression after RP (McNeal, Villers et al. 1990). There is substantial evidence in the literature supporting a correlation between the amount of cancer in biopsies and biochemical recurrence after RP (Freedland, Aronson et al. 2003; Villamon-Fort, Martinez-Jabaloyas et al. 2007). Freedland et al demonstrate that the percentage of needle biopsy tissue with cancer was more predictive of PSA relapse after RP or advanced pathology than the Gleason score or preoperative PSA (Freedland, Csathy et al. 2002). The same author further revealed that the amount of cancer in a biopsy can be used to preoperatively stratify patients into low, intermittent and high-risk for PSA relapse after RP (Freedland, Csathy et al. 2002).

Angiogenesis and tumour vascularity (TVC)

The metabolic need of a tumour is the main reason that the growth of a tumour above a certain volume is essentially dependent on the formation of new blood vessels, neoangiogenesis (Folkman, 1974; Folkman, 1976).

The different markers of angiogenetic activity measured in the blood or in tumour tissue have been suggested as being prognostic markers in PC (Silberman, Partin et al. 1997; Borre, Offersen et al. 1998; Strohmeyer, Rossing et al. 2000).

An increased density of capillaries has been demonstrated in PC tissue in radical prostatectomy specimens compared to benign prostate tissue (Bigler, Deering, et al. 1993). Because of the limited amount of the cancer tissue in a biopsy, in the majority of published studies the prostate tissue used for assessing TVC was originated from a prostatectomy specimen or TURP.

Proliferation marker, Ki-67

Ki-67 is a nuclear protein that is present in all the active phases in the cell cycle but is absent in the resting cells. This is the reason why this protein is strictly associated with cell proliferation and consequently the expression of Ki-67 in the cells is used as a proliferation marker.

An increased proliferation index measured by Ki-67 had been reported to be correlated with biochemical recurrence after radical prostatectomy (Cowen, Troncoso et al. 2002),

distant metastasis and mortality in men who have undergone radiotherapy and hormonal treatment (Pollack, DeSilvio et al. 2004) and cause-specific survival (Stattin, Damber et al. 1997).

New promising markers

There are a number of different molecules and genes that have been evaluated as prognostic markers in PC. Vascular Endothelial Growth Factor (VEGF) has been suggested as being a predictor of biochemical disease progression after radical prostatectomy (Shariat, Anwuri et al. 2004). Bcl-2, P53 and E-cadherin are some of the most investigated and promising markers. These markers often demonstrate a significant prognostic value in a univariate analysis. The problem is that the majority of these markers appear to have limited additive prognostic information in the multivariate analysis, including the clinically used prognostic markers such as PSA, T-stage and Gleason score (Buhmeida, Pyrhonen et al. 2006). However, none of these factors have been accurate enough to serve routinely as a prognostic marker.

Classification and staging

TNM is the classification system based on the local extension of the tumour, the presence of the tumour in lymph nodes and the existence of distance metastasis**.** The 2002 TNM classification is generally used worldwide. (Sobin L.H 2002)

T-stage

Digital Rectal examination (DRE) is a subjective test and there is a substantial risk of over- and under-staging. Carter and Partin made a summary based on four articles which compare pathological stage with clinical stage based on DRE. In this summary only 54% and 46% of T1c and T2 tumours were organ-confined at the pathological evaluation. Of patients with a T3 tumour at DRE 19% had organ-confined disease. The authors conclude that DRE represents a sensitivity of 52% and a specificity of 81%. In other words the DRE is a more reliable predictor of progression when DRE suggests the presence of advanced disease but T-stage based on DRE is an unreliable predictor of progression when the DRE suggests localized disease (Schröder F.H. 1997).

N-stage

Staging based on the presence of lymph node metastasis in the obturator fossa by lymphadenectomy should be performed only when the finding will influence the treatment decision. This Pretreatment staging has limited value in low-risk patients (PSA <20 or stage T2 or less and a Gleason score of 6 or less). Generally, the presence of lymph node metastasis leads to discontinuation of the planned curative irradiation (Aus, Abbou et al. 2005).

M-stage

To investigate the presence of the skeletal metastasis a bone scan is performed. However, in asymptomatic patients with well or moderately differentiated tumours and PSA <20, the risk of harbouring bone metastasis is extremely low and the bone scan does not add any prognostic information in these patients. In a study of newly diagnosed PC patients all 237 patients with PSA <15ng/ml, a Gleason score of 2-7 and T stage <= 2 had a negative bone scan (Lee, Fawaaz et al. 2000).

In conclusion, the TNM classification is correlated with the prognosis in PC. However, in the cohort of men with early PC (T1c N0 M0 diseases), this classification seems not to offer any prognostic information.

The prognostic dilemma with early-detected prostate cancer

Traditionally, T-stage, amount of cancer in the core biopsy, the Gleason grade and PSA at diagnosis have been used to evaluate the prognosis in localized PC. The therapy discussion and decision is strongly affected by this estimated prognosis. In a heterogeneous cohort of patients each of the above factors has been demonstrated as harbouring valuable prognostic information. Several authors have reported that better accuracy could be gained by a combination of these markers in a neural network or nomogram (Graefen, Karakiewicz et al. 2002; Stephenson, Scardino et al. 2006).

The problem is that men with early-detected PCs have in 50% of cases a homogeneous pattern regarding these prognostic markers (PSA <10, T1c, Gleason score <7) (Klotz 2006). In a biennial PSA screening programme the number of these patients increased to

80% of cases (Hugosson, Aus et al. 2004). In this growing group of patients the traditional prognostic markers seem to lose their efficacy and subsequently the therapy decision becomes very complicated because of a lack of accurate prognostic information. This situation puts the patients with early-diagnosed, low-risk PC in a very stressful situation, particularly younger patients.

To prevent overtreatment in early, low-risk PC the active surveillance approach has been popularized during the past decade. An active surveillance approach can be considered as a “prolonged prognostic test”, during the test period the patient is evaluated by repeated PSA and biopsies. The new information that originates from repeated PSA and biopsies can improve the evaluation of the aggressive potential of the tumour and thereby offer a superior platform for a therapy decision.

There is no prognostic marker that can reduce the risk of overtreatment of PC without increasing the risk of undertreatment. It is more a question of which level of overtreatment can be tolerated. It is essential to emphasize that different prognostic markers provide altered information. In other words a marker that can recognize the aggressive potential in a tumour cannot consistently exclude all the harmless tumours.

A marker which in the early stage of the disease can identify the aggressive PC decreases the risk of undertreatment and a marker that can identify the harmless PC prevents overtreatment. The active surveillance approach seems to be an excellent tool to decrease overtreatment but a longer follow-up in the ongoing studies is mandatory to explore whether this approach can also decrease the risk of undertreatment in aggressive PC tumours.

The aims of the present studies

Study I

To evaluate whether initial surveillance followed by prostatectomy impairs the pathological stage compared to immediate surgery in men with prostate cancer detected as a result of early screening.

Study II

To evaluate whether tumour vascularity by Chalkley counting (TVC) in prostate core biopsies can be a predictor of PSA recurrence after radical prostatectomy in prostate cancer and to estimate the concordance between the TVC in core biopsies and the subsequently examined prostatectomy specimen.

Study III

To evaluate the outcome of active surveillance in men with PSA screening-detected prostate cancer (PC), PSA doubling time (PSADT) was evaluated as a predictor for selecting patients for active treatment or surveillance.

Study IV

To evaluate the Ki-67 as a prognostic marker in early-detected PC and to explore the relationship between PSADT and Ki-67.

Study V

To explore the influence of different factors on PSADT in men who participated in a PSA screening programme.

Patients and methods

The patient cohort for Studies I, III, IV and V originated from the Gothenburg randomized PSA screening study. The patients in Study II were selected from a cohort of all the patients who underwent radical prostatectomy at Sahlgrenska University Hospital, Gothenburg, between 1990 and 1997.

(I) In Study I, 26 of the patients in the initial surveillance group had undergone radical prostatectomy as of December 31, 2000. For each case, two control cases were randomly selected from those patients who were operated on without prior surveillance. The mean period of surveillance was 23.8 months (9 - 55.5).

The 26 patients in the surveillance group and the 52 patients in the control group were matched for PSA, age, T stage and Gleason score at biopsy (Table I).

Table 1

Characteristic of the age, preoperative serum PSA, prostate volume and clinical stage in the surveillance group and control group

	Surveillance group (n = 26)	Control group (n = 52)	***P-value***
Age mean; median (range)	60.9; 62 (51-67)	63.1; 63 (51-69)	
T-stage, T2/T1c	5/21	10/42	
PSA ng/ml	5.35(3.05-13.2)	4.83(3.03-9.92)	*0.71*
Prostate volume, ml	39.8 (17.9-79)	37.6 (18.7-70)	*0.54*
PSA density ng/ml/cc	0.15 (0.06-0.43)	0.13 (0.07-0.27)	*0.76*

There were no statistical differences in age, T-stage, preoperative PSA, PSA quote, prostate volume or PSA density between the surveillance group and the control group (Mann-Whitney test).

The time between biopsy and surgery was registered. One pathologist (C-G. P.) re-examined all biopsies and RRP specimens without knowing which group the patients belonged to. In biopsies, the total length of the cancer in millimetres (core cancer length) and the Gleason score were registered and in prostatectomy specimens the tumour volume, presence of extracapsular tumour growth (pT3) and Gleason score were registered. PSA relapse was defined as two postoperative values exceeding 0.1 ng/ml. The significance of differences between the two groups was tested using Mann–Whitney and χ^2 tests.

(II) To obtain a longer follow-up, the original cohort in Study II consists of 363 patients who underwent radical prostatectomy between 1990-1997 at our hospital. Of these patients, 171 experienced PSA recurrence during the mean follow-up time of 93.7 months (68.6-148.7). All the patients with neoadjuvant hormonal treatment, stage T3, a Gleason score >7 as well as the patients who had TURP or cytology as a source of diagnosis were excluded. Of the remaining 77 patients, 25 had PSA recurrence during the follow-up. Another 25 patients were randomly selected from the group without PSA recurrence.

The final cohort for evaluation consisted of 50 patients with T1-2, a Gleason score <8 and who did not receive neoadjuvant hormonal treatment. This patient's selection resulted in one group of 25 patients with PSA recurrence (two consecutive PSA values above 0.1 ng/ml) and the other without PSA recurrence.

All the biopsies and the prostate specimens were examined by one pathologist (C-G P), who had no knowledge of the clinical outcome of the patients. Tumour vascularity (TVC) in biopsies was added to the prior database, including the preoperative prognostic markers. The characteristics of the preoperative prognostic markers and TVC in biopsies are presented in table 2.

Table 2
Comparison of preoperative prognostic markers in the PSA recurrence and non-recurrence groups, number of patients*, [median], (range)

	Total	Non-recurrence	Recurrence
PSA ng/ml	9.7[8]	5.48(2-9.7)	13.4(3.6-28.7)
Gleason score 6	32*	21*	11*
in the core biopsy 7	18*	4*	14*
T-stage T1	23*	18*	7
T2	27*	7*	18*
Core cancer length, mm	10.4[8]	8.1[7]	11.9[8.9]
TVC; mean vessel hits in biopsy	5.00(2.75-13.40)	4.39(2.75-6.80)	5.52(3.10-13.40)

(III) The cohort in Study III consists of men with screen-detected PC who were initially managed by active surveillance. As of December 2004, 660 men were diagnosed with PC in the study. Of these, 270 were managed primarily with surveillance. Surveillance was defined as an active standpoint to postpone the treatment until at least six months after diagnosis.

The reasons for choosing surveillance were comorbidity, small-volume cancers in biopsies or the patient's wish. In many cases, there was more than one reason for choosing surveillance. Small-volume cancers in biopsies were classified as one or two adjacent cores with a total core cancer length of less than 2 mm and where rebiopsies of the area did not reveal more cancer.

The patient's age, PSA, free/total PSA and PSA density at diagnosis were registered. The core biopsy information, including the total number of cores, number of cores with cancer, total length of cancer in the biopsy, the GS and the clinical tumour stage according to the TNM classification (1992), were prospectively registered in the database. During follow-up, new information, including the PSA at the time of active treatment and the type of treatment following surveillance, were registered.

The characteristics of the entire surveillance group at diagnosis, stratified according to age group, are presented in Table I. The median age was 64.6 (51.2-70.0) years; the median PSA at diagnosis was 4.2 (3.0-27.8) ng/ml. Of the patients, 87% had T1c and none of the patients were N1 or M1.

Table 3
Distribution of PSA, ratio of free PSA, PSA density (PSAD) and TNM stage in different age categories

Age	**50-54**	**55-59**	**60-64**	**65-70**	**All**
Number of patients	6 (2%)	44 (16%)	99 (37%)	121 (45%)	270 (100%)
PSA, mean/median (range)	4.7/3.6 (3.1-9.9)	5.0/4.5 (3.0-15.6)	4.7/3.9 (3.0-16.4)	5.8/4.3 (3.0-27.8)	5.2/4,2 (3.0-27.8)
PSAD, mean/median (range)	0.16/0.14 (0.06-0.37)	0.16/0.14 (0.06-0.56)	0.14/0.11 (0.04-1.02)	0.16/0.13 (0.04-0.74)	0.15/0.12 (0.04-1.02)
Ratio of free PSA mean/median (range) **Range**	11.1/8.9 (6.7-21.2)	16.5/15.7 (6.4-28.9)	19.3/18.2 (5.5-44.9)	19.6/18.5 (5.4-47.4)	18.8/17.8 (5.4-47.40)
T1c (No. of patients) **T2 (No. of patients)** **T3 (No. of patients)**	6 0 0	37 7 0	84 14 1	108 13 0	235 (87%) 34 (12.6%) 1 (0.4%)

PSA doubling time (PSADT) was calculated based on PSA measurements at least three months apart. PSADT was estimated for each patient as the reciprocal of the slope from regression of log-2 PSA on time. We used the PSA at diagnosis and the latest PSA value before any active treatment was received or at the last follow-up for the patients still under surveillance (Schmid, McNeal et al. 1993).

Differences in continuous prognostic markers (PSA, ratio of free-to-total PSA, PSA density, PSADT, total cancer length in biopsies and age) between groups were tested using the Mann-Whitney U test. The Kaplan-Meier estimates were used for calculating time to active treatment (patients were considered censored at the time of the last follow-

up or death due to other causes) and to calculate PSA-free survival in men treated with RRP.

The influence of possible preoperative prognostic covariates on PSA-free survival after RRP was tested in a Cox proportional hazard model. A p-value of <0.05 was regarded as significant. All analysis was performed using the SAS statistical program.

(IV) Patients in Study IV are a subgroup from Study III. Of the 270 patients in the surveillance group within the screening study, 70 discontinued the surveillance and received radical prostatectomy during the follow–up. All these 70 patients had a Gleason score of 3+3 in the core biopsy. These patients were stratified into three PSADT groups: PSADT <2years, 2-4 years and >4 years. Fifty of these 70 patients were selected for this study; all 14 patients who had PSADT <2 years, all 18 patients who had PSADT = 2-4 years and of the remaining 38 patients with PSADT > 4 years, 18 were randomly selected for this study. A total of 50 patients were selected for further investigation. Nine cases of PSA relapse (two consecutive PSA values above 0.1ng/ml) were observed during the mean postoperative follow-up of 63 months.

The correlation between Ki-6 and PSADT as well as Ki-67 and Gleason grade was tested. A Spearman test was used to calculate the correlation between Ki-67 and PSADT. A Mann-Whitney U test was used to test the possible differences in expression of Ki-67 in different Gleason grades. A Cox proportional hazard model was used to evaluate the influence of Ki-67 and other markers on the risk of PSA relapse after radical prostatectomy. A p-value of less than 0.05 was considered to be statistically significant.

(V) The entire cohort of the Gothenburg screening study was explored in Study V. Patients in the screening group were invited to the first PSA testing during 1995 and 1996, and were then invited for PSA testing every second year until they reached the age of 70.

Men with PSA levels >= 3 ng/ml were offered a laterally directed, TRUS-guided sextant biopsy of the prostate. Around 90% of men with elevated PSA have accepted further investigation with biopsy. A total of 7,510 men have participated at least once during these six rounds of screening and 6,387 have participated at least twice. These 6,387 men

made up the Study V population. In cases with more than two PSA tests the two latest tests were used for PSADT calculations.

A Gleason score was obtained from the pathological examination of core biopsies and split into two categories: Gleason 2-6 and Gleason 7-10. The amount of cancer in the biopsies was the total mm cancer length calculated as the sum of cancer extension in the sextant biopsies.

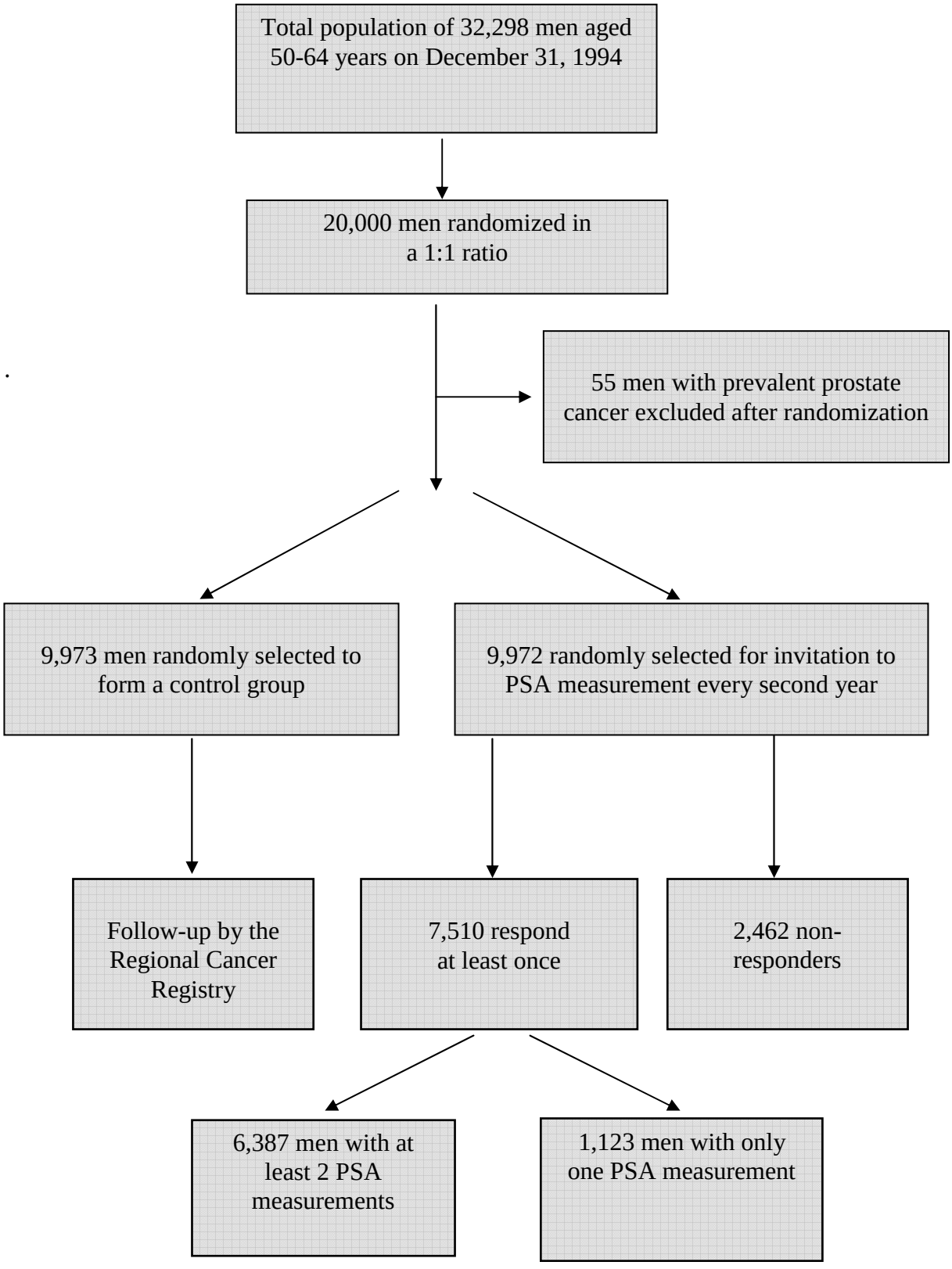
In the entire cohort (6,378 men) the influence of the PSA, F/T-PSA and age on PSADT was analyzed.

In men with PC (n = 582), 564 had complete data (PSA, F/T-PSA, age, prostate volume, amount of cancer in biopsies and Gleason score) and were included in the analysis.

PSADT was calculated as the reciprocal of the slope from regression of log-2 PSA on time $PSADT = log(2)t / log(final\ PSA) - log(initial\ PSA)$ (Schmid, McNeal et al. 1993).

An ordinal regression analysis was used to explore the influence of different parameters on PSADT. The SAS software version 9.1.1 was used. A p-value of less than 0.05 was regarded as a significant result. The algorithm of the Gothenburg screening study is presented on the next page.

The Gothenburg PSA screening algorithm



Statistical methods

Study I: Mann-Whitney U test, Fisher exact test and Mantel-Haezel test were used to calculate the significance of the differences between the two groups for PSA, PSA density, age, prostate volume, tumour volume, total core cancer length, Gleason score and number of biopsy cores with cancer.

Study II: The influence of each preoperative prognostic marker on PSA relapse was analysed using a univariate Cox regression model and odds ratio was calculated.
The Kaplan-Meier estimate was used to determine the PSA-free survival in different TVC-quartiles.

Study III: Mann-Whitney U test was used to calculate the differences between surveillance group and active treatment group regarding prognostic markers.
The Kaplan-Meier estimates were used to calculate the PSA-free survival and the treatment-free survival.
Cox proportional hazard model was used to evaluate each parameter (PSA, f/t- PSA, total cancer length in biopsy and PSADT) as a predictor of PSA relapse after radical prostatectomy.

Study IV: Spearman correlation coefficient test was used to calculate the correlation between Ki-67 and PSADT. To explore the differences in expression of Ki-67 in different Gleason grade, Mann Whitney U test was used. Cox proportional hazard model was applied to evaluate the influence of Ki-67 and other markers on the risk of PSA relapse after radical prostatectomy.

Study V: Ordinal logistic regression analysis was used to explore the influence of prognostic markers on PSADT in different levels. The analyses were performed for the entire screening cohort and for the subgroup of men with cancer in the biopsies.
In the all of the studies a *p*-value of less than 0.05 was considered to be statistically significant. All the tests were two-sided.

Results

Study I

The surveillance group SG and control group CG were well matched for age, PSA and clinical stage. All the biopsies had a Gleason score of 3 + 3 = 6. Evaluation of the biopsy results between the groups showed no statistically significant differences in terms of core cancer length or number of biopsy cores with cancer between the two groups (Table 4).

Table 4
Comparison of the biopsy results in the surveillance group the and control group

	Surveillance group (n = 26)	Control group (n = 52)	P-value
Core cancer length (mm)	4.74(0.6-11)	4.76(0.4-22.1)	0.56
Number of biopsy cores with cancer (1/2/3/4/5/6)	14/5/5/1/1/0	30/14/4/2/2/0	0.65
Gleason score in biopsy (5/6/7)	0/26/0	/0/52/0	

Of the patients in the SG, 54% had cancer in only one biopsy core and 19% had cancer in two biopsy cores. In the CG, 58% of the patients had cancer in one biopsy core and 27% had cancer in two biopsy cores.

The mean time between biopsy and operation was 23.4 months (range 9–55.5 months, median 20.4 months) in the SG. In the CG the mean time to operation was 4.7 months (range 1.8–8.2 months, median 4.6 months). The results from the pathological examination of PC specimens are shown in Table 5.

Table 5

The results from the pathological stage in the surveillance group and the control group.

	Surveillance group (n = 26)	Control group (n = 2)	P-value
Tumour volume, ml Mean (range) median	1.35 (0.14-3.54) 0.99	1.04(0.05-4.17)0.85	0.18 (MW)
pT3 number of patients	3	10	0.39 (cs)
Gleason score in RRP 5 / 6 / 7	6 / 15 / 5	2 / 42 / 8	0,50(cs)

There were no statistically significant differences in tumour volume, capsular penetration (pT3) or Gleason score between the groups (MW = Mann-Whitney U test, cs = chi square test).

There were 11.5% and 19.2% of pT3 tumours in the SG and CG respectively. The Gleason score was upgraded from 6 to 7 in 19% and 15% of patients in the SG and CG, respectively. The Gleason score was downgraded from 6 to 5 in 23% and 3% of patients in the SG and CG respectively. This small difference was not statistically significant. The mean follow-up after RP was 23.3 months and during this time only two PSA relapses occurred in each group.

Study II

All of the preoperatively prognostic markers, except core cancer length, were related significantly to PSA relapse after radical prostatectomy in a univariate analysis (Table 6). Patients with the lowest TVC quartile had a PSA-free survival of 67% compared to patients with the highest TVC quartile who had a PSA-free survival of 17%.

Table 6

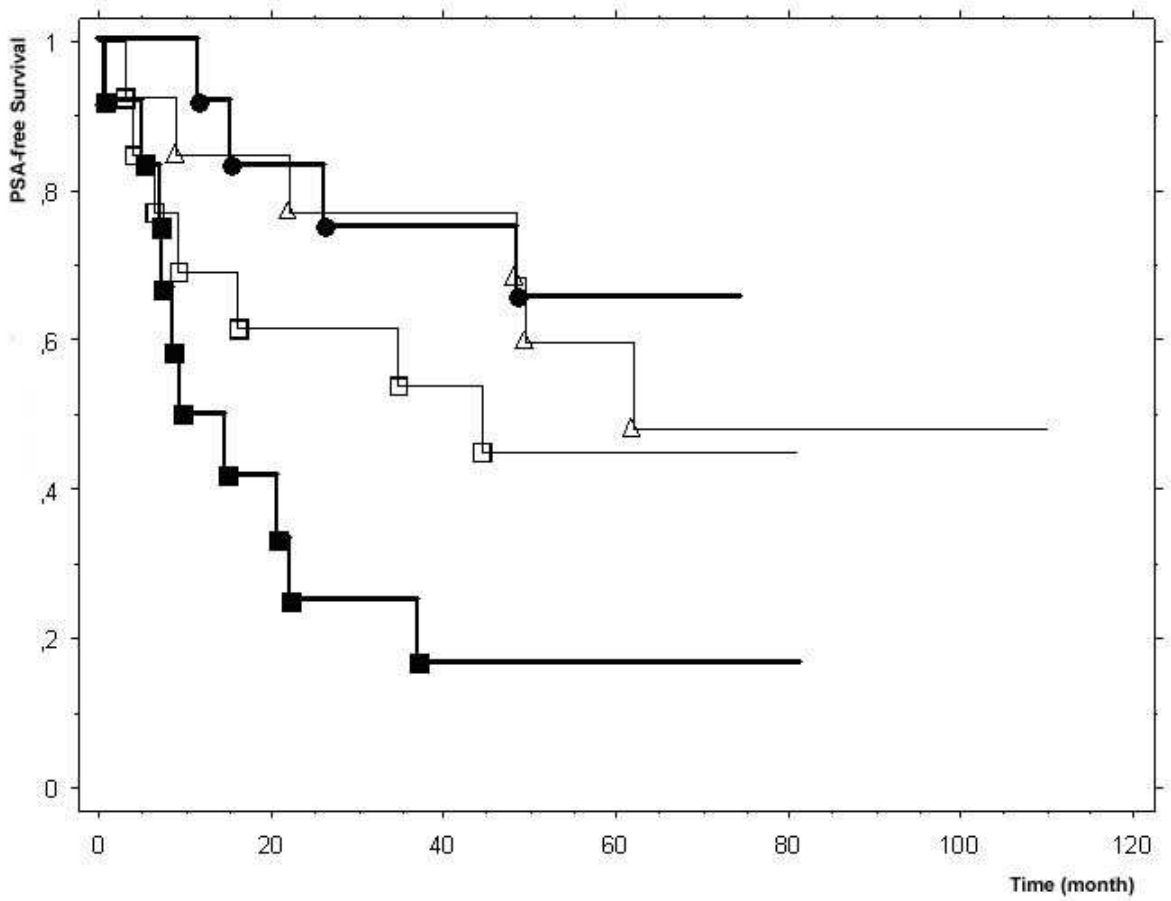
The results of univariate analysis. The odds ratio for PSA recurrence is calculated for each preoperative prognostic marker.

Prognostic marker	**Odds ratio**	**p-value**
PSA	1.69 [1.36 - 2.12]	<0.0001
Gleason score biopsy	1.05 [1.02 - 1.10]	0.0075
T-stage	6.53 [2.72 - 15.66]	<0.0001
TVC biopsy	1.97 [1.11 - 3.48]	0.02

The Kaplan-Meier diagram demonstrates PSA-free survival in different TVC quartiles (Figure 2).

Figure 2

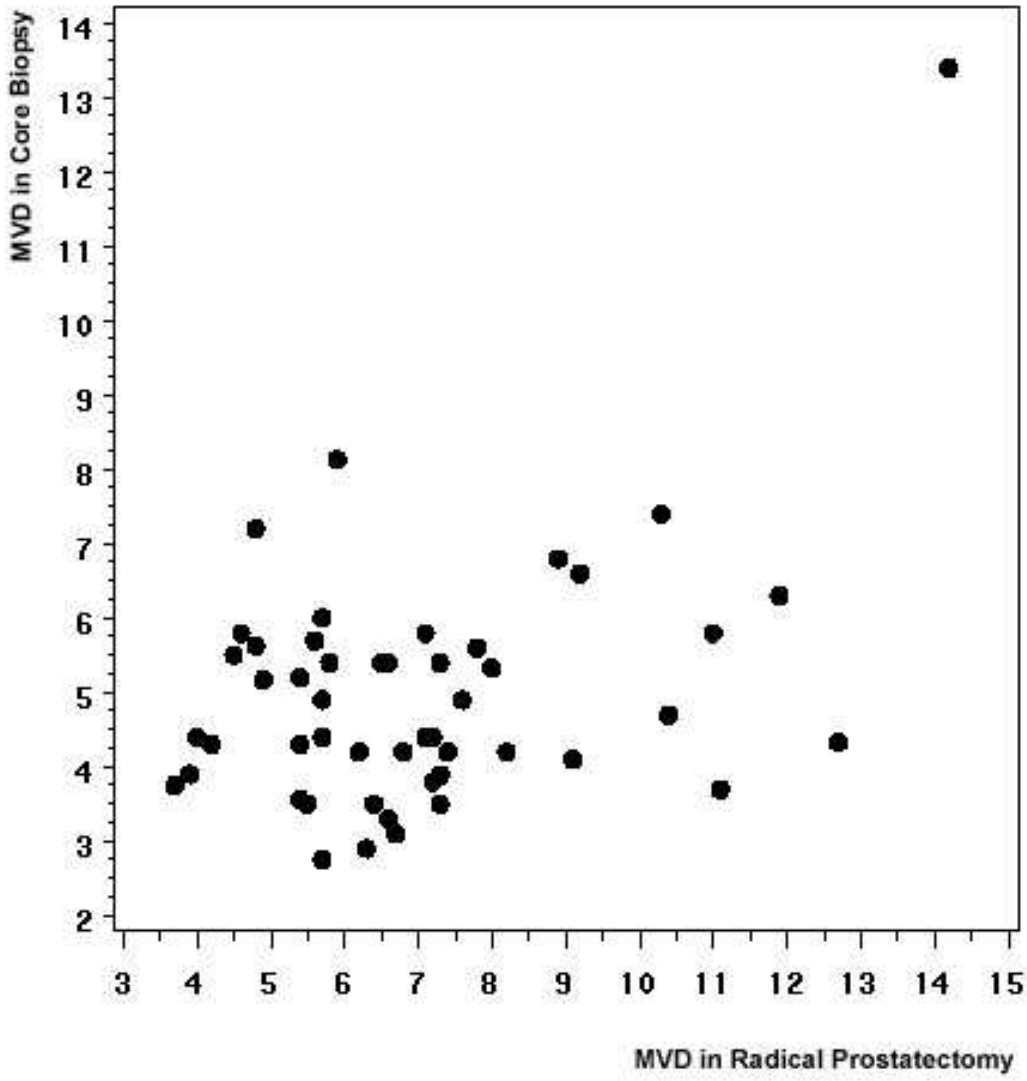
Kaplan-Meier analysis of time to PSA relapse in relation to different quartiles of MVD in core biopsy. Patients with the highest TVC are in first quartile. The PSA-free survival rate was 17% in the first quartile ■, 46% in the second quartile □, 54% in the third quartile △ and 67% in the fourth quartile ● (P = 0.020)



The correlation between TVC in the core biopsy and the radical prostatectomy specimen is presented in Figure 3.

Figure 3

The relationship between TVC in the preoperative core biopsy and the postoperative prostate specimen. A correlation coefficient of 0.41 was found (P = 0.003)



Study III

The mean follow-up time was 63 (11-120) months. During this time period, 104 (39%) patients changed from surveillance to active treatment, with RRP in 70 (67%) patients, radiation therapy in 24 (23%) patients and hormonal therapy in 10 (10%) patients. During the follow-up, another ten patients had been treated due to urinary obstructive symptoms; three with TURP, one with TUMT and six patients with 5-α reductase inhibitors.

Table 7
Distribution of prognostic markers between the active treatment group and the surveillance group.

	All	Continued surveillance	Active treatment			Treatment	p-value#
			RRP	Radiation	Hormonal		
PSA at diagnosis	5.2 (4.2)	5.2 (4.1)	4.9 (4.2)	5.0 (3.9)	8.2 (7.0)	5.3(4.2)	0.41
Ratio free PSA %	18.8 (17.8)	19.9 (19.3)	17.3 (16.7)	17.0 (15.7)	15.5 (13.9)	17 (16)	0.007
PSA density	0.15 (0.12)	0.15 (0.12)	0.15 (0.12)	0.16 (0.13)	0.21 (0.17)	0.16 (0.13)	0.52
TCL* in biopsy	3.4 (1.9)	2.9 (1.6)	3.2 (2.0)	6.9 (4.6)	6.4 (6.6)	4.3 (2.5)	0.003
Age	63.8 (64.5)	64.8 (65.5)	61.6 (62)	63.0 (64.3)	64.4 (65.5)	62.1 (62.6)	<0.0001
PSA at treatment	-	-	6.7 (5.9)	7.7 (5.4)	11.9 (9.9)	7.4 (5.9)	-
Time to treatment	-	-	28.4 (21.0)	21.2 (15.4)	36.6 (24.6)	-	-
PSADT	7.1	11.8	3.8	2.5	5.0	3.7	<0.0001

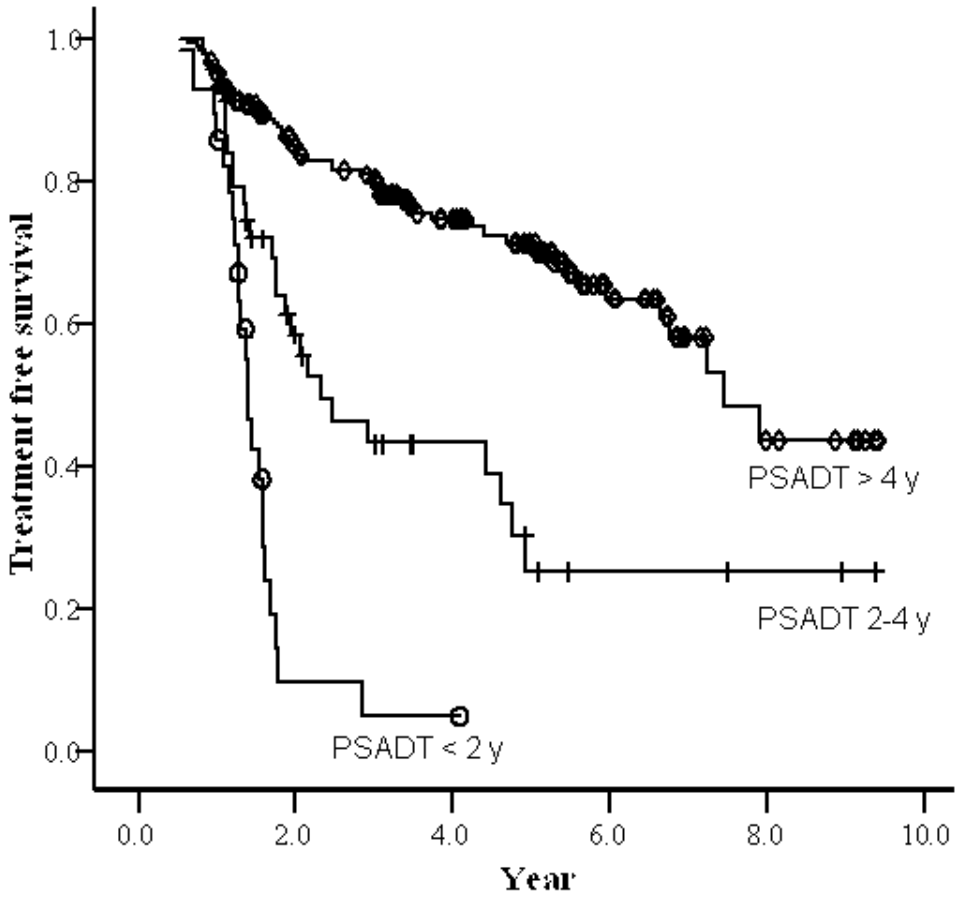
Mean (median), PSADT = median, TCL =Total cancer length, # =Active treatment vs surveillance*

The distribution of different prognostic markers in those who received active treatment and those who were still under surveillance is presented in Table 7. Patients who received active treatment were significantly younger ($p < 0.0001$), had a lower free PSA ratio ($p = 0.007$) and a higher amount of cancer in the biopsies ($p = 0.033$). The median PSADT in patients still under surveillance was 11.8 years compared to 3.7 years ($p < 0.0001$) in those who changed to active treatment. There was no statistically significant difference in PSA at diagnosis or PSA density between the two groups.

We observed that the PSADT was decisive for the patients to discontinue surveillance. Figure 4 illustrates the time to active treatment stratified for different PSADT intervals.

Figure 4

Patients who received active treatment during the follow-up, stratified according to PSADT <2 years (28), PSADT 2-4 years (49), and PSADT >4 years (188). (No. of patients)



Patients at risk:

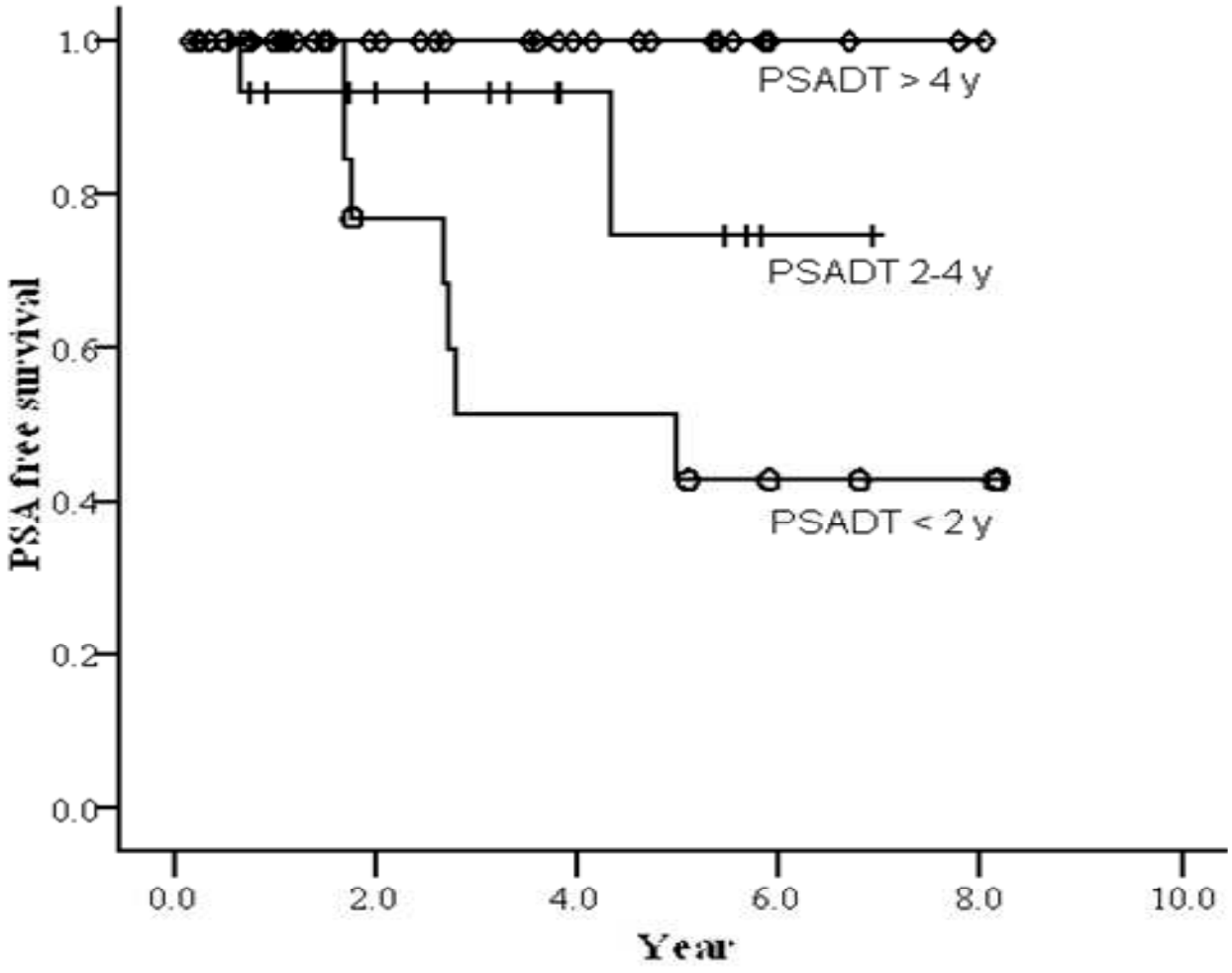
270	212	14	80	51	7

During a mean follow-up of 37 months, nine (13%) of the patients in the RRP group had a PSA relapse. Of the nine patients who had relapsed, seven had a preoperative PSADT of less then two years and the other two had a PSADT of between two and four years. There

was not a single PSA relapse in the patients with a preoperative PSADT of more than four years (Figure 5).

Figure 5

PSA-free survival after radical prostatectomy in different PSADT categories. PSADT <2 years (14), PSADT 2-4 years (18) and PSADT >4 years (38). (No. of patients)



Patients at risk:

70	40	23	8	5

The result of the Cox regression analysis, including PSA at diagnosis, free-to-total PSA and total length of cancer in biopsy, confirmed that the preoperative PSADT was the only parameter that significantly correlated with PSA relapse after RRP (p = 0.031, Table 8).

Table 8
Each variable is tested as a predictor for risk of PSA relapse after radical prostatectomy. Cox regression analysis, including PSA, ratio free PSA, total cancer length in biopsy-TCL and PSADT.

Variable	Parameter estimate	Standard deviation	Risk ratio	P-value
PSA	0.23	0.18	1.27	0.18
Ratio free PSA	-0.08	0.07	0.92	0.29
TCL* in biopsy	0.14	0.12	1.15	0.26
PSADT	**-0.96**	**0.45**	**0.38**	**0.03**

'Parameter estimate' is the logarithm of the risk ratio; a negative value illustrates that a long PSADT reduces the risk of PSA relapse.

During the follow-up, 14 patients in the study population died for reasons other than PC. None of these patients had received active treatment. At the most recent PSA assessment, only five patients had a PSA exceeding 20 ng/mL and all of these patients were still in the surveillance group. In spite of the fact that not all the patients had undergone a bone scan, there was no patient who clinically manifested with bone metastasis or other metastasis during the follow-up.

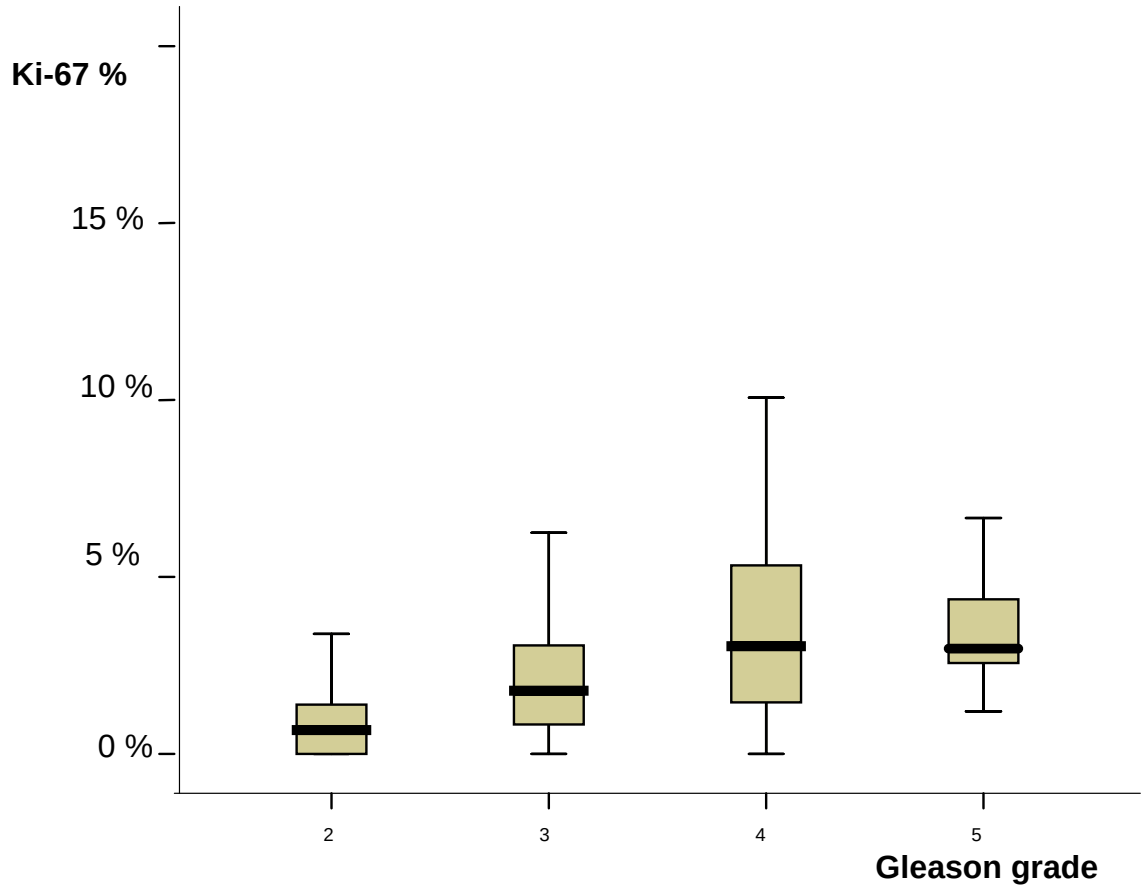
Study IV

The pathological evaluation of the prostatectomy specimen resulted in pT3 in seven cases and 11 cases had positive margins. No cases of lymph node invasion were reported. The Gleason score in biopsies was 3+3 in all patients while the Gleason score in the prostatectomy specimen was upgraded in 18 patients, in 15 patients to Gleason score 3+4, in two patients to Gleason score 4+4 and in one patient to Gleason score 4+5. The Gleason score was downgraded in eight patients to Gleason score 3+2. Due to the small

amount of tumour the percentage of Ki-67 could not be assessed in four patients and these patients were excluded, leaving a total of 46 patients who formed the study base.

The percentage of Ki-67 in different areas of the tumours increased significantly with the Gleason grade. The increase in the percentage of Ki-67 was statistically significant between Gleason grade 2 and Gleason 3 to 4 ($p<0.0001$). (Figure 6)

Figure 6



Ki-67%				
Median (mean)	0.7 (1.0)	1.8 (2.2)	3.0 (3.7)	3.0 (3.6)

There was no statistically significant correlation between PSADT and the average percentage of Ki-67 (p = 0.45). Nor was there any correlation between the total number of Ki-67 staining cells and PSADT (p = 0.23)

The results from the Cox proportional hazard analysis, including PSA, PSADT, percentage Ki-67, tumour volume and the Gleason score in the prostatectomy specimen, are presented in Table 9. In this model total PSA and Ki-67 were the only statistically significant predictors of PSA relapse after radical prostatectomy.

Table 9

Cox regression analysis. Total PSA and Ki-67 in the prostatectomy specimen were significant predictors of PSA relapse after radical prostatectomy, n = 46.

Variable	Parameter estimate	Standard error	P-value	Hazard ratio	HR (95%CI)
PSA, ng/ml at diagnosis	0.62	0.23	0.0068	1.86	1.19 - 2.92
PSA DT	-0.68	0.39	0.0816	0.50	0.24 - 1.09
Ki-67 prostatectomy	0.91	0.43	0.0346	2.49	1.07 - 5.80
Tumour volume, ml	0.62	0.50	0.2221	1.85	0.69 - 5.02
Gleason score in prostatectomy	0.21	0.73	0.7717	1.23	0.29 - 5.22

Study V

The distribution of PSADT in a cohort of randomly selected men who participated in a biennial PSA screening showed that 58% of these men had a PSADT >10 years, only 5% and 14% of patients had a PSADT of <2 years and 2-4 years. Men with elevated PSA and cancer in the biopsies had generally shorter PSADT than men with elevated PSA without cancer in the biopsies (Table 10).

Table 10
Distribution of PSADT in the entire screened population. (*Men with PSA<3ng/ml were not biopsied.)

PSADT, years	PSA < 3 No. of men	PSA >3 Benign bx No. of men	PSA >3 Cancer No. of men	Total No. of men (%)
0-<2	143 (3%)	94 (8%)	78 (13%)	315 (5%)
2-<4	499 (11%)	220 (19%)	177 (30%)	896 (14%)
4-<10	994 (21%)	295 (25%)	165 (29%)	1,454 (23%)
>=10	2,988 (65%)	575 (48%)	159 (28%)	3,722 (58%)
Total	4,624 (100%)	1,184 (100%)	579 (100%)	6,387 (100%)

The results from the ordinal regression analysis for the entire cohort is presented in Table 11, F/T-PSA showed the most significant association with PSADT ($p<0.0001$) followed by age ($p = 0.002$). The total PSA did not reach a significant level in this analysis. The negative sign in the estimate column means that men with a higher F/T-PSA had a shorter PSADT, and subsequently higher age is associated with longer PSADT. (Table 11)

Table 11
The results from logistic regression analysis in the entire screening cohort with at least two PSA measurements (6,378 men). The negative estimate means that a parameter is inversely associated with PSADT.

Parameter	Estimate	Chi-Square	p-value
F/T PSA	- 1.48	55.53	<0.0001
Age	0.02	9.68	0.002
Total PSA	- 0.01	1.94	0.16

Men with cancer in the biopsy were analyzed separately and the results are presented in Table 12. Total PSA and prostate volume were positively correlated with PSADT. This means that in men with cancer the higher the PSA and/or the larger the prostate the longer the PSADT. Patients with a Gleason score of >6 had significantly shorter PSADT compared to men with a Gleason score of 2-6. (Table 12)

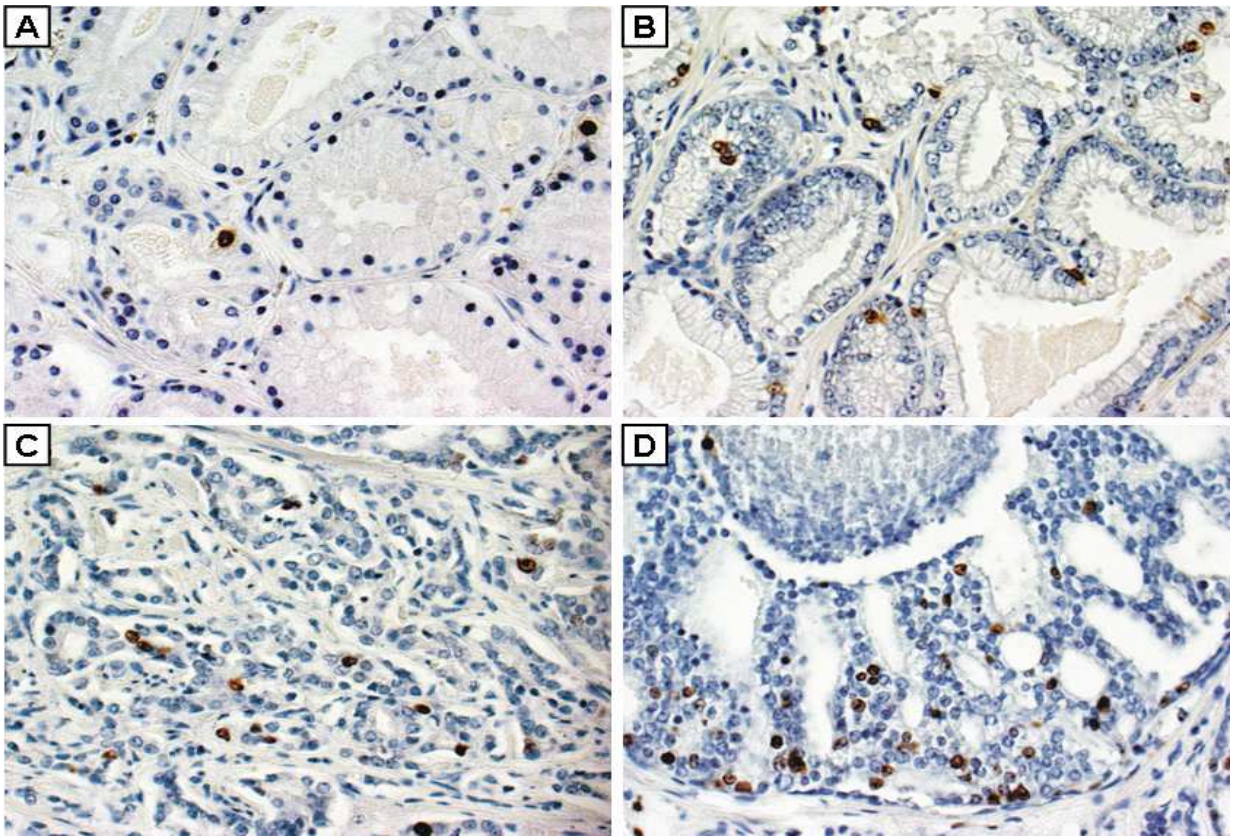
Table 12
The results from regression analysis for all men with cancer in the biopsies (564 men). The negative estimate means that a parameter is inversely associated with PSADT.

Parameter	Estimate	Chi-Square	P-value
Total PSA	0.42	39.76	<0.0001
Gleason score 7-10	- 0.75	8.96	0.0028
Prostate volume	0.02	6.80	0.0091
Total cancer length	- 0.02	1.46	0.23 ns
Age	- 0.02	1.05	0.30 ns
F/T PSA	- 0.95	0.73	0.39 ns

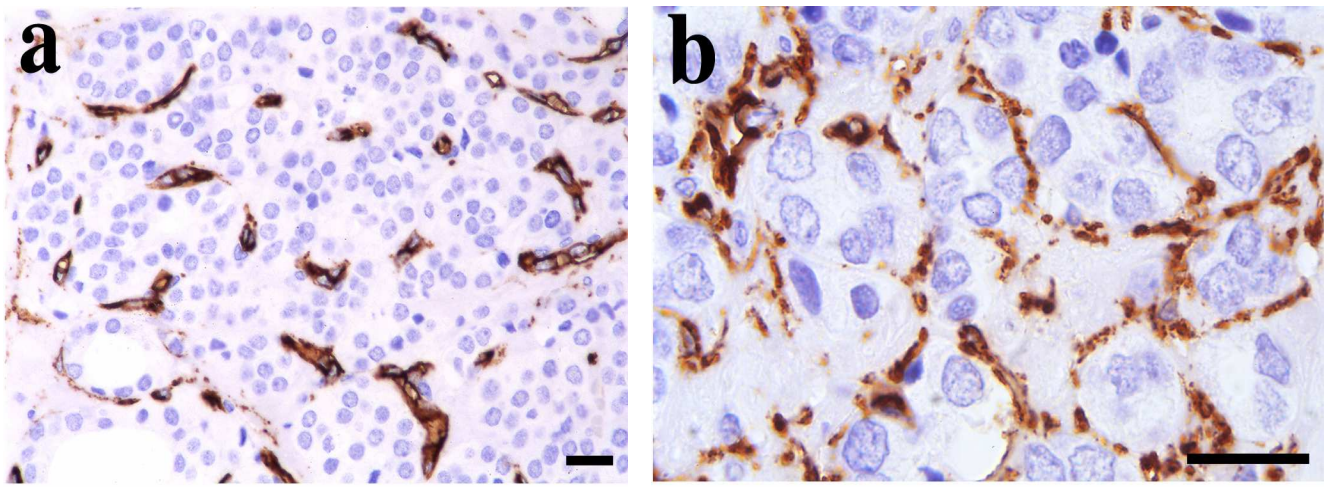
Ki-67 and TVC - microscopic view field

Figure 7

I) Ki 67 nuclear immunostaining in various Gleason grade prostate carcinoma. A, Gleason grade 2; B, Gleason grade 3; C, Gleason grade 4; D, Gleason grade 5.



II) a. Area of prostate cancer with clearly identifiable microvessels (400x).
b. Detail of another viewfield showing many small microvessels (1000x). Bar denote 10 micrometer. Immunostaining with anti CD34.



Methodological considerations

Histopathological analysis and considerations

In **Studies I, III and V** the routinely used pathohistological methods was used to assess the Gleason score in biopsies, total length of cancer in each core biopsy, tumour volume, extracapsular extension of the tumour, positive margin and the Gleason score in prostatectomy specimen.

Study II starts with an experimental study to identify the best marker for assessment of microvessels. Immunohistochemical staining was made to highlight microvessels. Anti-CD31, anti-CD34 and anti-Factor VIII, were used initially for staining three randomly selected tumours to clarify which of the antibodies gave the best result.

Staining with anti-Factor VIII (DAKO, Glostrup, Denmark) revealed relatively few stained microvessels and rather prominent unspecific staining in the stroma. Anti-CD31 (DAKO) also visualized relatively few microvessels but a clean background. AntiCD34 (DAKO) stained a larger number of microvessels than the other immunostainings but often showed some slight, unspecific stromal reaction.

Based on this comparison, anti-CD34 was chosen for the investigation.

Moreover, it is common for investigators to use different criteria, different optical magnification, when defining microvessels and different ways of quantifying microvessels. This may render comparisons between different studies difficult. In the present study, the general criteria set by Weidner for staining and identifying microvessels in formalin-fixed, paraffin-embedded tumour tissue were followed. Instead of assessing MVD we assessed TVC by Chalkley counting. The reason is that the Chalkley point overlaps morphometric technique, removing one of the highly observer-dependent steps when measuring MVD, namely the frequent decision an observer has to make about whether two immunostained and adjacent structures are the reflection of one single or two separate blood vessels. TVC is the objectively assessed relative area that the vessels cover, a measurement that is strongly and significantly associated with vessel number (MVD), i.e. the number of discrete microvessels, and with vessel area per unit of tissue area. In the prostatectomy specimen ten measuring areas were examined for each tumour, which corresponds to a total area of 0.75 mm^2, an area that is close to the one previously recommended for MVD measurements.

In the biopsies, the cancer tissue was often limited, which did not always allow ten view fields to be measured within one and the same hotspot. In these cases, the remaining view field was examined within a second, somewhat less richly vascularized hotspot.

In **Study IV**; Ki-67 positive staining was identified by the presence of brown nuclear (DAB) staining in prostate cancer cells. Any nuclear staining, regardless of intensity, was considered positive for Ki-67. The quantification of Ki-67 positive staining cells was performed by counting 5 to 15 randomly selected microscopic fields measuring at least 1,000 tumour cells using an eyepiece graticule at 400× magnification. The percentage of Ki-67 staining cells was calculated for each tumour area. The percentage of Ki-67 staining cells in each patient was also calculated and mirrored the average percentage in the total tumour area. In each patient the total number of Ki-67 cells was also calculated as the average percentage multiplied by the total tumour volume.

Ki-67 is a proliferation marker and the strong association between Gleason grade and Ki-67 resulted in substantial heterogeneity in the percentage of Ki-67 in different areas of the tumour in the same patient. The major consideration is to decide the true proliferation rate (percentage Ki-67) in the tumour. In this study we used the average percentage Ki-67 although the Ki-67 can be assessed in the 'hotspot', which means the highest level of Ki-67 assessed in an area in the tumour. If a tumour consists of 95% Gleason 3 and 5% Gleason 5 the percentage Ki-67 calculated from the Gleason 5 area is considerably higher than the average percentage Ki-67. The question is which of the measurements mirrors the true proliferation rate in the entire tumour. This is one of the limitations of using Ki-67 as a proxy of proliferation rate in prostate cancer tumours, which in the majority of cases consist of an area with a different Gleason grade.

The considerations in PSADT calculation

The PSADT has been established as a prognostic marker of disease progression in patients with PC. (Roberts, Blute et al. 2001; Freedland, Humphreys et al. 2005; Lee, Levy et al. 2005; Semeniuk, Venner et al. 2006; Khatami, Aus et al. 2007; Freedland, Humphreys et al. 2007). There are several methods for calculating PSADT. The pros and cons of each method have been discussed by several authors (Daskivich, Regan et al. 2006; Svatek, Shulman et al. 2006). To our knowledge a calculation method that

describes the PSA kinetics best has not been established. The two commonly used methods are the log slope method and the first and last or two-point method (Schmid, McNeal et al. 1993), which is the method that has been adapted in this thesis. In both these methods a separate log-linear model for the growth of a patient's PSA over time determine the patient's PSADT. The limitation of these methods is mainly in calculating the PSADT in patients with decreased PSA during the time, which can result in a negative slope. This could be due to the variation in PSA on a day-to-day basis. However, in patients with early PC the prognostic information that generates from PSADT needs to be accurate within a range of 0-10 years. The precision of the PSADT value above a limit of 10 years does not generate any relevant prognostic information. In fact a PSADT >10 years has been reported to recognize the tumours with low aggressive potential. In Study III none of the patients with PSADT >10 years experienced PSA relapse after radical prostatectomy after a follow-up period of four years compared to 50% PSA relapses in patients with PSADT <2 years. The precision of the method of PSADT calculation is most important within a PSADT range up to 10 years.

Both methods of PSADT calculation have been used in different studies, the log slope method and the two-point method. It has been suggested that these two methods generate the same clinical prognostic information regarding PSA relapse after radical prostatectomy (Patel, Dorey et al. 1997; Pound, Partin et al. 1999). Roberts et al present a statistical comparison between these two methods and the author concludes that the methods were equivalent (Roberts, Blute et al. 2001).

Daskivich et al investigate the shortcomings of different methods for calculating the PSADT in a review article. To optimize the accuracy of the two-point method the author recommended 1) a well-defined starting point, 2) similar time interval between the measurements 3) a well-defined end point 4) the same PSA measurement method (Daskivich, Regan et al. 2006). In this thesis we have used the same laboratory for PSA analysis. The starting point and the end point are well defined for the entire cohort as well as the time interval.

In general there is always a risk of false high or low PSA as is the case with all other measurement of markers and the physician must be aware of this risk. A false PSA obviously results in a false PSADT. To improve the accuracy of the PSADT in everyday

clinical work it is important to critically evaluate every PSA value as one does with all other tests. An unreasonable PSA in a patient must be rechecked before the PSADT is calculated.

Statistical considerations

Study I is a case control study with a limited number of patients and we could not find any statistically significant differences in the form of tumour progression in the surveillance group compared to patients who had been operated on immediately after diagnosis. However, this was not a randomized prospective study and despite the proper matching procedure the study has the limitation of a case control study. The major consideration in this study is the limited number of patients in the study and the question is whether a significant difference between the groups would have been revealed with a larger number of patients. However, as we have explained in the study we included all the eligible patients who were operated on at our hospital after an initial period of surveillance. This fact reveals the importance of and the need for a multicentre study to improve the power of such a study.

In **Study II** we used only univariate analysis due to the limited number of patients, which is the reason why we do not compare the prognostic value of TVC with other markers. To investigate deeply the value of a new prognostic marker a multivariate analysis, including the clinically used markers, is preferable. We performed such an analysis but due to the low number of patients in this exploratory study the significance of the multivariate analysis result is of limited value.

In **Study III**, Cox regression analysis has been used to compare PSADT as a marker of PSA relapse to PSA, F/T-PSA and total amount of cancer in the biopsy. To calculate the time to active treatment and PSA-free survival among the patients with an altered PSADT interval, the Kaplan-Meier estimate was used. The shortcoming is once again the small number of patients with PSA relapse in this group of patients with early PC. Because of the natural history of early PC, a larger cohort of patients or a longer follow-up could improve the power of this study.

In **Study IV**, a Cox regression analysis was used to evaluate the Ki-67 as a prognostic marker. This was an explorative study and the results from this analysis must be

interpreted with caution, especially in markers which have not been shown to be significant predictors of PSA relapse. However, despite the limited number of patients the PSA and percentage Ki-67 were predictors of PSA relapse after RRP.

The analytic method used in **Study V** was an ordinal logistic regression to evaluate the association between PSADT and other prognostic markers. We used all the eligible markers from the screening study. It is important to underline that the results show the association not the correlation between the parameters included in the analysis. However, there could be other factors which are unknown to us that could interfere with the results.

General discussion and clinical applications

Managing men with low-grade/low-stage and early PC is very complex. Various factors apart from the tumour behaviour influence the treatment decision. The patient's assumed remaining length of life, co-morbidities, the patient's expectation and his attitude to the potential side effects of treatment, the prognostic insecurity and risk of suffering from metastasis and dying from PC are significant factors that influence the patient's choice of therapy.

In the studies in this thesis we investigate certain parameters which might have the potential to improve prognostic accuracy in early PC and thereby facilitate the patient's choice of therapy.

In Study I we demonstrated that initial close surveillance up to two years after diagnosis did not reduce the chance of cure. This is important information for the patients and the urologist. Receiving a PC diagnosis is very stressful for the patient. For some patients, there is the fear of the word "cancer" which leads to active treatment more than an accurately calculated risk analysis. For the urologist the lack of a reliable and accurate prognostic marker means it is safer to recommend active treatment to the patient. The knowledge that initial active surveillance does not reduce the chance of cure, both the patient and the urologist gain valuable time. The patient can use the time to reflect on his situation, evaluate the relevant information and make his therapy decision based more on solid facts rather than fear. The urologist can through close surveillance, including PSA and rebiopsies, increase the accuracy of the prognostic information.

There is one recent prospective randomized study which compares radical prostatectomy to watchful waiting in men with early PC, SPCG-4. The study demonstrated that active treatment in the form of radical prostatectomy reduces disease-specific mortality, overall mortality, the risk of distant metastasis and local progression compared to watchful waiting (Bill-Axelson, Holmberg et al. 2005). The authors of this study had calculated that the number of patients needed to be treated to save one patient from dying from PC was 19. This study was started in 1989 and the definition used by the authors of the study of "early prostate cancer" was different from today's "early prostate cancer". It has been discussed before that about 80% of the patients with PC detected in a screening scenario have T1c, a Gleason score <7and PSA <10ng/ml disease (Hugosson, Aus et al. 2004).

These patients have considerably less advanced disease than the patients that were included in the SPCG-4 study. In other words, if all the screen-detected, potentially curable PC patients received active treatment the number of men who needed to be treated to save one from dying from PC would be much higher than 19. The SPCG-4 study demonstrates that radical prostatectomy can save lives but the price is overtreatment.

There is an urgent need for more accurate prognostic tools to identify the aggressive PC at an early stage when the disease is still curable and at the same time avoid overtreatment of patients with "innocent" PC.

PSADT had been suggested as a prognostic tool in early PC to identify patients with low aggressive potential PC. This approach could result in decreasing overtreatment in patients with early PC. Study III investigates the value of PSADT as a prognostic marker in patients during active surveillance. There was not a single PSA relapse in the group of patients with PSADT >4 years. A PSADT >4 years in the patients with early, low-grade, low-stage PC can be interpreted as such that the patient's disease has low aggressiveness and continuing surveillance can be recommended. On the contrary, a PSADT <2 years reveals that there is a high risk of aggressive disease and active treatment is a better option than continuing surveillance. The patients with a PSADT between two and four years are classified in an intermediary group. This is valuable prognostic information that is not available at the time of diagnosis and can only be attained following an initial period of surveillance.

Another interesting finding in Study III was that a minor PSA increase was the reason for changing to active treatment in the majority of patients. This finding mirrors the anxiety that some patients experience during the surveillance. On the other hand, during a mean follow-up of 63 months, 61% of the patients did not receive any treatment and were still under surveillance. During the follow-up only five out of 270 patients reached a PSA value above the 20ng/ml. Fourteen patients died for reasons other than PC and none of these patients received active treatment. Despite the fact that 63 months is a short period of follow-up, it is obvious that because of active surveillance overtreatment had been prevented in these 14 patients.

The concept of active surveillance has been discussed by several authors during the last few years. Klotz et al have an ongoing, prospective, non-randomized study which has

included 299 patients with early PC in an active surveillance programme. At eight years 65% of the patients remained free from treatment and the prostate cancer-specific survival was 99.3% (Klotz 2006).

The major problem with the active surveillance concept is still the lack of an accurate marker of progression during the follow-up. In Study III, PSA relapse after radical prostatectomy was observed in 19% of patients during a follow-up of 37 months. PSADT was less than two years in 14/70 patients who received radical prostatectomy. However, 50% of these patients with a PSADT <2 years experience PSA relapse. These patients probably had a better chance of cure through immediate active treatment.

The active surveillance approach seems to have the potential to identify the PCs with low aggressive potential and thereby prevent overtreatment to some extent. However, patients with short PSADT were possibly at risk of losing the window of cure through active surveillance.

The question that needed to be addressed is whether there is any marker that can identify the patients with a high risk of PSA relapse (PSADT< 2 years) at diagnosis.

The observation from Study III that a small increase in PSA during the follow-up leads to disruption of the surveillance has been reported by several authors before (Zietman, Thakral et al. 2001; Carter, Donahue et al. 2003). These therapy changes were not based on the rebiopsy. If the majority of patients change the therapy based on a small decrease in PSADT, it is essential to investigate the rationale behind PSADT in these patients. In other words, how accurately does the PSADT mirror tumour activity in these early tumours. Studies IV and V were an attempt to better understand the rationale behind PSADT as a prognostic marker.

In Study IV we tested whether PSADT was correlated to the proliferation rate in the tumour measured by Ki-67 and whether Ki-67 could predict the PSA relapse after radical prostatectomy. This was an explorative study with a limited number of patients. The results revealed that percentage Ki-67 in the prostatectomy was correlated with PSA relapse. However, we could not find a statistically significant correlation between percentage Ki-67 and PSADT. It could be because of the limited number of patients that we did not find such a correlation. A significant correlation was observed between the Ki-67 and Gleason grade. This fact reveals the major problem with tissue markers such as

Ki-67. The heterogeneity of the prostate cancer due to Gleason grade is a well-accepted fact. The Ki-67 was associated with the Gleason grade and there was substantial variation between the levels of the Ki-67 in different parts of the tumour in the same patients. On the other hand it is a well-known that the Gleason score can be upgraded and downgraded in up to 50% of cases between the core biopsy and the prostatectomy specimen. These facts substantially decreased the precision of the Ki-67 as a prognostic marker from the core biopsy. Because of the limited amount of cancer in the biopsy in patients with early PC, the Ki-67 value that is generated from the biopsy depends on which parts of the tumour have been hit by the biopsy needle. These limitations decrease substantially the value of Ki-67 as a clinically useful prognostic marker in early PC.. However, to evaluate in depth the usefulness of Ki-67 as a prognostic marker in early PC a prospective biopsy-based study can be recommended.

In Study V the factors that influence PSADT were explored. We conclude that high-grade disease and prostate volume are the two major factors that influence PSADT. The first clinical application is that because of the presence of high-grade cancers is associated with shorter PSADT, it is mandatory to have repeated biopsies in patients with short PSADT regardless of initial biopsy results .The threshold to change to active treatment in these patients should be reduced.

The second application is that in patients with BPH and a large prostate volume harbouring a small cancer the PSADT could be very long. This finding also highlights the importance of repeated biopsy in patients with a PC and large-volume prostate participating in an active surveillance programme despite a long PSADT.

The idea behind Study II originated from the concept of angiogenesis, which was initially presented by J. Folkman in 1977. Tumour growth is depended on the formation of new blood vessels. The hypothesis was that the number of microvessels in a tumour is correlated to the aggressiveness of the tumour. The result of the study revealed a significant correlation between the number of microvessels and PSA relapse after radical prostatectomy. It was a small study and a larger prospective study is needed to confirm our results. Nevertheless, we observed several problems with the histological evaluation. The limited amount of cancer tissue in biopsies and the lack of concordance between the

biopsy and the prostatectomy specimen because of the heterogeneity of the PC in the same patient are the major limitation of this marker.

Management of men with early prostate cancer has undergone a revolution during the last three decades. The introduction of PSA, the use of TRUL-guided core biopsy and the popularization of radical prostatectomy are the main aspects that are responsible for the reformation in the treatment of these men.

There are two major, ongoing, randomized screening studies: the ERSPC trial in Europe (Schroder, Denis et al. 2003) and the PLCO trial in the United States (PLCO andriole GL 2005). The results from these studies will reveal whether PSA screening would reduce mortality from PC or not. At the same time, the introduction of PSA has contributed greatly to non-systematic screening of asymptomatic men for PC.

Regardless of the results from ongoing, randomized screening studies, non-organized PSA screening is adapted in the majority of the developed countries and the problem of early-detected, often non-life-threatening PC is going to involve the urologist and their patients for many years ahead. Until there is a prognostic marker which can with acceptable precision separate the potentially life-threatening PC from the harmless ones, the concept of active surveillance is promising. Active surveillance should be presented to patients with low-grade, low-stage, early PC as an alternative to active treatment. This approach has the potential to decrease overtreatment of early PC patients.

To reduce mortality from prostate cancer it is important to identify and treat the tumours with aggressive potential in the early stages during the window of cure. There are a substantial number of articles that discuss the problem of overdiagnosis and overtreatment in PC (Etzioni, Penson et al. 2002; Bangma, Roemeling et al. 2007). However, the issue of undertreatment of men with aggressive PC is actually a more significant problem if the aim is to reduce PC mortality. During the past 20 years approximately 2,500 men have died in Sweden each year from PC. Mortality is not decreasing regardless of the substantial increase in the curative and the non-curative treatment of men with PC in Sweden. This information should be interpreted in such a way that there is ongoing undertreatment of men with lethal PC and subsequently there is an urgent need for markers that can identify these tumours at an early stage when the cure is achievable.

One of the central questions that needs to be explored is whether the approach of active surveillance can even prevent the undertreatment of aggressive tumours or if there is a risk that because of the lack of accurate progression markers during active surveillance, the window of cure is going to be closed for some of these patients by the time the progression is detected.

Future perspective

To study the real value of any prognostic markers, prospective longitudinal studies are mandatory. By including men with low-grade, low-stage, early-detected PC in an active surveillance programme in a multicentre study, the accuracy of different serum or tissue markers can be evaluated during the natural history of the disease. The marker/markers that could increase the accuracy of the prognosis in the early-detected PC can be recognized by such a study.

As long as we do not have an accurate prognostic marker which can separate the potential lethal PCs from the “innocent” ones, every centre with a PSA screening programme, or clinics who manage men with early, low-stage, low-grade PC, should have a well-defined, active surveillance programme, including repeated PSA and biopsies. The programme offers patients an alternative to active treatment as well as watchful waiting.

Conclusions of the studies in brief

Study I

In selected patients with early, low-grade, low-stage PC (T1c–T2 tumours, a Gleason score of <7 at biopsy, PSA <10 ng/ml), close surveillance for almost two years followed by RRP did not lead to a more aggressive pathological stage in subsequent prostatectomy specimens compared to what was found in patients who received immediate surgical treatment.

Study II

Tumour vascularity in a core biopsy of PC is a predictor of PSA recurrence after radical prostatectomy.

Study III

PSADT seems to be a useful, reliable and discriminating prognostic marker of disease progression and active treatment during the follow-up of patients with screening-detected early PC who opt for initial active surveillance.

Younger, screen-detected men who opt for an initial period of surveillance with a PSADT >4 years still have an excellent chance of cure by RRP. However, patients with a short PSADT (<4 years) should be informed about the risk of disease progression using this approach.

Study IV

In screen-detected, low-grade, low-stage PC patients in whom the traditional prognostic markers have limited efficacy, the Ki-67 is a significant predictor of PSA relapse after radical prostatectomy.

Study V

In men with early screen-detected prostate cancer the PSADT is associated significantly with high-grade cancer. However, the BPH component of the gland seems to have a significant impact on the PSADT. The physician must be aware of the impact of BPH on PSADT to avoid misinterpretation of PSADT in these patients.

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