From the Centre for Surgical Sciences, Division of Urology, Karolinska Institute, Department of Urology at Huddinge University Hospital, the Sahlgrenska Academy at Göteborgs University, Institute of Surgical Sciences, Department of Urology and the Karolinska Institute at Söderhospital, Department of Urology, Karolinska Institute, Stockholm, Sweden

The Diagnostic Performance of Prostate-Specific Antigen (PSA) in Early Detection of Prostate Cancer:

Considerations of Sensitivity, Specificity, Lead-Time and Survival

Magnus Törnblom

Stockholm 2003

Published and printed by Karolinska University Press
Box 200, SE-171 77 Stockholm, Sweden

ISBN 91-7349-660-X

The Diagnostic Performance of Prostate-Specific Antigen (PSA) in Early Detection of Prostate Cancer: Considerations of Sensitivity, Specificity, Lead-time and Survival

Magnus Törnblom, Centre for Surgical Sciences, Division of Urology, Karolinska Institutet, and Department of Urology, Karolinska Institutet at Huddinge University Hospital Stockholm, Sweden.

Abstract

Background Prostate cancer (PC) is the most common form of cancer, as well as the single major cause of cancer-related mortality among Swedish males. Recent reports indicate that screening of the serum levels of prostate-specific antigen (PSA) in asymptomatic men may result in decreases in both the incidence of metastatic disease and PC-specific mortality. However, the diagnostic performance of PSA-based screening is limited by rather low sensitivity and specificity. An improved understanding of the natural course of PC in relationship to PSA levels at the time of diagnosis is required in order to calculate how much earlier diagnosis can be achieved by screening (the lead-time) and when the beneficial effect in the form of improved survival can be expected to become apparent.

Patients and methods This study is based on two well-defined cohorts of men both selected randomly from the general population. One of these cohorts (the Stockholm South PC study) included 1782 men who participated in a single intervention study for evaluation of early diagnostic methods in PC detection in 1988-89, whereas the other cohort (n=657) was involved in a screening of general health in men, not including PC, in 1980 (The Study of Men Born in 1913). The diagnostic performance of percentage free PSA (%fPSA) was evaluated in the Stockholm cohort. For estimations of lead-time and PC-specific survival, men from the two cohorts, with a median age of 67 years, were monitored for 12 and 20 years, respectively, following PSA testing. Individuals diagnosed as having PC as well as causes of mortality were ascertained utilizing the national Cancer Registry together with the Cause of Death Registry.

Results and conclusions Retrospective application of a %fPSA "cut-off" value of $\leq$18% improved the diagnostic sensitivity among men with normal PSA levels (i.e., < 3.0 ng/ml), whereas a "cut-off" of 22% increased the specificity at slightly elevated PSA levels (3.0-9.9 ng/mL). In men without PC the %fPSA ratios were influenced by age and prostate volume, which at PSA levels in the interval of 4.0-9.9 ng/mL together accounted for much of the variation in this ratio.
Men with PSA levels < 1.0 ng/mL were at a very low risk of being diagnosed as having, or of dying from PC during 20 years of follow-up.
In the PSA interval of 3.0-9.9 ng/mL the various estimates of median lead-time obtained ranged from 4.5-11.2 years. Successful screening for PC (i.e., decreased mortality) should be discernible after 6 years, since men with PSA levels < 10.0 ng/mL accounted for 62%, 78% and 100% of all PC deaths 6-10, 11-15 and 16-20 years later, respectively.
The results of this study support the diagnostic value of %fPSA for early detection of PC. Furthermore, this thesis indicates that intense PSA screening should result in a reduction in PC mortality already before the median lead-time is reached.

Keywords: PSA, free PSA, prostate cancer, sensitivity, specificity, lead-time, survival, epidemiology

LIST OF PUBLICATIONS

I. Tornblom M. Norming U. Adolfsson J. Becker C. Abrahamsson PA. Lilja H. Gustafsson O. Diagnostic value of percent free prostate-specific antigen: retrospective analysis of a population-based screening study with emphasis on men with PSA levels less than 3.0 ng/mL. Urology. 53(5):945-50, 1999 May.

II. Tornblom M. Norming U. Becker C. Lilja H. Gustafsson O. Variation in percentage-free prostate-specific antigen (PSA) with prostate volume, age and total PSA level. BJU International. 87(7):638-42, 2001 May.

III. Tornblom, M., Eriksson H., Gustafsson O., Franzén S., Norming U., Lilja H., Hugosson J. Lead-time of screen detected Prostate Cancers. Accepted: International J of Cancer.

IV. Tornblom, M., Eriksson H., Gustafsson O., Franzén S., Norming U., Lilja H., Hugosson J. When is reduced prostate cancer mortality to be expected provided that efficacious PSA screening is introduced? Submitted: International J of Cancer.

CONTENTS

LIST OF ABBREVIATIONS

ANN	Artificial neural network
BPH	Benign prostatic hyperplasia
DPCP	Detectable preclinical phase
DRE	Digital rectal examination
ERSPC	European Randomized Study of Screening for Prostate Cancer
FNAB	Fine needle aspiration biopsy
NPV	Negative predictive value
%fPSA	Percent free PSA
PC	Prostate cancer
PLCO	Prostate, Lung and Colorectal and Ovarian Cancer Screening Trial
PP	Primary prevention
PPV	Positive predictive value
PSA	Prostate-specific antigen
SCB	Statistiska centralbyrån
SPCG-4	Scandinavian Prostate Cancer Group study number 4
SP	Secondary prevention
TNM	Tumour, node, metastasis classification
TP	Tertiary prevention
TRUS	Transrectal ultra-sonography
TURP	Transuretral resection of the prostate

1 BACKGROUND

1.1 QUANTITATIVE ASPECTS OF PROSTATE CANCER

During the past few decades there has been steady improvement in the health status of men and women in Western countries. As a consequence, life expectancy has increased and greater awareness has been focused on diseases that cause morbidity and death after middle-age. With a peak incidence during the eighth to ninth decades of mans life, prostate cancer (PC) is a major form of fatal cancer among elderly men in the Western world [1].

In the case of Sweden, the 7611 new cases of PC diagnosed in 2000 accounted for 32.6% of all male cancers diagnosed that year [2]. The mean age at the time of diagnosis was 72.7 years. After a slow, but steady rise in the age-standardized incidence of PC during the last 30 years (with an average of 1.6% per year from 1970-1997) a trend towards a more rapid increase has been observed during the last few years (on average of 6.5% per year from 1997-2000) [3].

As a consequence of the steep decrease in cardiovascular mortality among Swedish males since the 1980's, PC accounts for a steadily increasing percentage of all age-standardized mortality among men, from 3.6% in 1980 to 5.6% in 2000 (Fig. 1), despite only a modest rise in absolute age-standardized PC-related mortality [4].

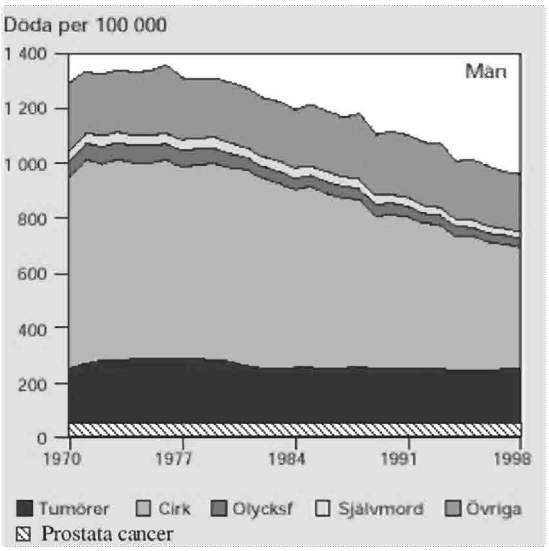

Fig. 1. The different causes of deaths in Swedish males between 1970 and 1998. Age-standardized and calculated per 100,000 men. "Folkhälsorapporten 2001", page 41. Socialstyrelsen. Reprinted with permission and slightly modified.

The increasing life-expectancy among males, a large number of whom are about to enter the age at risk for developing PC (Fig. 2), together with the increased attention directed towards this disease, will most probably lead to an increase, in the number of men living with this disease (from 36,983 in 1999) [2], rendering it an even greater health problem in the near future.

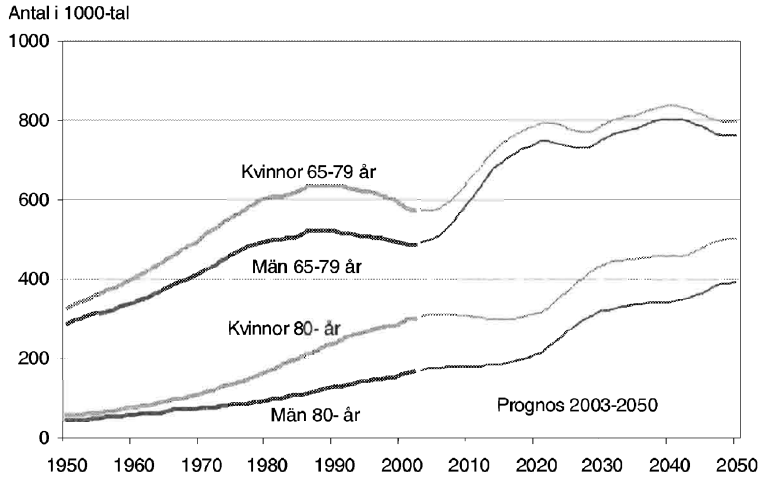

Fig. 2. The Swedish population in the age-groups 65-79 and 80- by sex (females grey lines and males black lines). Registered numbers 1950-2002 and forecasted numbers 2003-2050. Thousands of individuals. From "Sveriges framtida befolkning –Befolkningsframskrivning för åren 2003-2050" SCB 2003. Reprinted with permission.

Since in 1999 the mean age at the time of PC-related death was 79.1 years and the life expectancy of a Swedish man was 77.1 years [3], it might seem reasonable to regard PC almost as a disease associated with a longer-than-average lifetime. Nonetheless, in most cases PC causes severe pain and discomfort during its final stages. Furthermore, younger men also die from this disease, which in males less than 75 years of age was second in incidence only to lung cancer as a cause of cancer-related death. In this context it is also worth considering that the additional mean life-expectancy of a man who reached 72 years of age in 1999 was 11.5 years [3].

The consequences of early detection and treatment of this form of cancer are evident from reports from the US [5]. Following its introduction in this country 1987, determination of serum levels of prostate-specific antigen (PSA) rapidly became a widespread and common tool for early detection of PC. In 1992 when approximately 35% of American US men above the age of 65 had undergone PSA testing [6], the recorded incidence of PC had doubled and the number of radical prostatectomies performed tripled (to approximately 100,000 that year) [7]. However, whether the decrease in PC-related mortality in the US, which started in 1993 and was 21.6% by the year 1999 [8], is a result of this early diagnostic procedure remains an open question.

In the case of Sweden, the policy concerning early diagnosis of PC based on PSA screening has been rather restrictive, due to an assumed risk of overdetection and overtreatment [9]. The recent increase in the number of Swedish men undergoing PSA testing at the initiative of individual doctors is the most probable cause for the increased incidence of PC recorded in recent years. However, no decrease in mortality has yet been observed.

In spite of refinements in early diagnostic procedures and the availability of improved treatment options, several questions remain as to whether introduction of early screening for PC is the correct approach for decreasing mortality from this disease. Fortunately, two extensive screening studies, the ERSPC [10] and PLCO [11] studies, presently being performed will most likely provide better understanding of the risk versus gain associated with screening for PC. Evaluation of these studies will, however, take at least five more years and in the meantime, while waiting for clearer directives concerning which course to choose, individual physicians will have to follow their own judgement.

1.2 STRATEGIES FOR PREVENTING DEVELOPMENT OF AND MORTALITY DUE TO PROSTATE CANCER

1.2.1 Primary prevention

Primary prevention (PP) of PC involves all strategies designed to prevent the development of this disease. The known risk factors for PC are advanced age, a familial history of the disease, adequate androgen levels, a western life style and race [12]. Although these factors are strong predictors of the development of this disease in certain men, they do not seem to explain why among the majority of men, at ordinary risk, clinical symptoms of PC develop in some, but not in others. The virtually random distribution of the clinical appearance of this form of men at ordinary risk supports the belief that PC arises as the result of a complex cascade of genetic events influenced by several environmental factors [13].

1.2.1.1 Genetic factors

As is the case for other forms of cancer, genetic changes are a prerequisite for the development, of PC. These changes can be inherited or acquired. Hereditary impacts on the aetiology of PC include autosomal dominant or X-linked mode inheritance of predisposition with high penetrance [14], as well as familial and sporadic PC in which mutations in several genes (genetic polymorphism) are involved [15].
Hereditary PC with three or more close relatives developing the disease accounts for approximately 5-10% of all cases of this disease. In 1996, Grönberg et al. [16] mapped the first locus associated with a predisposition for PC to chromosome 1 (HPC1). However linkage of this locus, as well as of 6-7 others discovered later, to PC has not been conclusive in subsequent studies [17].

Of the other 90-95% of cases of PC approximately 10-15% can be classified as familial [18], with more than one family member being afflicted by the disease, but without dominant inheritance. In such cases the relative risk for prostate cancer ranges from 2-7, with an increase in the risk when several relatives are afflicted. Sporadic cases account for the remaining 75-85% of prostate cancers. As in the case of familial cancers, the aetiology of these sporadic cases could involve genetic polymorphism, with additional exposition to environmental mutagens (with or without a genetic predisposition). Several different kinds of genetic polymorphism have been proposed to be of significance in this connection, but their association with an increased risk for developing PC has, for the most part, not been observed consistently [15].

Thus, despite considerable research, the genetic changes involved in the development of PC are still largely obscure. New methodological approaches, such as the use of DNA microarrays to analyze the expression of thousands of genes in each individual subject, should help to clarify this issue [19]. The possibility of preventing PC by genetic manipulation still seems rather far-fetched.

1.2.1.2 Environmental risk factors and protective agents

Examples of PP in connection with PC include minimizing exposure to carcinogens, as well as the use of substances that protect against the development of this disease (chemoprevention). Potential environmental risk factors that can be avoided include saturated fat, animal meat and high levels of calcium or potentially beneficial eg. vitamin D [20, 21]. Possible candidates for chemoprevetion include, among other things, finasteride [22], selenium [23], vitamin E [23], lycopens[24] and phytoestrogens including soy proteins [25].

To date, studies designed to specifically identify avoidable risk factors as well as agents for chemoprevention have not been successful [26]. In one recently reported study, the PC Prevention Trial (PCPT) [22], the potential preventive effect of finasteride was evaluated. Among the 18,882 randomly subjected to treatment and follow-up for seven years, more receiving the placebo were diagnosed as having PC, whereas the frequency of high grade cancers (Gleason 7-10) was higher among the men who were actually diagnosed as having PC in the finasteride group.

Another major on going study, the SELECT study [23], includes 32,400 men and is designed to determine whether selenium and/or vitamin E can reduce the risk for PC. In spite of the large number of participants involved, this study will only be ready for final analysis 10 years from now. The stepwise and often lengthy development of PC, prior to the appearance of clinical symptoms, complicate evaluation of such PP trials.

Modern techniques for early detection allow PC to be diagnosed during the detectable preclinical phase (DPCP), giving rise to difficulties in evaluating whether the cancers thus prevented are clinically significant. Consider the fact that in the PCPT study described above, 18,4% of the men receiving finasteride and 24.4% of those administered the placebo were diagnosed as having PC. These rates are extraordinarily high, especially in the light of the low PSA levels (< 3.0 ng/mL), young age (with a

median of approximately 63 years) and benign DRE present at the start of this study, as well as the short follow-up period of seven years.

It is possible that finasteride may prevent certain cancers from entering the DPCP, but from this particular study it cannot be determined whether there is a reduction in PC destined to cause clinical symptoms and/or death. More refined and aggressive opportunistic screening (e.g., employing twelve instead of six biopsies and lowering the PSA level "cut-off"), fuelled by a rapidly increasing awareness of PC among men at risk, tends to complicate evaluation of PP. In summary, at present no reliable recommendations can be made concerning the avoidance or use of specific agents in order to decrease the risk of developing PC.

1.2.2 Secondary prevention

Secondary prevention (SP) of PC is designed to eradicate the established disease before it becomes incurable. In most cases, PC is curable only as long as the cancer remains localised in the prostate. Since the majority of PCs detected on the basis of clinical symptoms have already passed the localized stage, effective SP requires identification and treatment of men in the DPCP before the disease presents clinically, i.e., screening is necessary. Strategies for curative treatment following early detection include radical prostatectomy and radiation therapy. The recently described SPCG-4 study [27] was the first to provide evidence for the much discussed assumption that treatment of localised PC with intention to cure (radical prostatectomy) improves survival and attenuates metastatic disease.

1.2.3 Tertiary prevention

To date, there is no treatment that has been demonstrated to cure PC when the disease has spread outside the prostate gland (TP). However the waste majority of men afflicted in this manner, respond positively to androgen deprivation, which results in temporary arrest of cancer growth and exerts beneficial palliative effect on the symptoms. Unfortunately, with time such PC enters a hormone-independent state, where palliative treatment alone is provided and the survival time is likely to be less than 18 months. In spite of the large number of trials performed in order to evaluate various chemotherapeutical agents in this context, it has only rarely been possible to prolong survival after this hormone-independent state has been reached [28, 29].

In summary, the aetiology of most cases of PC appears to involve both a vast number of different genetic alterations, the majority of which are still unknown, and several, largely unidentified environmental factors. This multifactorial origin probably explains the difficulties involved in choosing effective PP strategies and the consequent highly variable phenotype associated with PC may also explain the difficulties involved in determining how to achieve early diagnosis of the disease and in identifying chemical agents which are effective for SP and TP. The only proven strategy for decreasing PC mortality at present is SP including surgery and, if such treatment is to be efficacious,

the disease must be detected and treated while still in a localised stage, thus necessitating screening for early PC.

1.3 THE DETECTION AND TREATMENT OF LOCALISED PROSTATE CANCER

Early detection and curative treatment thus appear to be the strategy of choice for defeating PC. However, in spite of great improvements in both respects, this approach still has major drawbacks.

There are several reasons why screening for PC in asymptomatic men is a highly debated and complex procedure. The most important of these is, of course, the lack of randomised studies that supply a definitive answer to the question as to whether early detection really can lead to a reduction in PC mortality. Furthermore, several other questions, some of which are listed below, remain unsolved:

- Uncertainty in the screening procedure may give rise to over- as well as underdetection, the former especially in the case of latent cancers and due to the obscure natural history of localised PC.

- Uncertainty in connection with prediction and unpredictiveness of prognosis leads to a risk for overtreatment, especially since our understanding of the clinical significance of PC detected at an early stage and of preoperative prognostic factors is incomplete.

- Uncertainty at the level of treatment involves the choice of a treatment option, adverse effects of this treatment and the key question as to whether early detection and treatment actually reduce PC mortality.

1.3.1 Uncertainity in the screening procedure and the risk for over and underdetection

1.3.1.1 Latent carcinoma

The prostate gland is prone to develop cancer with a greater frequency than perhaps any other human organ. As early as 50 years ago, when Franks [30] performed a histological examination of the prostate gland in connection with autopsies of men, he observed small, focal PC in 69 (31%) of those cases. Since these cancers were histologically identical to lethal PC, but would obviously not acquire aggressive biological features during the men's life-times, they were designated as latent carcinoma. Further investigations have confirmed these findings and it has been estimated that 30% of 50-year-old and 50% of 80-year-old men have latent carcinoma [31].

The presence of such small focal PC even before the age of 50 was demonstrated by Sakr et al. [32], who found in the prostates of autopsied men small (mean maximal

dimension of approximately 2 mm) foci of histological cancer in 27% and 34% of these men in the fourth and fifth decades of life, respectively. Since the projected probability of being diagnosed with clinical PC during a mans lifetime was only 9.5% in the US in 1985 and since the probability of dying of this disease was only 3% [33], it is apparent that only a few of these PC will ever lead to the development of clinical symptoms.

Therefore, there has been much concern that the introduction of PSA-based early detection would also result in identification of these latent carcinomas, thus causing major overdetection and, possibly, overtreatment.

1.3.1.2 The natural course of localised prostate cancer

Localised PC was frequently diagnosed even before diagnosis based on PSA levels became possible. Most of these PCs were diagnosed unintentionally in resected prostatic tissue from men undergoing transuretral resection of the prostate (TURP) due to symptomatic benign prostatic hyperplasia (BPH). Histological examination of resected prostate tissue samples, resulted in a diagnosis of PC, often of small size and highly differentiated, in approximately 10% of the cases. In addition, localized PC was commonly detected in connection with digital rectal examination, performed as part of a general health examination.

Several observational studies, both retrospective and prospective, have evaluated the natural course of localised PC during the era prior to determination of PSA levels. For example, a much debated study by Johansson et al. [34] from Örebro County, Sweden, revealed that men with, predominantly, highly differentiated PC and a mean age of 72 years had an excellent disease-specific survival of 89% after 15 years. This report is in disagreement with the SPCG-4 study [27], in which even after a median follow-up period of 6,2 years, the cumulative PC-specific mortality in men subjected to "watchful waiting" was 8.9%. Possible explanations for this difference might be the greater percentage of highly differentiated PC (66%) and higher mean age at the time of initiation of the study (72 years) in the former investigation than in the latter (48% and 64.7 years respectively). The significance of these two factors for the natural course of localised PC has also been demonstrated, by among others, Adolfsson et al. [35] and Albertsen et al. [36]. In the later study, by estimating retrospectively total and PC-specific survival in men diagnosed with clinically localised PC, the authors could demonstrate that the state of differentiation of the cancer at the time of diagnosis, together with the remaining life-expectancy exert a decisive influence on the risk of dying from PC (Fig. 3, see next page).

1.3.1.3 Overdetection and competing causes of death

Overdetection, (used here synonymously with overdiagnosis) is a crucial and probably inevitable problem encountered in connection with all interventions designed to achieve early diagnosis of cancer. There is no consensus with respect to how overdetection in connection with PC should be defined and several different definitions have recently appeared in the literature:

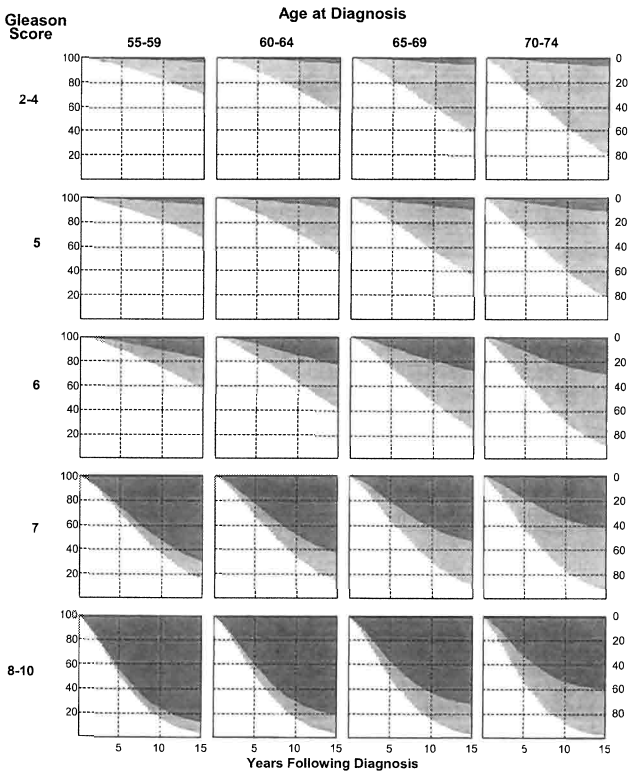

Figure 3. Survival (white lower band) and cumulative mortality (dark gray upper band) and other causes (light gray middle band) up to 15 years after diagnosis stratified by age at diagnosis and Gleason score. Percentage of men alive can be read from the left-hand scale, and percentage of men who have died from prostate cancer or from other causes during this interval can be read from the right-hand scale. Reprinted with permission from JAMA 1998;280: 975-980. Copyrighted 1998, American Medical Association.

Perhaps the most common definition of overdetection is the ratio of the incidence following screening to the incidence in the absence of screening (i.e., PC detected clinically). Applying this definition, Zappa et al. [37] estimated that the overdetection in connection with biennial screening in Italy of 60 and 65 years old men was 44-55% and 85-101%, respectively. Schröder et al. [38] employed this same definition when comparing the PC incidence in a screened population in the Netherlands to that of a control population and obtained, a ratio of 6.5/1 indicating an overdetection of 85%.

On the other hand, Etzioni et al. [39] defined overdetection as the proportion of the men in whom PC was detected on the basis of PSA screening who would not survive long enough to present clinical symptoms. Using this definition, these investigators estimated that among US Caucasian and African 60-84 year-old men, 18-39% and 20-44%, respectively, of the PCs detected would represent overdetection.

Alternatively, McGregor et al. [40] use the term overdetection for all non-lethal PCs detected by screening. Their reason to use this definition is that only men who are destined to die from the disease may benefit from early detection. These investigators calculated the mean annual ratio between the frequency of *lethal*, screen-detectable PC (1.3/1000 men) and of *all* screen-detectable PC (8/1000 men) to be 0.16, indicating that 84% of all screen-detected PC in men between 50 and 70 years of age would not be fatal before the age of 85 years. An even higher value was estimated by Schröder et al. [38] by comparing the projected incidence/mortality ratio (case-fatality ratio) of a

screened population (14.6) to that of its control (2.2) concluding that 93% of all screen-detected cancers represent overdetection. In the latter study the lead-time associated with screen-detected PC was not taken into account. Such, case-fatality ratios may also be estimated for an entire country. Thus, in the year 2000 this ratio was estimated to be 5.3 in the US, compared to 2.6 in Western Europe [1] In comparison the age-standardized incidence/mortality ratio increased from 1.9 to 2.4 in Sweden between 1988 and 1999 [3].

Despite the somewhat divergent findings described above, all of these definitions of overdetection can be considered to be appropriate. The choice of which one to apply depends on what is being evaluated. However, shortly after the introduction of PSA screening in the US, it became apparent that testing men with a short life-expectancy is seldom beneficial [41, 42]. Ever since, it has been recommended that asymptomatic men with a life-expectancy shorter than 10 years should not be tested. As a consequence it seems appropriate to use the definition applied by Etzioni et al. [39]; see above.

Since achievement of early diagnosis is the goal of a screening procedure for cancer, it might be argued that evaluation of the consequences of screening should be postponed until eventual benefits (i.e., prolonged post-lead-time survival) can be properly determined, for example at the time chosen for evaluation of on-going randomised screening trials. Furthermore, additional effects of screening, such as a possible decline in the number of men diagnosed as having bone metastases, can also be evaluated then and perhaps the issue of overdetection can be put into better perspective.

1.3.1.4 Underdetection

In addition to the risk of overdetection, it must be assumed that screening will not result in identification of all cases of PC that are destined to lead to clinical symptoms and, possibly, even death. The rapid growth of many such cancers entails that they remain for only a short time in a detectable but preclinical stage. A short interval between repeated testing and/or a highly sensitive screening procedure is required to identify men with such cancers. In the ERSPC study, inter-screening intervals of different lengths as well as different PSA "cut-off" values are being applied to different cohorts [43]. The incidence of PC detected clinically between interventions (interval cancers) in different cohorts of this study will most probably improve our knowledge in this area.

1.3.2 Uncertainity in connection with prediction and prognosis: the risk for overtreatment

1.3.2.1 The clinical significance of prostate cancer diagnosed on the basis of early intervention: evaluation of tissue samples obtained in connection with radical prostatectomy

PSA screening has been estimated to enable detection of PC on an average of more than 10 years prior to the development of clinical symptoms (lead-time) [44] and, due to resulting treatment, this naturally makes it very difficult to decide which cancers

would have become clinically significant in the absence of screening. In order to be able to relate PC detected on the basis of PSA to its assumed clinical behaviour in the absence of such screening, Stamey et al. [45] in 1993 introduced the term "clinically significant PC". Definition of this term was based on the sizes of prostate tumours detected unintentionally in connection with cystoprostatectomy of the bladder and the prostate as treatment for bladder cancer. At that time approximately 8% of the male population developed symptomatic PC in the US. Therefore, Stamey and co-workers stated that among all cancers detected unintentionally those with the 8% largest tumour volumes were prone to become clinically significant. By applying this hypothesis they estimated that a tumour volume > 0.5 cm^3 was likely to be clinically significant. Employing this measure, several extensive studies on PCs detected by screening have reported that the vast majority (i.e., 70-90%) of these cancers in men with PSA levels in the interval of 4-10 ng/mL at the time of screening and later subject to radical prostatectomy are clinically significant [46, 47].

Another definition, this time of "clinically insignificant cancer" was proposed by Dugan et al. [48], who related significance to life-expectancy and the time required for the volume of the cancer to double. The findings of these investigators indicated that a doubling time of either 4 or 6 years was associated with a rate of 7.4% or 14.5% of clinically insignificant PC among the cancers subjected to operation.

Further evaluation of tissue samples obtained in connection with prostatectomy has revealed that the tumour volume is correlated to the risk for metastases. Thus, tumour volumes of 5, 13, and 20 cm^3 are associated with on the average, 10%, 50% and 87% risks, respectively, for metastases [49].

Post-operative measurement of tumour volume takes into consideration neither the growth rate of the tumour nor the life-expectancy of the patient. In addition to cancer volume, the pathological stage, surgical margin status and grade have also been employed to define clinically insignificant PC [50].

Unfortunately, no reliable method for measuring tumour volume preoperatively is currently available, so that the preoperative prediction of the clinical significance of a given PC must be based on other prognostic factors.

1.3.2.2 Prognostic factors prior to treatment

When diagnosis of a localised PC has been established, a decision must be made concerning subsequent management of the disease. This decision must be based on often limited information regarding the clinical significance of the localised cancer. Employing a combination of several risk factors, tables and nomograms have been constructed to allow prediction of the stage of the cancer [51] and the prognosis with respect to treatment [52]. The only factors that independently provide this information [53, 54] are the clinical stage (extension of the cancer as decided before) and Gleason grade of the cancer and the PSA level. Ideally these tables should make it possible to decide which tumours are insignificant and require no treatment, which will benefit from treatment and which are no longer amenable to treatment with curative intent. However, the imprecise nature of the information obtained from each of these factors,

together with the large heterogeneity in clinical behaviour even among PCs determined to be of equal potential danger, make these predictions unreliable. Other factors that have been examined for prognostic information include microvessel density [55] and the percentage of PSA in serum that is free (%fPSA) [56].

1.3.3 Uncertainity at the level of treatment

1.3.3.1 The influence of early detection and treatment on prostate cancer mortality

No properly designed randomised screening study has been evaluated for the effect of early detection and treatment on PC-specific survival. However, the decreasing PC mortality in the US following the widespread introduction of PSA testing has been considered to be indirect evidence for its effectiveness [57]. PSA testing was introduced in the US in 1988 and by the end of 1994 the majority of men above the age of 65 years had been tested. The parallel increase till 1992 in incidence and in the number of radical prostatectomies performed during this period emphasise the prevailing ambition to identify and treat PC at an early stage [7]. The subsequent rapid decrease in the incidence of metastases at the time of diagnosis since 1991, followed by a decrease in the incidence of early-stage disease since 1992 and a continuous decrease in mortality since 1994 have been interpreted as positive effects of this early diagnostic effort [57].
Critics of this interpretation have argued that in light of the long lead-time achieved, the observed decrease in PC-specific mortality came too early to be a consequence of PSA-based screening. Other arguments against a causal relationship between the screening and decreases mortality in the US include the declines in PC mortality also observed in countries where early detection and treatment have been much more limited [58]. Furthermore, similar declines in mortality have occurred in different parts of the US, despite large regional differences in diagnostic and treatment activities [59]. Hopefully, this issue will be resolved when the presently on-going, extensive, randomised screening studies for PC [10, 11] are mature for evaluation.

1.3.3.2 Clinical management of localized prostate cancer

Today, decisions concerning management of a man diagnosed as having localised PC are often made by the patient and his doctor together. In order to obtain complete information concerning the treatment options, the patient should be invited to consult with both the urologist and the oncologist. In addition to the personal preferences of the patient, his general health status and the known prognostic factors associated with the cancer should also be part of the basis for this decision.
Radical prostatectomy and radiation therapy are the two major forms of treatment with curative intent in connection with early PC. So far, long-term follow-up on PC mortality indicate that radical prostatectomy is preferable [60]. However, often these evaluations appear to be biased in terms of patient selection, as well as with regards to the analytical [61] and the treatment procedures employed (especially in the case of radiation therapy).

Radical prostatectomy
The proportion of men in the US with clinically localised PC who underwent radical prostatectomy increased from 10 to 25% during the period of 1983-1989 [62]. Improvements in surgical techniques resulting in reduced blood loss and an improved rate of postoperative continence together with improvements in early detection due to the development of TRUS [63] are the most probable reasons for this increase even prior to the introduction of PSA testing. Furthermore, a nerve-sparing technique developed by Walsh [64] in the 1980s has increased post-operative potency rates in cases detected early. Recently, laparoscopic prostatectomy [65] allows a minimally invasive operation, a better visualisation and, most probably, a reduced blood loss. Only a few studies designed to compare this new technique to the traditional open retropubic operation have been described.

Radiation therapy
In the 1980s external-beam radiotherapy was still limited by severe side-effects on the bladder and rectum, in spite of the low, and often insufficient, dosages delivered. Later developments in this area, including three-dimensional conformal radiotherapy and intensity-modulated radiotherapy, have improved precision, as well as enabled much higher dosages to be applied to the PC [66].
Treatment of PC by brachytherapy involves transperineal implacement of radioactive isotopes in the prostate gland under TRUS guidance [67]. This placement, which can be either permanent (with low-energy isotopes) or temporary (high-energy isotopes) is performed under general or spinal anaesthesia and is often combined with external-beam radiation. The use of brachytherapy to treat localised PC has increased considerably during the past few years [68].
Neoadjuvant treatment in the form of androgen deprivation prior to (neo-adjuvant) radiation therapy is considered to improve efficacy [69].

Watchful-waiting
Long-term follow-up studies on the natural course of localised PC in men with a good prognosis and/or short life-expectancy have revealed that a considerable number of men diagnosed as having localised PC will not benefit from treatment with curative intent[34, 70, 71]. If they could be correctly identified, conservative treatment (watchful-waiting) would be the management strategy of choice for these men. If the cancer progresses, most of these men can receive effective, but temporary palliation from endocrine treatment. A study begun recently [72] proposes a more active watchful-waiting approach and considers indications for active therapy of localised PC of low or intermediate grade to be either a doubling of the PSA value in less than 2 years, clinical progression and/or histological progression observed in connection with regularly repeated examinations.

1.3.3.3 Adverse effects associated with treatment of prostate cancer at an early stage

All treatments with curative intent are associated with side-effects and the incidence of such morbidity is probably the major obstacle to introduction of PSA screening of the general population. The frequency of reported side-effects differs between different studies, as well as between individuals. A recent report on the Netherlands arm of the

ERSPC study [73] revealed that one year after treatment by radical prostatectomy or external beam radiation, 80-90% and 41-55% erectile dysfunction, 39-49% and 6-7% urinary incontinence, and 6-7% and 30-35% gastrointestinal problems respectively, were observed. The side effects associated with brachyterapy are likely to resemble those from external beam radiation.

Investigations have been designed to evaluate the extent to which such side-effects lower the quality of everyday life. Quality of life [74], as well as the economics [75], of screening are fundamental factors involved in evaluating the total cost benefits of screening for PC. However, both of these issues are outside the scope of the present study.

1.4 PSA AND ITS MOLECULAR ASPECTS

1.4.1 Historical background

In 1970 the glycoprotein later referred to as prostate-specific antigen (PSA) was first described by Ablin et al. [76], and PSA was further purified and characterised by Wang et al in 1979 [77]. Stamey et al. [78] found that PSA level in serum increases with an increasing volume of the PC, thus enabling detection of PC at an early-localised stage. This approach is, however hampered by the fact that even benign disorders of the prostate lead to elevated PSA levels. Further investigations of specimens obtained in connection with radical prostatectomy by Stamey et al. [79] revealed that each ml increase in the volume of a PC causes an elevation in the PSA level which is approximately 10-fold greater than the increase associated with each ml of benign prostatic hyperplasia (BPH). Population-based studies also revealed that the serum PSA concentration is directly correlated with patient age [80]. In spite of its limitations, including low specificity, a relatively low sensitivity and a substantial risk for overdiagnosis, opportunistic PSA-based screening for PC became widespread in the US from 1988 onwards, resulting in the most dramatic rise in cancer incidence ever recorded [58].

1.4.2 Molecular aspects

PSA is one of 15 known members (hK1-15) of the tissue kallikrein family of proteins the genes for which are all located on chromosome 19q13.3-q13.4 [81]. As is the case for the gene encoding PSA (hK3), the genes coding for hK2 and hK4 are also expressed primarily in the prostate gland under the regulation of androgens.
PSA is a serine protease with a molecular weight of approximately 33 kDa. It is normally first synthesized by secretory cells of the prostatic epithelium in the form of an inactive 244-aminoacid precursor protein (proPSA) [82]. This is after secretion into the excretory ducts cleaved by hK2, which removes the first 7 N-terminal amino acid, to produce the active, 237-amino acid enzyme [83]. The major function of active PSA appears to be proteolysis of the seminal coagulum, thus enhancing sperm motility.

However, not all of the PSA in the semen is free and enzymatically active. Recent investigations [84] have revealed that in addition to binding of a minor proportion (5%) of seminal PSA to the protein C inhibitor, approximately 25% of this enzyme is inactive, even though uncomplexed. This inactive free PSA contains internal cleavages at various positions, leading to an alteration of the PSA molecule from a single to a multi-chain state.

On the other hand, PSA in the circulation has no known biological function and cell walls and the basal membrane at the dorsal surface of the epithelial cells form a barrier, which efficient prevents PSA from entering the circulation. Thus, when this barrier is intact, as is the case in both most young men, and many older men, only small amounts of PSA leak out and serum levels remain below 1 ng/mL i.e., $1/1000^{th}$ of the concentration present in seminal fluid [85].

When the number of epithelial cells, in the prostate increases, as seen in connection with BPH, serum PSA levels often rise in proportion to the concomitant increase in prostate volume. However, damage to this barrier by prostatic disorders results in the leakage of even more PSA into the circulation. If the nature of this leakage is more acute, e.g. associated with prostate biopsy or prostatitis, the PSA level will usually return to normal within 4-6 weeks after termination of the damage, although recent result indicate that this normalisation may last as long as one year [86]. In contrast chronic disruption of the epithelial cells and basal membranes, as caused by prostate cancer, lead to an elevation in PSA levels that is surprisingly stable. Studies on prostatic tissue itself [87] have revealed lower PSA levels in malignant than in benign tissue, emphasising the fact that the increase in serum PSA is due to increased leakage, not to increased production.

Once in the blood, the enzymatically active PSA rapidly forms complexes with alpha-1-antichymotrypsin and, to a lesser extent, with alpha-2-macroglobulin and the alpha-1-proteinase inhibitor. However, some 10-30% of this circulating PSA remains uncomplexed (free PSA,) with lower levels of free PSA being observed in the serum of men with prostate cancer than of men with BPH. Circulating, free PSA is not enzymatically active. Recent publications have concluded that at least three different forms of inactivated, free PSA are present in serum. One of these forms is the precursor proPSA [88], which is either intact, with all 7 amino acids at the N-terminal end in place, or truncated, with only 1-6 of these amino acids still present. Another form is the so called BPSA [89], in which internal cleavages have rearranged the molecule and thus inactivated its enzymatic activity. The third form is referred to as inactive PSA, and is similar to intact PSA, but with only minor structural changes.

1.4.3 Early detection of prostate cancer based on determination of serum levels: diagnostic limitations and efforts to overcome them

1.4.3.1 The accuracy of PSA testing

Despite the fact that among tumours markers, PSA exhibits an outstanding ability to indicate the presence of cancer, the most effective clinical application of this marker is

not diagnostic, but rather for monitoring the clinical course PC. When employed to diagnose early PC, the usefulness of PSA levels is limited by the fact that PSA is also produced by benign tissue which might be present, as well as by its ability to detect PC that will never cause clinical symptoms. As is the case for other diagnostic tests as well, the usefulness of PSA testing depends on the degree to which it correctly identifies those who are ill–i.e., *sensitivity*- and those who are healthy–*specificity*,- employing a given "cut-off" level. Since there is a reciprocal relationship between sensitivity and specificity (i.e., if one increases the other will decrease), the choice of "cut-off" point will always be arbitrary and dictated by whether false negatives or false positives are most harmful.

Factors that influence the prevalence of PC have to be considered when the performance of PSA testing is decided on. Such factors are: age; presence or absence of micturition problems; findings on digital rectal examination and whether it is single or repeated testing [90]. Once the test results become available, it is possible to evaluate the performance of PSA screening on both sides of the "cut-off" chosen. The *positive predictive value* (PPV) is determined from the group with a value above the" cut-off" level (i.e., positives). In this group the ratio between those with the disease and all men who test positive provides the probability of being diagnosed on the basis of a positive test or the PPV. Similarly, the *negative predictive value* (NPV) is determined from the men with a value below the "cut-off" level (negatives) by dividing the number without the disease by the total number of those testing negative.

As discussed above, the aim of early detection of PC is to diagnose and treat this disease while it is still localised to the prostate gland. It soon became evident that the risk for residual cancer following surgery was directly proportional to the pre-operative serum PSA level. In men with a level between 4 and 10 ng/mL, 75-90% of the PC was localized [90-93] and this interval thus became "the window of opportunity" for early detection. However, as many as 20-30% of PC detectable by digital rectal examination or transrectal ultrasonograhy were associated with PSA levels below 4 ng/mL [94-96] and consequently not diagnosed when this "cut-off" for biopsy was employed. Strategies for increasing the sensitivity of PSA screening includelowering the "cut-off" level[97], combining such screening with digital rectal examination, repeating the PSA testing (velocity of change)[98]: application of age- standardised "cut-off" levels [99] and determination of the percentage of the total serum PSA which is free [100]. In addition analysis of complexed PSA [101], hK2, BPSA, proPSA and/or truncated proPSA has been proposed to provide useful information although this proposal has not yet been adequately evaluated or tested in clinical practice.

The clearest limitation associated with determination of PSA levels as a diagnostic test for localised PC is the relatively large number of false positives obtained, even employing rather high "cut-off" levels. Choosing a "cut-off" value of 4 or 10 ng/mL will, at the expense of impaired sensitivity, provide a specificity of approximately 60% or 95%, respectively [102]. Nonetheless, if men with PSA levels in the interval 4.0 to 10.0 ng/mL are subjected to sextant biopsy, only one in 4 or 5 will prove to have PC (PPV =20-25%). Lowering the "cut-off" level to 3 ng /mL will reduce this PPV value even further [103]. Strategies proposed for increasing this specificity include, adjustment of the PSA value to account for prostate volume (PSAD); use of age-

standardised "cut-off" values (age PSA); and determination of %fPSA. In addition, in this case as well, analysis of complexed PSA, hK2, BPSA, proPSA and/or truncated proPSA have been proposed to provide useful information, although as for sensitivity this proposal has not yet been adequately evaluated or tested in clinical practice.

1.4.3.2 PSA density (PSAD)

The ten-fold greater increase in serum PSA observed per gram PC tissue in comparison to gram BPH [79] indicates that the ratio between the PSA level and the volume of the prostate might be a useful tool for distinguishing between benign and malignant prostate tissue when the PSA level is only slightly elevated (i.e., 4-10 ng/mL) [79, 104, 105]. However, employing a "cut-off" ratio of 0.15 in this PSA interval, increased specificity is only achieved at the expense of an unacceptable reduction in sensitivity [106, 107]. With lower "cut-off" ratios the sensitivity would be higher, but the test will then be less specific.

There are several explanations for the poor diagnostic performance of PSAD. One is the relatively large inter-individual variation in the number of PSA-producing epithelial cells present per ml BPH tissue. Another explanation involves the relatively poor agreement between estimations of prostate volume performed by TRUS by different examiners, as well as, a rather poor correlation between estimated prostate volume and prostate weight (r=0.61) [106]. In addition, estimation of prostate volume employing TRUS is time-consuming and relatively expensive.

Furthermore, the originally reported clear-cut difference in PSAD between PC and BPH, described by Stamey was recently questioned by this same investigator, who now finds only weak correlation between PC volume and PSA at levels below 9 ng/mL [108]. In retrospect, it can be seen that the original study, in which the correlation was 0.70, included men with PSA levels as high as 266 ng/mL with the majority of participants having a PSA value greater than 10 ng/mL. Apparently, the correlation between the PSA level and PC volume is only strong in the case of larger PC volumes. Recently, TRUS measurement of the volume of the prostatic transition zone and subsequent calculation of its PSA density has been proposed as an approach to increasing specificity [109]. However, use of the PSA density of the transition zone appears to involve much of the same problems as does the use of PSAD.

1.4.3.3 Age-standardised PSA "cut-off" levels (Age PSA)

Serum PSA levels increase slowly throughout the adult life of men, even in the absence of benign and/or malignant growth. Considering this age-related increase, Oesterling [80] concluded that normal values are lower than 4.0 ng/mL in younger and higher in elder men. Application of these age-standardised "cut-off" levels will increase the sensitivity of PSA testing in young men and decrease the number of biopsies performed on elderly. However, this will be achieved at a cost of more biopsies being performed on the younger men and fewer cancers being successfully diagnosed in the elderly.

1.4.3.4 Lowering the "cut-off" levels

Although certain investigators have proposed that the majority (69%) of PC associated with PSA levels below 4 ng/mL are small and of low grade [98] other studies have reported a higher incidence of significant cancers, at least in the PSA interval of 3-4 ng/mL [97, 103, 110]. However, the decreasing PPV [103] associated with lower PSA "cut-off" values necessitates the use of additional tools to identify those individuals with PC. An alternative strategy is to repeat determination of PSA levels in hope of being able to diagnose developing PC while still curable.

1.4.3.5 Repetitive the PSA testing

The concept of repetitive PSA testing arose from the retrospective analysis of the absolute change in PSA levels with time performed by Carter el al. [111], who observed a more rapid increase in those men who were subsequently diagnosed as having PC compared to those without PC. These studies also indicated a change from slow to exponential PSA velocity on the average of 7-9 years prior to clinical diagnosis. This approach requires determination of PSA in least three serum samples over a period of 1-2 years and its use is further limited by the relatively large day to day variations in the PSA levels of any given individual [112]. As an alternative,the time required for the PSA value to double [113] may be estimated, which has the possible advantage of measuring the change observed relative to a previous measurement.

1.4.3.6 Percentage of the total serum PSA that is free (%fPSA)

Introduction of analysis of %fPSA opened the possibility of improving the diagnostic performance of PSA screening at the cost of just one additional serum analysis [100, 114]. Evaluations has demonstrated that in the PSA interval of 4-10 ng/mL this approach yields, increases in specificity with up to 20-30% at the expense of not detecting 5% of cancers [115]. In connection with lower PSA levels, determination of % fPSA may improve sensitivity while requiring fewer unnecessary biopsies than DRE or the use of age standardised "cut off" levels [97]. The problems associated with this approach include the instability of the free component, large variations in inter-assay performance and difficulties in choosing an appropiate "cut-off "level. In addition there are contradictory reports on the variation in % fPSA with age and changing prostate volume.

Also the use of complex PSA, hK2, BPSA, proPSA and truncated proPSA have been proposed and shown promising results. Although, they are still not sufficiently evaluated to be used in clinical practice.

1.4.3.7 Artificial neural networks

Clinical data on a large number of patients who have undergone prostate biopsy have been collected and subjected to computerized processing in order to construct models for use in making decisions about whether or not to perform a biopsy. These models, called artificial neural networks (ANN), are predominantly based on the variables discussed above. A recent study [116] showed that in comparison to basing such decisions on %fPSA values, application of ANN would have increased specificity from 21% to 41%, with a sensitivity of 95%, in the PSA interval of 4-10 ng/mL. In the PSA

interval of 2-4 ng/mL, sensitivity would have increased from 10% to 65% at a specificity of 95%. Problems connected with the use of ANN include unsatisfactory reproducibility between different populations and the need to determine multiple variables each with its own risk of misclassifications.

1.5 DIGITAL RECTAL EXAMINATION (DRE) AND TRANSRECTAL ULTRSONOGRAPHY (TRUS)

1.5.1 DRE

Digital rectal examination (DRE) was till the mid 1980's the method of choice for early detection of localised PC [117]. The shortages of this diagnostic method became evident with the introduction of PSA. A meta-analysis comparing the sensitivity of DRE to that of all cancers diagnosed due to DRE, TRUS and or an elevated PSA level revealed a high specificity (0.94) but a low sensitivity (0.59) and a very low PPV (0.28). [118]. Furthermore, several studies [119-121] have shown even lower PPV (6-10%) for DRE in early detection of PC in men with normal PSA levels (< 4.0 ng/mL). At PSA levels in the interval < 1.0 ng/mL, which included 38% of all studied men, Schroder et.al. [122] found a PPV of only 2% for DRE i.e., 46 biopsies were needed per detected PC. The poor performance of DRE in men with PSA levels < 4.0 ng/mL made the authors question DRE as a primary test for annual PC screening as is recommended in the US **[123]**.

The introduction of PSA and systematic sextant biopsies have enabled detection of PC long before they become palpable. This is underlined by the increase of inpalpable (T1C cancers), which now are estimated to constitute 80% of all new PC in the US [124]. Thus, DRE is unspecific at normal PSA levels and in men with moderately elevated PSA its diagnostic value is limited due to a low sensitivity. Nevertheless, DRE is still often used as an adjunct test to PSA.

1.5.2 TRUS

Transrectal ultrasonography (TRUS) of the prostate was first described in 1957 but it was not until the mid 1980s that it became accepted as a diagnostic tool for PC [63]. The imaging is performed by insertion of an ultrasound transducer into the rectum. Different parts of the prostate can be visualised due to differences in echo-densities. In comparison to kidney and testicular cancer the echogenic pattern of PC is more variable and less specific. Compared to DRE, TRUS has a better diagnostic accuracy since 80%-90% of all palpable tumors are visible by TRUS, but only 50%-60% of all tumors visible by TRUS are palpable. Furthermore TRUS enable ultrasoundguided biopsies towards palpable and/or visible lesions in the prostate as well as randomised, systematic-quadrant or sextant-biopsies–[125, 126]. However, with the introduction of systematic biopsies it became apparent that PC often is neither visible on TRUS [127] nor palpable with DRE. Since TRUS also lack specificity [126] this method is today rarely used as a primary tool in early detection of PC.

Recent attempts to improve the diagnostic ability of TRUS include 3 dimensional colour doppler imaging and injection of contrast agents. Also prostate volume is measurable with TRUS although the accuracy of formulas for this estimation are limited by the varying shape of the prostate gland as well as variability in results between examiner.

During the late 1980's and early 1990's, there was considerable optimism concerning the use of TRUS as a compliment to DRE to achieve improved pre-treatment staging of PC. However, the efficacy of this approach was never established and today routine use of TRUS has been abandoned in favour of the use of various tables and nomograms [51]. Despite all its shortcomings, TRUS is still necessary for examination and systematic biopsy of the prostate gland, and few contemporary urologists would be satisfied to perform their work without access to this diagnostic tool.

1.6 BIOPSY

Examination of a sample of prostate tissue under the light microscope is absolutely necessary to establish a diagnosis of prostate cancer. Such an examination can involve cytology on single prostate cells or, as is more common today, by histopathology. The cytological or histological evaluation also provides the pathologist with highly important information concerning the nature and future behaviour of the cancer, leading to the assignment of a grade of malignancy.

In the 1980's fine needle aspiration cytology (FNAB) of the prostate [128] was still the method of choice for diagnosing PC in Sweden. Evaluation of the findings is based on the World Health Organisations criteria for highly, moderately and poorly differentiated malignant cells. Compared to the prostatic core biopsy, which allows histopathology and was introduced in the 1980's, FNAB appears to exhibit equal sensitivity [129, 130] and a lower risk for septic complications. However, during the past decade core biopsy has become the method of choice, probably because it is supposed to allow better estimation of the extent of the tumour and the possibility to evaluate premalignant lesions (PIN), as well as due to a lack of pathologists trained in cytological evaluation. The Gleason grading system is widely applied to the analysis of core biopsies [131]. With this system the predominant and second most prevalent architectural patterns are identified and assigned a grade from 1-5, with 1 being the most differentiated and 5 the least. A sum (score) of these grades greater than 7 is characteristic of a highly aggressive tumour; a sum of 7 indicates a lower degree of aggressiveness; and PC with a sum below 7 usually have the best prognosis. Unfortunately, the vast majority of PC detected by biopsy receive a Gleason score of 6 or 7, which provides rather limited prognostic information.

With the improvements in TRUS and procedures for systematic biopsy it became evident that a considerable number of significant PC are neither detectable by ultrasound nor palpable. Randomly directed quadrant biopsies in men exhibiting elevated PSA levels were replaced by sextant biopsies [126] in order to enhance the

diagnostic performance. Although the average palpable PC is larger than the average non-palpable [132], questions have been raised concerning whether PCs detectable by TRUS are larger than those not detectable by this procedure [133].

In studies where systematic sextant biopsies are employed in combination with lesion-directed biopsies alone or together with a PSA "cut-off" value of 3.0 ng/mL, the improvement in diagnostic outcome is surprisingly small 3.9% and 4.4%, respectively [121, 134], compared to studies employing only lesion-directed biopsies (3.6%) [105]. Several new biopsy strategies have been proposed in attempt to further improve this performance. Norberg et al. [135] found that an additional 15% of cases of PC could be diagnosed employing eight or ten biopsies instead of six. Other investigators have been concerned with the use of as many as 32 biopsies in certain cases, the optimal sites for systematic biopsying [136] and the importance of repeated biopsying in the case of negative findings [137]. The common philosophy of biopsy studies can be summarised as "seek and you shall find". At present, the most generally accepted biopsy strategy in Sweden involves taking lateral sextant biopsies, as proposed by Hodge et al.[126].

1.7 THE EPIDEMIOLOGICAL METHODOLOGY EMPLOYED

1.7.1 Analysis of survival time and censoring

Analysis of survival time is concerned with the time that elapses before an individual reach an endpoint of interest (often, but not always death), starting from a given time-point [138, 139]. Two characteristic features of survival analysis are the length of time required to reach the endpoint and lack of complete data, i.e., censoring. As used here, censoring means that only partial information is available for a participant, because he is not followed-up before he reaches the endpoint of interest, or before the study is terminated.

Survival time, even for censored individuals prior to censoring, can be evaluated utilizing the Kaplan-Meier procedure and the cumulative probability that the endpoint has not been reached by an individual at any given period of time subsequent to baseline can then be illustrated in the form of survival curves. Thereafter, the log-rank test, which compares events occurring at all time-points on the survival curves, can be employed to test for differences in survival between the groups studied. The use of survival curves involves two assumptions: first, that the risk for the event remains constant throughout the entire follow-up period and, secondly, that individuals who are censored during the follow-up are at the same risk as those who are not censored.

1.7.2 Lead-time

Lead-time is the period of time, which is gained by basing diagnosis on early detection rather than clinical symptoms. If early detection is achieved at the very beginning of the detectable preclinical phase (DPCP), then the lead-time will be the same as the DPCP or sojourn time. If no previous (prevalence) screening for PC has been conducted,

stages of the cancers detected at the time of the screening will be randomly distributed. Lead-time will then, on the average be, equal to half the sojourn time, whereas repeated (incidence) screening will result in a lead-time that is somewhat longer, especially when short screening intervals are employed (due to the comparatively fewer number of cancers detected which are at the end of the DPCP).

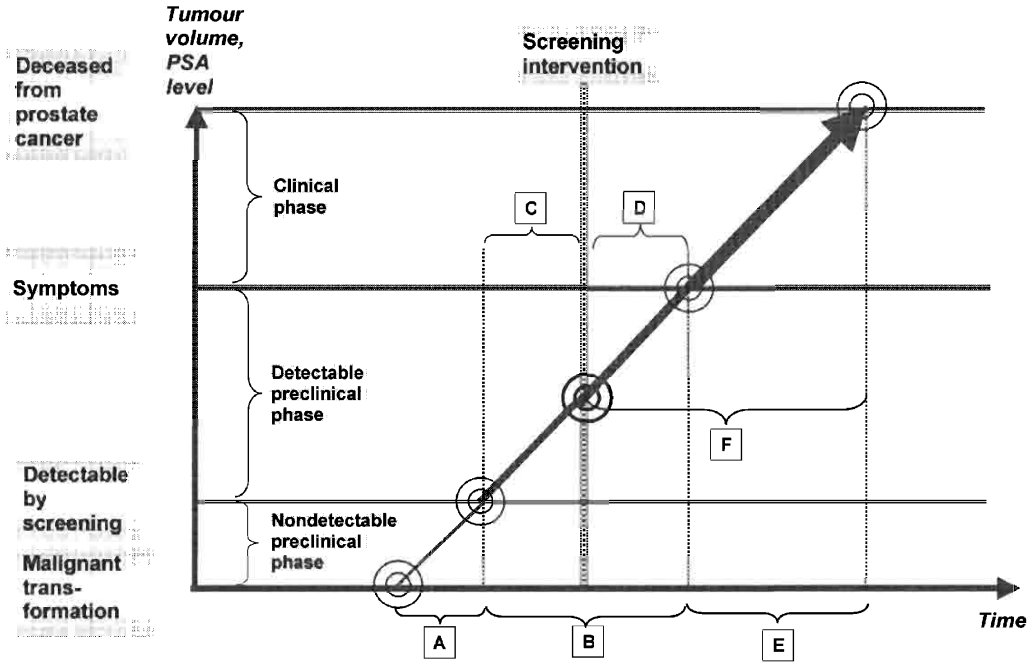

Figure 4. This schematic figure is an attempt to explain the terms used to describe the progression of PC over time. The large arrow pointing obliquely to the right represent this process in a man. The three phases highlighted to the left indicate both characteristic steps in the cancer development as well as defined periods of time. (The scale in this figure is not defined.)

A. **The non-detectable preclinical phase**. This phase starts with the malignant transformation of a dividing cancer cell and includes the time until the cancer is detectable by screening.
B. **The detectable preclinical phase**. The cancer has now become detectable with the screening method employed eg. a PSA level $\geq$ 3.0 ng/mL. This phase lasts until the cancer is diagnosed due to symptoms. Another name for this period is sojourn time
C. **The delay-time** is the time from the entrance of the DPCP till diagnoses. In this figure the delay time is shortened due to screening.
D. **The lead-time.** The time gained by screening. If early detection of PC is introduced, e.g. by screening, the time of diagnoses is advanced from the time of clinical diagnoses due to symptoms.
E. **The post lead time survival.** Is the period of time from diagnose due to symptoms until death from PC or death from other cause (or in a study until censoring).
F. **Total survival.** This expression is used to consider the false improvement in cancer survival due to early detection. It estimates the time between diagnose and death

including both lead-time and post-lead-time survival.

1.7.3 Attributable mortality

Attributable risk used in epidemiology to evaluate how much of the incidence of a disease can be attributed to a certain factor, e.g., the attributable risk of lung cancer in smokers or the relationship between incidence of acute myocardial infarction and blood cholesterol levels. In a similar fashion the attributable mortality in connection with a disease can be employed to indicate how much of the total mortality can be attributed to a certain parameter, e.g., how much of the total PC-related mortality is accounted for by men with a baseline PSA level ≥ 10.0 ng/mL. This measure can then be utilized to estimate when and to what extent a reduction in PC mortality may be expected if successful curative treatment of men with PSA levels in this interval is achieved.

1.8 METHODOLOGICAL ASPECTS OF SCREENING

Screening for a disease in asymptomatic men is associated with responsibilities beyond those encountered in connection with a patients visit to the doctor to due to symptoms. Screening introduces considerations such as harm, anxiety [140], loss of time and unreasonable cost [75], which are usually not otherwise present. Consequently, in order to justify screening, the physician must be convinced that the benefits for certain men outweigh the negative consequences for all of the others. Furthermore, since the prevalence of clinically significant PC is relatively low in asymptomatic men, the screening test employed must possess a high degree of sensitivity in order to detect all men with the disease and, perhaps even more important, this test must exhibit pronounced specificity in order to minimize the number of false positives.

According to Gann [141] four factors decide the pattern of incidence following the introduction of a screening method i.e., the rate of dissemination of the screening technique, the lead-time, the background level of incidence and the extent of overdiagnosis.

In 1968 Wilson and Jungner [142] listed some principle criteria which must be fulfilled before population screening is to be recommended. A simplified version of their list includes the following major criteria:
1) The disease should represent a substantial burden in terms of public health.
2) The asymptomatic, non-metastatic phase of the disease should be detectable by the procedure employed.
3) The screening procedure employed should demonstrate satisfactory sensitivity, specificity and PPV.
4) The potential for successful curative treatment should be substantially greater during the early than during the late stage of the disease.
5) Treatment of screen-detected patients should lead to a reduction in cause-specific mortality.
These criteria will be commented upon briefly here in relationship to our current knowledge concerning PC.

The first criterion is probably fulfilled, considering the high rate of PC-specific mortality in Sweden and the significant morbidity associated with the large number of PC detected clinically. Likewise, the second criterion can also be considered to be fulfilled, with the present availability of serum PSA testing and systematic biopsy procedures guided by ultrasound. In contrast, it is less evident that the third criterion is fulfilled with respect to PC. The sensitivity of 70-90% obtained with these screening procedures [143] is acceptable. However, specificity is a major weakness, since 75-80% of the men who test positive for PC in connection with such primary screening (i.e., who exhibit PSA levels of 3-10 ng/ml) are not diagnosed as having PC on the basis of subsequent biopsy. The PPV associated with this PSA interval is approximately 15-25% [134]. The recent evaluation of the SPCG 4 study [27] indicates that the final two criteria above may also be considered as being fulfilled, even though most of the patients involved in that study were diagnosed on the basis of clinical symptoms, rather than screening.

Thus, most of the criteria discussed above are fulfilled and, furthermore, screening appears to be the only realistic strategy currently available for reducing PC mortality. Nonetheless, before deciding that such screening is justified, certain other major disadvantages must also be taken into consideration, i.e., the potential harm associated with curative treatment, the risk of overdetection and the prolonged anxiety of living with a diagnosis of cancer (as a result of the additional lead-time obtained). More information about such considerations, as well as an improved understanding with respect to who to screen, who to biopsy, and who to treat and how are required before a well-founded and definite decision about PC screening of the general population can be reached. Fortunately, the two large randomised screening studies [11, 43], designed to answer these questions, will hopefully give an answer to the crucial issue whether the sacrifice of many will justify the benefits in a few.

2 THE STUDIES

2.1 AIMS OF THE PRESENT INVESTIGATION

- To decide the diagnostic performance of percent free PSA with regard to sensitivity at normal PSA levels and specificity at moderately elevated PSA levels (I)
- Study the influence of PSA, prostate volume and age of the patient on the percent-free PSA ratio (II).
- Evaluate the lead-time to prostate cancer diagnosis in relation to PSA levels (III).
- Evaluate the risk of overdiagnosis in relation to PSA levels at a single intervention (III).
- Study the total PC-specific survival at different PSA levels (IV)
- Evaluate whether the decrease in PC mortality in the US is in accordance with the lead-time of the present study. (IV)

2.2 METHODOLOGICAL ASPECTS

2.2.1 The populations studied

The men studied here belong to one of two well-defined cohorts that are described further below. In both cohorts the men were selected randomly, on the basis of their age, from the general population and invited to participate in health examinations.

The Stockholm South Prostate Cancer Study (the SÖS study) conducted in 1988-1989, involved a single intervention designed to evaluate early detection of PC. Among the 26,830 men born between 1918 and 1933 and living in the southern region of Stockholm in January of 1988, 2,400 (every eleventh man in each annual age group from 55-70 years of age) were chosen randomly on the basis of the day of their birth to receive an invitation to undergo PC screening. Of these 2,400 men, 1,782 without a prior diagnosis of PC accepted this invitation.

All participants underwent a digital rectal examination (DRE) and transrectal ultrasonography (TRUS), irrespective of their serum PSA levels, which were also determined (Tandem-R Hybritech). Prostate biopsies were taken in cases involving abnormal findings upon either DRE and/or TRUS and/or a PSA level $\geq$ 10.0 ng/mL. As a result of these procedures for early detection, 65 men were diagnosed as having PC [120].

The study of men born in 1913. In 1963 all men living in Gothenburg, Sweden, who were born on dates that were divisible by 3 during 1913 were invited to a health screening. The total number was 973, and the attendance rate was 88% [144]. These men were subsequently followed and re-examined for, above all, risk factors for cardiovascular disease. In 1980 serum was sampled and stored frozen from 657 participating men without PC diagnosis. The frozen sera were analysed for PSA in 1995. During follow-up no man underwent PSA testing for early detection of PC and no man diagnosed as having PC underwent treatment with curative intent. Two

previous publications have evaluated this population with regard to the risk of being diagnosed with or to die from PC [134, 145].

Paper I
The population examined in Paper I included those 1,748 men (98.1% of all participants) from the SÖS study from whom frozen serum samples were available for determination of the percentage of serum PSA that was free (%fPSA). Eligible for inclusion were men with prostate cancer diagnosed either at the time of the early-detection intervention (n =64) or clinically (on the basis of symptoms) during the five-year period immediately following this intervention, referred to here as interval cancers (n =7).

Paper II
The population studied in Paper II consisted of 1622 men who, after participating in the SÖS study, fulfilled four criteria: no diagnosis of PC at the time of the early screening nor during the first 5 years of follow–up; a PSA level of less than 10.0 ng/mL; determination of their prostate volume in connection with the intervention; and availability of a serum sample for determination of %fPSA.

Papers III and IV
Two cohorts of men, designated as the reference and screening populations, were investigated in these studies. The reference population included 657 men who had participated in the study of men born in 1913 and for whom serum samples were available for PSA analysis. The screened population was selected by age-matching of the men in the SÖS study to participants in the study of men born in 1913 (the reference population). This matching resulted in a group of 946 men born between 1918 and 1925 with available serum PSA values. The reference population was monitored for 20 years, whereas the follow-up period for the screened population was 12 years. As background controls for these two populations we used all age-matched men living in the Stockholm and Gothenburg communities during the same time period.

2.2.2 The methodology employed

In the SÖS study, serum samples was taken from all participating men at the beginning of study and stored frozen at –70° C thereafter. These frozen serum samples were assayed in 1995 for total PSA content and %fPSA using a commercially available, dual-label, time-resolved immunofluorometric assay (Delfia Prostatus PSA free/total, Wallac Oy, Turku, Finland).

In the study of men born in 1913, serum samples were taken from all participants in 1980, stored frozen and analysed for total PSA content with the same assay as employed in the SÖS study (Delfia Prostatus PSA free/total, Wallac Oy, Turku, Finland).

In all cases (Papers I-IV) the Swedish Cancer Registry was employed to identify those of the men who were diagnosed as having PC during the follow-up period. In a similar

manner the Swedish Cause of Death Registry was utilized to identify those men who died during this same period, as well as the assigned causes of deaths. Information from medical charts was used to carry out TNM classification of the clinically detected PCs. In the cases of men with PC who died during the follow-up period, their medical journals provided detailed information concerning the cause of deaths. These independent conclusions concerning cause of death were then compared to those stated on the death certificates.

Paper I

Total levels of serum PSA (Tandem-R Hybritech PSA assay) and %fPSA (Delfia Prostatus PSA free/total, Wallac Oy assay) were analyzed with the Mann-Whitney U test in order to detect possible differences between the men with PC and those without. The Kruskal-Wallis analysis was used to determine the statistical significance of differences in median %fPSA values between patients with cancer of different stages and grades. The outcome of the screening intervention was evaluated with respect to %fPSA in relationship to PSA levels using a "cut-off" value of 3.0 ng/mL and the findings from DRE and TRUS, as well as the biopsy results. A more detailed descriptive analysis was performed on %fPSA values and the clinical features of those men who were diagnosed as having PC during the follow-up period. Different assay procedures were employed to determine total PSA levels (Tandem-R Hybritech PSA assay) and %fPSA values (Delfia Prostatus PSA free/total, Wallac Oy assay) in this study. We chose to utilise the Tandem-R assay for determination of total serum PSA content since we wished to compare the results obtained to previous reports on the SÖS study group, in which the Tandem-R procedure was employed.

Paper II

In this study the Prostatus assay procedure was utilized to determine both total and %fPSA. To allow evaluation of the influence of prostate volume (measured by TRUS), PSA level and age on the %fPSA value, the men were classified arbitrarily into 4 groups with different PSA levels (0-1.9, 2.0-3.9, 4.0-6.9 and 7.0-9.9 ng/mL). For the men in each PSA interval, as well as for all of the men combined, comparison between %fPSA values and prostate volumes, age and PSA levels were undertaken employing Pearson correlation analysis. Fishers r-to-z transformation was employed to determine the significance of the results thus obtained. Multiple regression analysis was used to calculate the proportion of variability (adjusted R^2) in %fPSA values that was accounted for by prostate volume and age. The correlation values determined in our study were also compared to those reported previously by others.

Paper III

The cumulative incidence from the time of PSA determination at the beginning of the study to the time-point when PC was diagnosed was calculated for the different PSA intervals in the two populations employing survival curves (Kaplan Meier). Lead-time, defined as the time gained by basing diagnosis on early intervention (after adjusting for censoring) was here determined by two different approaches. Both of these approaches assume that in men who later developed PC, the disease was already present at the time when the PSA level reached a value $\geq$ 3.0 ng/mL.

In the case of the reference population, lead-time was estimated as the period of time that clasped from determination of an elevated PSA level to the clinical diagnosis of PC. In the case of the screened population, on the other hand, the men had undergone an early diagnostic intervention, so that the time that elapsed from determination of an elevated PSA level to clinical detection was unknown. Utilising the other approach with this population, we considered the observed cumulative incidence for the reference population to represent the expected incidence for the screening population. The lead-time was then calculated as the advancement in time from the expected incidence to that reached by the early diagnostic intervention. The median lead-time, defined as the time after which the cumulative incidence reaches 50% of its maximum value, was then calculated for the PSA intervals ≥3.0, 3.0-9.9 and ≥ 10 ng/mL. Confidence intervals for the median lead-time and the 80th percentile for the screened population were then calculated employing the percentile bootstrap approach.

Paper IV

The men in the two populations were evaluated for risk of PC death. Survival analysis revealed a similar cumulative PC-specific mortality at 12 years in the populations, which together with a comparable cumulative PC incidence allowed us to merge the two populations for further analysis. The total survival, here including lead-time and post-lead-time survival, was calculated in relation to PSA levels at blood sampling. Men who were diagnosed with PC during follow-up were then considered to have PC already at the time of sampling of an elevated PSA. We also studied how different PSA intervals attributed to PC specific mortality during various time periods. The attribution of PC deaths was calculated both in relation to the number of person-years at risk in the actual PSA interval as well as in the total population (population attributable PC mortality). Finally, also the population attributional ratio was calculated as the percentage of population attributable PC mortality that was attributable to different PSA levels during the specified time periods.

2.3 FINDINGS

Paper I

Retrospective analysis of the diagnostic performance of %fPSA values at the time of the screening intervention for men with PSA levels < 3.0 ng/mL indicated that a "cut-off" ratio of <18% correctly identified all nine men with PC and 75% of all men diagnosed as having PC at the time of screening or within the first 5 years of follow-up. In this same PSA interval and during the same period of time the large group of 1109 men (63% of the total) with %fPSA ratios >18% were at very low risk of being diagnosed as having PC. In this later group, 159 biopsies (49% of the total) were performed on the basis of positive findings with DRE and/or TRUS and no cancers were detected. In the PSA interval of 3.0-9.9 ng/mL, a "cut-off" ratio of 22% correctly identified 24 (96%) of the 25 detected cancers, resulting in a 24% decrease in the biopsy rate. If PSA and %fPSA values had been used in combination, a PSA "cut-off" level of ≥ 3.0 ng/mL and/or a %fPSA ratio of < 18% would have correctly identified all 64 men with PC.

Regarding the two different assays utilized in this paper, simple linear regression analysis of the total PSA values for our samples determined using the Prostatus® and the Hybritech® assays revealed a close correlation (Y=-0.544+0.933X; r=0.984).

Paper II

The estimated correlation coefficients for %fPSA in relationship to prostate volume, patient age and PSA level are summarised in Table I.

PSA level (ng/mL) Variable	**All combined*** (n=1622)	**<2.0** (n=1144)	**2.0-3.9** (n=331)	**4.0-6.9** (n=112)	**7.0-9.9** (n=35)
Prostate volume	r = –0.13 P < 0.001	**r = –0.04** **P = 0.18**	**r = 0.26** **P < 0.001**	**r = 0.39** **P < 0.001**	**r = 0.60** **P < 0.001**
Age	r = –0.01 P = 0.74	**r = 0.02** **P = 0.45**	**r = 0.22** **P < 0.001**	**r = 0.24** **P = 0.009**	**r = 0.56** **P < 0.001**
PSA level	**r =–0.46** **P < 0.001**	**r = -0.50** **P < 0.001**	r = -0.14 P = 0.01	r = -0.08 P = 0.39	r = –0.10 P = 0.56

Table I. The correlation-coefficients for *%fPSA* in relationship to prostate volume, patient age and total PSA level. *The median PSA level for all of the men combined was 1.22 ng/mL

As can be seen, %fPSA values increased with increasing prostate volume and age for men with PSA values ≥ 2.0 ng/mL, whereas %fPSA decreased with increasing PSA levels in the intervals of < 2.0 and 2.0-3.9 ng/mL. When all men with PSA levels of <10 ng/ml were combined and analysed, the correlation to prostate volume and age was not statistically significant, while the correlation to PSA levels was negative.
After grouping according to median PSA levels, the correlation coefficients observed in previously published studies are closely similar to those calculated here.

Further analysis (not included in II) revealed that in the case of *free* PSA, there was a direct correlation to prostate volume and age in all intervals (Table II). The correlation of the *total* PSA and *complexed* PSA values to these same parameters was, on the other hand, more variable (Tables III and IV).

PSA (ng/mL) Variable	**All combined** (No.=1622)	**<2.0** (No.=1144)	**2.0-3.9** (No.=331)	**4.0-6.9** (No.=112)	**7.0-9.9** (No.=35)
Prostate volume	**r = 0.63** **P < 0.001**	**r = 0.38** **P < 0.001**	**r = 0.35** **P < 0.001**	**r = 0.49** **P < 0.001**	**r = 0.63** **P < 0.001**
Age	**r = 0.22** **P < 0.001**	**r = 0.16** **P < 0.001**	**r = 0.24** **P < 0.001**	**r = 0.23** **P = 0.009**	**r = 0.58** **P < 0.001**

Table II -The correlation-coefficients for *free* PSA in relationship to prostate volume and patient age.

PSA level (ng/mL) Variable	**All combined** (No.=1622)	**<2.0** (No.=1144)	**2.0-3.9** (No.=331)	**4.0-6.9** (No.=112)	**7.0-9.9** (No.=35)
Prostate volume	**r = 0.53** **P < 0.001**	**r = 0.35** **P < 0.001**	**r = 0.20** **P = 0.0003**	r = 0.17 P = 0.07	r = 0.002 P = 0.99
Age	r = 0.16 P < 0.001	r = 0.11 P = 0.0001	r = 0.12 P = 0.02	r = 0.04 P = 0.65	r = 0.07 P = 0.68

Table III. -The correlation-coefficients for *total* PSA in relationship to prostate volume and patient age.

PSA (ng/mL) Variable	**All combined** (No.=1622)	**<2.0** (No.=1144)	**2.0-3.9** (No.=331)	**4.0-6.9** (No.=112)	**7.0-9.9** (No.=35)
Prostate volume	**r = 0.49** **P < 0.001**	**r = 0.22** **P < 0.001**	r = 0.037 P =0.50	r = 0.009 P =0.93	r = -0.25 P =0.15
Age	r = 0.14 P < 0.001	r = 0.09 P = 0.002	r = 0.10 P =0.09	r =-0.05 P = 0.57	**r = -0.47** **P = 0.005**

Table IV. -The correlation-coefficients for *complexed* PSA in relationship to prostate volume and patient age.

Multiple regression analyses, controlled for PSA levels, demonstrated that the proportion of variability (adjusted R^2) in %fPSA values for the *entire population* (all combined) that could be explained by variabilities in prostate volume and age was not significant (adjusted R^2 =0.02). With *PSA levels > 2.0 ng/mL*, the adjusted R^2 value was significant and in the PSA interval *7.0-9.9 ng/mL*, variations in prostate volume and age together could account for as much as 47% of the variation in %fPSA.

Paper III

The cumulative incidences for the two populations were in good agreement with those of their respective background populations. When the cumulative incidences for men with PSA levels ≥ 3.0 ng/mL in the two populations were compared, the incidence curves intersected after 10.6 years of follow-up. In the case of PSA levels ≥ 10.0 ng/mL, the curves approached one another after 5 years and intersected after 10 years. In the reference population a significantly higher cumulative incidence was associated with increasing PSA levels, and for men with PSA levels ≥ 10.0 ng/mL, this incidence curve reached 50% after 10 years of follow-up.

The median lead-time after 12 years of follow-up was shorter in the screened population compared to the reference population when all men with PSA levels ≥ 3.0 ng/mL were analysed (4.5 versus 7.8 years), which was also the case for men in the PSA interval 3.0-9.9 ng/mL (5.3 versus 8.5 years). In contrast, men in these two populations with PSA levels ≥ 10.0 ng/mL exhibited similar median lead-times (3.5 versus 3.6 years).

In the case of the reference group after 20 years of follow-up, even longer lead-times were associated with the PSA intervals of ≥ 3.0 and 3.0-9.9 ng/mL (10.7 and 11.2 years, respectively), whereas the lead-time for PSA values ≥ 10.0 ng/mL was unchanged (i.e., 3.6 years).

Paper IV

In men who developed PC during follow-up the total survival from sampling of an elevated PSA level was, due to the lead-time included, strongly dependent on the magnitude of that baseline PSA value. Thus, men with baseline PSA levels ≥ 10.0 ng/mL exhibited a significantly shorter survival time than to those with values of 3.0-9.9 ng/mL ($p<0.001$). Interestingly, men with baseline PSA levels in the range 1.0-2.9 ng/mL demonstrated the same PC-specific survival time as those in the interval 3.0-9.9 ng/mL. On the other in the large group of men (38% of all men) with PSA levels < 1.0 ng/mL only one man (0.02% of all those men) died from PC during follow-up. During the 6^{th}-10^{th} year period of follow-up men with PSA levels ≥ 10.0 ng/mL were at a 10 times higher risk of death from PC compared to men in the intervals 1.0-2.9 ng/mL and 3.0-9.9 ng/mL. However, when PC mortality of the entire population was considered the attributions from these intervals were similar to that from the interval ≥ 10.0 ng/mL. With follow-up beyond ten years the attribution to population attributable mortality was totally dominated by men with PSA levels < 10.0 ng/mL at base-line.

2.4 CONCLUSION AND FUTURE PERSPECTIVES

The diagnostic performance of determining serum levels of PSA as a screening procedure for early detection of PC can be improved by analysing the percentage free PSA (%fPSA) as well. In the present study choice of a "cut-off" value of 18% for this latter parameter results in correct identification of all nine men with screening-detected PC and a PSA level < 3.0 ng/ml. Thus, by also taking the %fPSA values for men with normal PSA levels into consideration, the sensitivity of the screening procedure can be enhanced.

Most cases of PC in men with slightly elevated PSA levels (i.e., in the interval 3.0 - 9.9 ng/ml) are localised and thus amenable to curative treatment. However, approximately 70% of all men with PSA levels in this interval on a single screening occasion did not have PC (false positives). By retrospective application of a %fPSA "cut-off" value of 22% in this PSA interval, the number of false positives was reduced by 24% leading to a total false positive ratio of approximately 55%.

The correlation between prostate volume, age and total PSA level, on the one hand, and %fPSA, on the other, was determined primarily by the serum PSA levels of the men examined. In men with PSA levels of 4.0-9.9 ng/ml, variations in prostate volume and age explained a significant proportion of the variation in %fPSA, indicating that an improvement in specificity might be achieved by controlling for these variables. Recently, possible approaches to achieving this task by employing artificial neural networks [116] or logistic regression analysis have been proposed.

Diagnosis of PC at a considerably earlier stage can be accomplished with PSA-based screening. The length of the lead-time thus obtained is dependent on the relative sensitivity of the screening procedure employed, the length of the follow-up period and the method used to calculate lead-time, as well as on the PSA levels at the time of screening. However, there are large variations in this lead-time for men with slightly elevated PSA levels. These variables should be taken into consideration if lead-time is used to determine the length of the period between screening occasions.

The PSA levels at the time of screening also determine, at least in part, the total survival time. Men with PSA levels $\geq$ 10 ng/ml accounted for most of the PC-specific mortality during the first 5 years of follow-up, but less thereafter. Consequently, men with PSA levels of < 10 ng/ml accounted for most of the PC-specific mortality in our population at longer follow-up times. Since most PC in men with these latter levels is amenable to curative treatment, the effect of successful screening for this disease should be discernible in the form of decreasing mortality after this initial 5-year period. A significant number of such men with rapidly progressing cancers will thus die from this disease even before the mean or median lead-time has elapsed. Since these men are the ones who may benefit most from screening for PC, a short screening interval and/or application of approaches that increase the sensitivity of PSA-based screening are of vital importance to them. Further improvement in the diagnostic performance of PSA-based testing is mandatory in order for such screening to be effective. Application of the %fPSA value in combination with other variables may improve the sensitivity and specificity of such early detection, a possibility which needs to be evaluated in studies involving large numbers of patients [116]. The number and clinical characteristics of the interval cancers detected in the large screening trials which are presently ongoing will provide further information concerning the length of the screening interval necessary to allow detection of these cancers as well.

3 ACKNOWLEDGEMENTS

Det är många som har hjälpt och stöttat mig under de år som jag ägnat åt detta arbete. Ett särskilt stort tack till:

Med. Dr. Ove Gustafsson, min huvudhandledare, som introducerade mig till detta outtömliga - men spännande - forskningsområde och som utöver bra handledning även lättat upp min tillvaro som doktorand.

Professor Jonas Hugosson, min bihandledare, vilkens stora arbetskapacitet och klarsynthet varit av mycket stort värde under sista hälften av arbetet.

Docent Tomas Berlin, min chef på Urologiska kliniken, Huddinge Universitetssjukhus, för hans utmärkta stöd och stora engagemang.

Docent Claes R Nyman, min andre bihandledare, och Med. Dr. Ulf Norming, vilkas entusiasm för urologi och urologisk forskning varit en inspirationskälla för mig.

Professor Jan Adolfsson och medarbetare vid Onkologiskt Centrum i Stockholm, för mycket god hjälp vid insamlande av datauppgifter.

Stefan Franzén, statistiker, för hans stora tålamod och utmärkta assistans.

Professor Hans-Göran Tiselius, för engagemang och hjälp.

Professor Hans Lilja and Dr Charlotte Becker, mina medförfattare för att ha bidragit med sitt stora kunnande inom PSA området.

Gun-Britt Eriksson, laboratorieassistent, för hjälp med serum analyser.

Docenterna, Mikael Häggman, Hans Wijkström och Lennart Öst för deras hjälp med det fjärde arbetet.

Gabriella Dillström, sekreterare, för mycket värdefull hjälp med att söka reda på patientjournaler.och till Marie Karlsson, Marie Lygdman och Sirpa Nuorala, sekreterare, för hjälp med administrativa uppgifter.

Mats Talbäck, statistiker, för hjälp med statistiska bakgrundsdata

Professor, Joe dePierre, för utmärkt språkgranskning med gott humör även när tiden varit knapp.

Stig och Marianne Abelin, mina svärföräldrar för ovärderlig hjälp under dessa år.

Dag och Ulla Törnblom, mina föräldrar, för all omtanke och support.

Susanne, Rasmus och Casper, min familj, “for being the sunshine of my life”.

4 REFERENCES

1. Parkin, D.M., F.I. Bray, and S.S. Devesa, *Cancer burden in the year 2000. The global picture.* Eur J Cancer, 2001. **37 Suppl 8**: p. S4-66.
2. *Cancer Incidence in Sweden 2000.* 2002, Health and diseases 2002:5. Official statistics of Sweden. National Board of Health and Welfare. Centre for Epidemiology. p. http://www.sos.se/fulltext/42/2002-42-5/summary.htm.
3. *Official statistics of Sweden. National Board of Health and Welfare. Centre for Epidemiology. Personal communication.*
4. *Causes of Death 2000.* 2002, Health and diseases 2002:5. Official statistics of Sweden. National Board of health and Welfare. Centre for Epidemiology. p. http://www.sos.se/FULLTEXT/42/2002-42-4/2002-42-4.pdf.
5. Etzioni, R., et al., *Cancer surveillance series: interpreting trends in prostate cancer-- part III: Quantifying the link between population prostate-specific antigen testing and recent declines in prostate cancer mortality.* J Natl Cancer Inst, 1999. **91**(12): p. 1033-9.
6. Legler, J.M., et al., *The role of prostate-specific antigen (PSA) testing patterns in the recent prostate cancer incidence decline in the United States.* Cancer Causes Control, 1998. **9**(5): p. 519-27.
7. Lu-Yao, G.L., M. Friedman, and S.L. Yao, *Use of radical prostatectomy among Medicare beneficiaries before and after the introduction of prostate specific antigen testing.* J Urol, 1997. **157**(6): p. 2219-22.
8. Ries L. A., E.M., Kosary C et al., *SEER Cancer statistics review , 1973-1999.* 2002, National Cancer Institute.
9. Johansson, J.E., *Masscreening for prostate cancer.* Int J Cancer, 1996. **Suppl 9**: p. 1-215.
10. de Koning, H.J., et al., *Prostate cancer mortality reduction by screening: power and time frame with complete enrollment in the European Randomised Screening for Prostate Cancer (ERSPC) trial.* Int J Cancer, 2002. **98**(2): p. 268-73.
11. Gohagan, J.K., et al., *Prostate cancer screening in the prostate, lung, colorectal and ovarian cancer screening trial of the National Cancer Institute.* J Urol, 1994. **152**(5 Pt 2): p. 1905-9.
12. Nomura, A.M. and L.N. Kolonel, *Prostate cancer: a current perspective.* Epidemiol Rev, 1991. **13**: p. 200-27.
13. Haas, G.P. and W.A. Sakr, *Epidemiology of prostate cancer.* CA Cancer J Clin, 1997. **47**(5): p. 273-87.
14. Monroe, K.R., et al., *Evidence of an X-linked or recessive genetic component to prostate cancer risk.* Nat Med, 1995. **1**(8): p. 827-9.
15. Coughlin, S.S. and I.J. Hall, *A review of genetic polymorphisms and prostate cancer risk.* Ann Epidemiol, 2002. **12**(3): p. 182-96.
16. Gronberg, H., et al., *Characteristics of prostate cancer in families potentially linked to the hereditary prostate cancer 1 (HPC1) locus.* Jama, 1997. **278**(15): p. 1251-5.
17. Ostrander, E.A. and J.L. Stanford, *Genetics of prostate cancer: too many loci, too few genes.* Am J Hum Genet, 2000. **67**(6): p. 1367-75.
18. Hayes, R.B., et al., *Prostate cancer risk in U.S. blacks and whites with a family history of cancer.* Int J Cancer, 1995. **60**(3): p. 361-4.
19. Mousses, S., et al., *Clinical validation of candidate genes associated with prostate cancer progression in the CWR22 model system using tissue microarrays.* Cancer Res, 2002. **62**(5): p. 1256-60.
20. Krishnan, A.V., D.M. Peehl, and D. Feldman, *The role of vitamin D in prostate cancer.* Recent Results Cancer Res, 2003. **164**: p. 205-21.
21. Corder, E.H., et al., *Vitamin D and prostate cancer: a prediagnostic study with stored sera.* Cancer Epidemiol Biomarkers Prev, 1993. **2**(5): p. 467-72.
22. Thompson, I.M., et al., *The influence of finasteride on the development of prostate cancer.* N Engl J Med, 2003. **349**(3): p. 215-24.

23. Klein, E.A., et al., *SELECT: the selenium and vitamin E cancer prevention trial.* Urol Oncol, 2003. **21**(1): p. 59-65.
24. Giovannucci, E., et al., *A prospective study of tomato products, lycopene, and prostate cancer risk.* J Natl Cancer Inst, 2002. **94**(5): p. 391-8.
25. Kolonel, L.N., et al., *Vegetables, fruits, legumes and prostate cancer: a multiethnic case-control study.* Cancer Epidemiol Biomarkers Prev, 2000. **9**(8): p. 795-804.
26. Wilkinson, S. and G.W. Chodak, *Critical review of complementary therapies for prostate cancer.* J Clin Oncol, 2003. **21**(11): p. 2199-210.
27. Holmberg, L., et al., *A randomized trial comparing radical prostatectomy with watchful waiting in early prostate cancer.* N Engl J Med, 2002. **347**(11): p. 781-9.
28. Martel, C.L., et al., *Current strategies in the management of hormone refractory prostate cancer.* Cancer Treat Rev, 2003. **29**(3): p. 171-87.
29. Tu, S.M., et al., *Bone-targeted therapy for advanced androgen-independent carcinoma of the prostate: a randomised phase II trial.* Lancet, 2001. **357**(9253): p. 336-41.
30. Franks, L.M., *Latent carcinoma of the prostate.* J Pathol, 1954. **68**: p. 603-16.
31. Franks, L.M., *Proceedings: Etiology, epidemiology, and pathology of prostatic cancer.* Cancer, 1973. **32**(5): p. 1092-5.
32. Sakr, W.A., et al., *The frequency of carcinoma and intraepithelial neoplasia of the prostate in young male patients.* J Urol, 1993. **150**(2 Pt 1): p. 379-85.
33. Seidman, H., et al., *Probabilities of eventually developing or dying of cancer--United States, 1985.* CA Cancer J Clin, 1985. **35**(1): p. 36-56.
34. Johansson, J.E., et al., *Fifteen-year survival in prostate cancer. A prospective, population-based study in Sweden.* Jama, 1997. **277**(6): p. 467-71.
35. Adolfsson, J., G. Steineck, and P.O. Hedlund, *Deferred treatment of clinically localized low-grade prostate cancer: actual 10-year and projected 15-year follow-up of the Karolinska series.* Urology, 1997. **50**(5): p. 722-6.
36. Albertsen, P.C., et al., *Competing risk analysis of men aged 55 to 74 years at diagnosis managed conservatively for clinically localized prostate cancer.* Jama, 1998. **280**(11): p. 975-80.
37. Zappa, M., et al., *Overdiagnosis of prostate carcinoma by screening: an estimate based on the results of the Florence Screening Pilot Study.* Ann Oncol, 1998. **9**(12): p. 1297-300.
38. Schroder, F.H. and M.F. Wildhagen, *Screening for prostate cancer: evidence and perspectives.* BJU Int, 2001. **88**(8): p. 811-7.
39. Etzioni, R., et al., *Overdiagnosis due to prostate-specific antigen screening: lessons from U.S. prostate cancer incidence trends.* J Natl Cancer Inst, 2002. **94**(13): p. 981-90.
40. McGregor, M., et al., *Screening for prostate cancer: estimating the magnitude of overdetection.* Cmaj, 1998. **159**(11): p. 1368-72.
41. Richie, J.P., et al., *Effect of patient age on early detection of prostate cancer with serum prostate-specific antigen and digital rectal examination.* Urology, 1993. **42**(4): p. 365-74.
42. Fleming, C., et al., *A decision analysis of alternative treatment strategies for clinically localized prostate cancer. Prostate Patient Outcomes Research Team.* Jama, 1993. **269**(20): p. 2650-8.
43. de Koning, H.J., et al., *Large-scale randomized prostate cancer screening trials: program performances in the European Randomized Screening for Prostate Cancer trial and the Prostate, Lung, Colorectal and Ovary cancer trial.* Int J Cancer, 2002. **97**(2): p. 237-44.
44. Draisma, G., et al., *Lead times and overdetection due to prostate-specific antigen screening: estimates from the European Randomized Study of Screening for Prostate Cancer.* J Natl Cancer Inst, 2003. **95**(12): p. 868-78.
45. Stamey, T.A., et al., *Localized prostate cancer. Relationship of tumor volume to clinical significance for treatment of prostate cancer.* Cancer, 1993. **71**(3 Suppl): p. 933-8.

46. Jhaveri, F.M., et al., *Declining rates of extracapsular extension after radical prostatectomy: evidence for continued stage migration.* J Clin Oncol, 1999. **17**(10): p. 3167-72.
47. Han, M., et al., *Era specific biochemical recurrence-free survival following radical prostatectomy for clinically localized prostate cancer.* J Urol, 2001. **166**(2): p. 416-9.
48. Dugan, J.A., et al., *The definition and preoperative prediction of clinically insignificant prostate cancer.* Jama, 1996. **275**(4): p. 288-94.
49. Bostwick, D.G., et al., *Staging of early prostate cancer: a proposed tumor volume-based prognostic index.* Urology, 1993. **41**(5): p. 403-11.
50. Epstein, J.I., et al., *Pathologic and clinical findings to predict tumor extent of nonpalpable (stage T1c) prostate cancer.* Jama, 1994. **271**(5): p. 368-74.
51. Partin, A.W., et al., *Combination of prostate-specific antigen, clinical stage, and Gleason score to predict pathological stage of localized prostate cancer. A multi-institutional update.* Jama, 1997. **277**(18): p. 1445-51.
52. Kattan, M.W., et al., *A preoperative nomogram for disease recurrence following radical prostatectomy for prostate cancer.* J Natl Cancer Inst, 1998. **90**(10): p. 766-71.
53. Hull, G.W., et al., *Cancer control with radical prostatectomy alone in 1,000 consecutive patients.* J Urol, 2002. **167**(2 Pt 1): p. 528-34.
54. D'Amico, A.V., et al., *A multivariate analysis of clinical and pathological factors that predict for prostate specific antigen failure after radical prostatectomy for prostate cancer.* J Urol, 1995. **154**(1): p. 131-8.
55. Brawer, M.K., *Quantitative microvessel density. A staging and prognostic marker for human prostatic carcinoma.* Cancer, 1996. **78**(2): p. 345-9.
56. Epstein, J.I., et al., *Nonpalpable stage T1c prostate cancer: prediction of insignificant disease using free/total prostate specific antigen levels and needle biopsy findings.* J Urol, 1998. **160**(6 Pt 2): p. 2407-11.
57. Hankey, B.F., et al., *Cancer surveillance series: interpreting trends in prostate cancer-- part I: Evidence of the effects of screening in recent prostate cancer incidence, mortality, and survival rates.* J Natl Cancer Inst, 1999. **91**(12): p. 1017-24.
58. Oliver, S.E., M.T. May, and D. Gunnell, *International trends in prostate-cancer mortality in the "PSA ERA".* Int J Cancer, 2001. **92**(6): p. 893-8.
59. Lu-Yao, G., et al., *Natural experiment examining impact of aggressive screening and treatment on prostate cancer mortality in two fixed cohorts from Seattle area and Connecticut.* Bmj, 2002. **325**(7367): p. 740.
60. Barry, M.J., et al., *Outcomes for men with clinically nonmetastatic prostate carcinoma managed with radical prostactectomy, external beam radiotherapy, or expectant management: a retrospective analysis.* Cancer, 2001. **91**(12): p. 2302-14.
61. Lu-Yao, G.L. and S.L. Yao, *Population-based study of long-term survival in patients with clinically localised prostate cancer.* Lancet, 1997. **349**(9056): p. 906-10.
62. Lu-Yao, G.L. and E.R. Greenberg, *Changes in prostate cancer incidence and treatment in USA.* Lancet, 1994. **343**(8892): p. 251-4.
63. Lee, F., et al., *Transrectal ultrasound in the diagnosis of prostate cancer: location, echogenicity, histopathology, and staging.* Prostate, 1985. **7**(2): p. 117-29.
64. Walsh, P.C. and J.L. Mostwin, *Radical prostatectomy and cystoprostatectomy with preservation of potency. Results using a new nerve-sparing technique.* Br J Urol, 1984. **56**(6): p. 694-7.
65. Guillonneau, B. and G. Vallancien, *Laparoscopic radical prostatectomy: the Montsouris experience.* J Urol, 2000. **163**(2): p. 418-22.
66. Zelefsky, M.J., et al., *High dose radiation delivered by intensity modulated conformal radiotherapy improves the outcome of localized prostate cancer.* J Urol, 2001. **166**(3): p. 876-81.
67. Blasko, J.C., et al., *Prostate brachytherapy: importance of technique.* J Clin Oncol, 1996. **14**(6): p. 1965-7.

68. Blasko, J.C., et al., *Brachytherapy for carcinoma of the prostate: techniques, patient selection, and clinical outcomes.* Semin Radiat Oncol, 2002. **12**(1): p. 81-94.
69. Bolla, M., et al., *Long-term results with immediate androgen suppression and external irradiation in patients with locally advanced prostate cancer (an EORTC study): a phase III randomised trial.* Lancet, 2002. **360**(9327): p. 103-6.
70. Adolfsson, J., L.E. Rutqvist, and G. Steineck, *Prostate carcinoma and long term survival.* Cancer, 1997. **80**(4): p. 748-52.
71. Albertsen, P.C., et al., *Long-term survival among men with conservatively treated localized prostate cancer.* Jama, 1995. **274**(8): p. 626-31.
72. Choo, R., et al., *Feasibility study: watchful waiting for localized low to intermediate grade prostate carcinoma with selective delayed intervention based on prostate specific antigen, histological and/or clinical progression.* J Urol, 2002. **167**(4): p. 1664-9.
73. Madalinska, J.B., et al., *Health-related quality-of-life effects of radical prostatectomy and primary radiotherapy for screen-detected or clinically diagnosed localized prostate cancer.* J Clin Oncol, 2001. **19**(6): p. 1619-28.
74. Miller, A.B., et al., *Health-related quality of life and cost-effectiveness studies in the European randomised study of screening for prostate cancer and the US Prostate, Lung, Colon and Ovary trial.* Eur J Cancer, 2001. **37**(17): p. 2154-60.
75. Gustafsson, O., et al., *Cost-effectiveness analysis in early detection of prostate cancer: an evaluation of six screening strategies in a randomly selected population of 2,400 men.* Prostate, 1995. **26**(6): p. 299-309.
76. Ablin, R.J., et al., *Precipitating antigens of the normal human prostate.* J Reprod Fertil, 1970. **22**(3): p. 573-4.
77. Wang, M.C., et al., *Purification of a human prostate specific antigen.* Invest Urol, 1979. **17**(2): p. 159-63.
78. Stamey, T.A., et al., *Prostate-specific antigen as a serum marker for adenocarcinoma of the prostate.* N Engl J Med, 1987. **317**(15): p. 909-16.
79. Stamey, T.A., et al., *Prostate specific antigen in the diagnosis and treatment of adenocarcinoma of the prostate. II. Radical prostatectomy treated patients.* J Urol, 1989. **141**(5): p. 1076-83.
80. Oesterling, J.E., et al., *Serum prostate-specific antigen in a community-based population of healthy men. Establishment of age-specific reference ranges.* Jama, 1993. **270**(7): p. 860-4.
81. Yousef, G.M. and E.P. Diamandis, *The new human tissue kallikrein gene family: structure, function, and association to disease.* Endocr Rev, 2001. **22**(2): p. 184-204.
82. Lundwall, A. and H. Lilja, *Molecular cloning of human prostate specific antigen cDNA.* FEBS Lett, 1987. **214**(2): p. 317-22.
83. Kumar, A., et al., *Expression of pro form of prostate-specific antigen by mammalian cells and its conversion to mature, active form by human kallikrein 2.* Cancer Res, 1997. **57**(15): p. 3111-4.
84. Steuber, T., et al., *Discrimination of benign from malignant prostatic disease by selective measurements of single chain, intact free prostate specific antigen.* J Urol, 2002. **168**(5): p. 1917-22.
85. Akiyama, K., et al., *The chymotrypsin-like activity of human prostate-specific antigen, gamma-seminoprotein.* FEBS Lett, 1987. **225**(1-2): p. 168-72.
86. Zackrisson, B., Ulleryd, Aus G., Lilja H., Sandberg T. and Hugosson J., *Evolution of free, complexed and total serum PSA and their ratios during 1-years follow-up of men with febrile urinary tract infection.* Urology. **in press**.
87. Stege, R.H., et al., *Tissue PSA from fine-needle biopsies of prostatic carcinoma as related to serum PSA, clinical stage, cytological grade, and DNA ploidy.* Prostate, 1999. **38**(3): p. 183-8.
88. Sokoll, L.J., et al., *Proenzyme psa for the early detection of prostate cancer in the 2.5-4.0 ng/ml total psa range: preliminary analysis.* Urology, 2003. **61**(2): p. 274-6.
89. Linton, H.J., et al., *Benign prostate-specific antigen (BPSA) in serum is increased in benign prostate disease.* Clin Chem, 2003. **49**(2): p. 253-9.

90. Smith, D.S., W.J. Catalona, and J.D. Herschman, *Longitudinal screening for prostate cancer with prostate-specific antigen.* Jama, 1996. **276**(16): p. 1309-15.
91. Partin, A.W., et al., *The use of prostate specific antigen, clinical stage and Gleason score to predict pathological stage in men with localized prostate cancer.* J Urol, 1993. **150**(1): p. 110-4.
92. Catalona, W.J. and D.S. Smith, *5-year tumor recurrence rates after anatomical radical retropubic prostatectomy for prostate cancer.* J Urol, 1994. **152**(5 Pt 2): p. 1837-42.
93. Wieder, J.A. and M.S. Soloway, *Incidence, etiology, location, prevention and treatment of positive surgical margins after radical prostatectomy for prostate cancer.* J Urol, 1998. **160**(2): p. 299-315.
94. Hudson, M.A., R.R. Bahnson, and W.J. Catalona, *Clinical use of prostate specific antigen in patients with prostate cancer.* J Urol, 1989. **142**(4): p. 1011-7.
95. Lange, P.H., et al., *The value of serum prostate specific antigen determinations before and after radical prostatectomy.* J Urol, 1989. **141**(4): p. 873-9.
96. Labrie, F., et al., *Serum prostate specific antigen as pre-screening test for prostate cancer.* J Urol, 1992. **147**(3 Pt 2): p. 846-51; discussion 851-2.
97. Lodding, P., et al., *Characteristics of screening detected prostate cancer in men 50 to 66 years old with 3 to 4 ng./ml. Prostate specific antigen.* J Urol, 1998. **159**(3): p. 899-903.
98. Carter, H.B., et al., *Recommended prostate-specific antigen testing intervals for the detection of curable prostate cancer.* Jama, 1997. **277**(18): p. 1456-60.
99. Oesterling, J.E., *Age-specific reference ranges for serum prostate-specific antigen.* Can J Urol, 1995. **2**(Supp1): p. 23-9.
100. Lilja, H., et al., *Prostate-specific antigen in serum occurs predominantly in complex with alpha 1-antichymotrypsin.* Clin Chem, 1991. **37**(9): p. 1618-25.
101. Brawer, M.K., et al., *Measurement of complexed PSA improves specificity for early detection of prostate cancer.* Urology, 1998. **52**(3): p. 372-8.
102. Brawer, M.K., *How to use prostate-specific antigen in the early detection or screening for prostatic carcinoma.* CA Cancer J Clin, 1995. **45**(3): p. 148-64.
103. Schroder, F.H., et al., *Prostate cancer detection at low prostate specific antigen.* J Urol, 2000. **163**(3): p. 806-12.
104. Benson, M.C., et al., *The use of prostate specific antigen density to enhance the predictive value of intermediate levels of serum prostate specific antigen.* J Urol, 1992. **147**(3 Pt 2): p. 817-21.
105. Gustafsson, O., et al., *Prostate-specific antigen (PSA), PSA density and age-adjusted PSA reference values in screening for prostate cancer--a study of a randomly selected population of 2,400 men.* Scand J Urol Nephrol, 1998. **32**(6): p. 373-7.
106. Catalona, W.J., et al., *Comparison of prostate specific antigen concentration versus prostate specific antigen density in the early detection of prostate cancer: receiver operating characteristic curves.* J Urol, 1994. **152**(6 Pt 1): p. 2031-6.
107. Babaian, R.J., et al., *Comparative analysis of prostate specific antigen and its indexes in the detection of prostate cancer.* J Urol, 1996. **156**(2 Pt 1): p. 432-7.
108. Stamey, T.A., et al., *Preoperative serum prostate specific antigen levels between 2 and 22 ng./ml. correlate poorly with post-radical prostatectomy cancer morphology: prostate specific antigen cure rates appear constant between 2 and 9 ng./ml.* J Urol, 2002. **167**(1): p. 103-11.
109. Kalish, J., W.H. Cooner, and S.D. Graham, Jr., *Serum PSA adjusted for volume of transition zone (PSAT) is more accurate than PSA adjusted for total gland volume (PSAD) in detecting adenocarcinoma of the prostate.* Urology, 1994. **43**(5): p. 601-6.
110. Noldus, J. and T.A. Stamey, *Histological characteristics of radical prostatectomy specimens in men with a serum prostate specific antigen of 4 ng./ml or less.* J Urol, 1996. **155**(2): p. 441-3.
111. Carter, H.B. and J.D. Pearson, *PSA velocity for the diagnosis of early prostate cancer. A new concept.* Urol Clin North Am, 1993. **20**(4): p. 665-70.

112. Bunting, P.S., et al., *Intraindividual variation of PSA, free PSA and complexed PSA in a cohort of patients with prostate cancer managed with watchful observation.* Clin Biochem, 2002. **35**(6): p. 471-5.
113. Schmid, H.P., J.E. McNeal, and T.A. Stamey, *Observations on the doubling time of prostate cancer. The use of serial prostate-specific antigen in patients with untreated disease as a measure of increasing cancer volume.* Cancer, 1993. **71**(6): p. 2031-40.
114. Stenman, U.H., et al., *A complex between prostate-specific antigen and alpha 1-antichymotrypsin is the major form of prostate-specific antigen in serum of patients with prostatic cancer: assay of the complex improves clinical sensitivity for cancer.* Cancer Res, 1991. **51**(1): p. 222-6.
115. Catalona, W.J., et al., *Evaluation of percentage of free serum prostate-specific antigen to improve specificity of prostate cancer screening.* Jama, 1995. **274**(15): p. 1214-20.
116. Stephan, C., et al., *Multicenter evaluation of an artificial neural network to increase the prostate cancer detection rate and reduce unnecessary biopsies.* Clin Chem, 2002. **48**(8): p. 1279-87.
117. Pedersen, K.V., et al., *Screening for carcinoma of the prostate by digital rectal examination in a randomly selected population.* Bmj, 1990. **300**(6731): p. 1041-4.
118. Hoogendam, A., F. Buntinx, and H.C. de Vet, *The diagnostic value of digital rectal examination in primary care screening for prostate cancer: a meta-analysis.* Fam Pract, 1999. **16**(6): p. 621-6.
119. Catalona, W.J., et al., *Comparison of digital rectal examination and serum prostate specific antigen in the early detection of prostate cancer: results of a multicenter clinical trial of 6,630 men.* J Urol, 1994. **151**(5): p. 1283-90.
120. Gustafsson, O., et al., *Diagnostic methods in the detection of prostate cancer: a study of a randomly selected population of 2,400 men.* J Urol, 1992. **148**(6): p. 1827-31.
121. Bangma, C.H., et al., *The value of screening tests in the detection of prostate cancer. Part I: Results of a retrospective evaluation of 1726 men.* Urology, 1995. **46**(6): p. 773-8.
122. Schroder, F.H., et al., *Evaluation of the digital rectal examination as a screening test for prostate cancer. Rotterdam section of the European Randomized Study of Screening for Prostate Cancer.* J Natl Cancer Inst, 1998. **90**(23): p. 1817-23.
123. Carroll, P., et al., *Prostate-specific antigen best practice policy--part I: early detection and diagnosis of prostate cancer.* Urology, 2001. **57**(2): p. 217-24.
124. Noguchi, M., et al., *Relationship between systematic biopsies and histological features of 222 radical prostatectomy specimens: lack of prediction of tumor significance for men with nonpalpable prostate cancer.* J Urol, 2001. **166**(1): p. 104-9; discussion 109-10.
125. Hodge, K.K., J.E. McNeal, and T.A. Stamey, *Ultrasound guided transrectal core biopsies of the palpably abnormal prostate.* J Urol, 1989. **142**(1): p. 66-70.
126. Hodge, K.K., et al., *Random systematic versus directed ultrasound guided transrectal core biopsies of the prostate.* J Urol, 1989. **142**(1): p. 71-4; discussion 74-5.
127. Carter, H.B., et al., *Evaluation of transrectal ultrasound in the early detection of prostate cancer.* J Urol, 1989. **142**(4): p. 1008-10.
128. Esposti, P.L. and S. Franzen, *Transrectal aspiration biopsy of the prostate. A re-evaluation of the method in the diagnosis of prostatic carcinoma.* Scand J Urol Nephrol Suppl, 1980. **55**: p. 49-52.
129. Norming, U., et al., *Fine-needle aspiration biopsy with a new automatic fine-needle gun versus histological core in ultrasonically-guided transrectal biopsy for detection of prostate cancer.* Acta Oncol, 1991. **30**(2): p. 155-7.
130. Hostetter, A.L., et al., *Diagnosis and localization of prostate carcinoma by fine-needle aspiration cytology and correlation with histologic whole-organ sections after radical prostatectomy.* Am J Clin Pathol, 1990. **94**(6): p. 693-7.

131. Gleason, D.F. and G.T. Mellinger, *Prediction of prognosis for prostatic adenocarcinoma by combined histological grading and clinical staging.* J Urol, 1974. **111**(1): p. 58-64.
132. Stamey, T.A., et al., *Histological and clinical findings in 896 consecutive prostates treated only with radical retropubic prostatectomy: epidemiologic significance of annual changes.* J Urol, 1998. **160**(6 Pt 2): p. 2412-7.
133. Ferguson, J.K., et al., *Prostate-specific antigen detected prostate cancer: pathological characteristics of ultrasound visible versus ultrasound invisible tumors.* Eur Urol, 1995. **27**(1): p. 8-12.
134. Hugosson, J., et al., *Would prostate cancer detected by screening with prostate-specific antigen develop into clinical cancer if left undiagnosed? A comparison of two population-based studies in Sweden.* BJU Int, 2000. **85**(9): p. 1078-84.
135. Norberg, M., et al., *The sextant protocol for ultrasound-guided core biopsies of the prostate underestimates the presence of cancer.* Urology, 1997. **50**(4): p. 562-6.
136. Bauer, J.J., et al., *Three-dimensional computer-simulated prostate models: lateral prostate biopsies increase the detection rate of prostate cancer.* Urology, 1999. **53**(5): p. 961-7.
137. Djavan, B., M. Remzi, and M. Marberger, *When to biopsy and when to stop biopsying.* Urol Clin North Am, 2003. **30**(2): p. 253-62, viii.
138. Petrie A, S.C., *Medical statistics at a glance.* 2000, Blackwell science.
139. Waldron, I., *The contribution of smoking to sex differences in mortality.* Public Health Rep, 1986. **101**(2): p. 163-73.
140. Gustafsson, O., et al., *Psychological reactions in men screened for prostate cancer.* Br J Urol, 1995. **75**(5): p. 631-6.
141. Gann, P.H., *Interpreting recent trends in prostate cancer incidence and mortality.* Epidemiology, 1997. **8**(2): p. 117-20.
142. Wilson, J.M. and Y.G. Jungner, *[Principles and practice of mass screening for disease].* Bol Oficina Sanit Panam, 1968. **65**(4): p. 281-393.
143. Catalona, W.J., D.S. Smith, and D.K. Ornstein, *Prostate cancer detection in men with serum PSA concentrations of 2.6 to 4.0 ng/mL and benign prostate examination. Enhancement of specificity with free PSA measurements.* Jama, 1997. **277**(18): p. 1452-5.
144. Tibblin, G., et al., *The pituitary-gonadal axis and health in elderly men: a study of men born in 1913.* Diabetes, 1996. **45**(11): p. 1605-9.
145. Tibblin, G., et al., *The value of prostate specific antigen in early diagnosis of prostate cancer: the study of men born in 1913.* J Urol, 1995. **154**(4): p. 1386-9.