Population Based Screening for Prostate Cancer

Tumor Features and Clinical Decision Making

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POPULATION BASED SCREENING FOR PROSTATE CANCER

TUMOR FEATURES AND CLINICAL DECISION MAKING

Bevolkingsonderzoek naar de
vroegopsporing van prostaatkanker

Tumor karakteristieken en
klinische besluitvorming

Proefschrift

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PART I

GENERAL INTRODUCTION

THESIS OUTLINES AND OBJECTIVES

The aggregate morbidity and mortality attributed to prostate cancer are certainly sufficient to justify a search for rational, effective and efficient screening strategies. Unfortunately, the outcome of randomized controlled trials (RCTs) that investigate the efficacy of prostate cancer screening is still awaited. Before this final analysis takes place at the end of this decade, and before screening for prostate cancer can be applied as a nation-wide health care measure, efforts should be made to optimize the validity of the screening tests, assess the quality of life in those screened, and evaluate (reduce) the costs associated with large scale screening programs. In other words, efforts should be made to make the screening regimen more effective, selective and efficient.

The current thesis provides further insight into the pathology of screen-detected prostate cancer, and into its role in the clinical management of patients with this potentially lethal disease. Despite our knowledge that a definite answer on the question which cancers we wish to detect in screening programs to decrease the mortality of the disease can only be answered after the completion of RCTs, potential measures to make the screening regimen more selective and efficient are presented. Most data were obtained from the screening arm of the European randomized study of screening for prostate cancer (ERSPC), a large multicenter RCT that investigates the impact of screening on prostate cancer mortality and quality of life.

PART I. General Introduction

Chapter 1 provides an overview of the burden of prostate cancer to the male population, and gives a detailed outline on the objectives, methodology and pitfalls of early detection programs. The current controversies in prostate cancer screening are addressed, and a comparison is made to previous screening trials initiated for lung and breast cancer. Recent reports on prostate cancer screening are set in a wider clinical perspective with a special interest in the tumor characteristics and prognostic factors of the cancers detected. A further attempt is made to assess how theoretically a beneficial outcome of prostate cancer screening might be achieved.

PART II. Towards Predicting the Outcome of Prostate Cancer Screening

Prostate cancer screening trials should be preferentially targeted at individuals who have future aggressive disease, though in whom the disease is still curable with the currently available treatment options. Moreover, those cancers should be detected that constitute a

high risk of mortality in the remaining lifespans of their hosts. These cancers have been defined the 'window of opportunity' in screening for prostate cancer. Otherwise, cancers which pose no threat to the lives or well being of their hosts (i.e. clinically insignificant disease) should be refrained from interventions, and preferably, even their detection. By examining well-established pathologic prognostic tumor features, ***Chapter 2*** questioned whether surgically treated patients could be stratified into prognostic subgroups by relating combined statistically independent tumor features to the recurrence of PSA after radical prostatectomy. ***Chapter 3*** also deals with an (intermediate) end-point of a screening trial. It provides a comparison between the screening group and the control group of ERSPC with an interest in the pathological characteristics of the cancers detected. Eventually, this same comparison will take place later on this decade to compare the mortality rates. A potential caveat in prostate cancer screening is the detection of a large number of cases with clinically insignificant tumor features. Otherwise, a screening methodology may be used that is ineffective to detect clinically significant disease. ***Chapter 4*** addresses this question and investigates whether other biopsy techniques might result in a higher yield for clinically significant prostate cancer, and conversely, is less likely to detect clinically insignificant cancers.

Part III. The Predictive Value of Precursor Lesions of Prostate Cancer

Premalignant lesions of the prostate are detected coincidentally on prostatic needle biopsies. Recently, several putative precursor lesions of prostate cancer have been proposed. ***Chapter 5*** reviews the current knowledge and understanding of these distinct histologic and diagnostic entities. As prostate cancer is a slow-growing disease in most cases, and as it is assumed that precursor lesions of prostate cancer progress only slowly, doubt is raised on the need for early diagnostic follow-up in men with a diagnosis of a premalignant lesion of the prostate. ***Chapter 6*** outlines the biopsy incidence rate and the predictive value for prostate cancer of the most acknowledged precursor lesion of prostate cancer, i.e. high-grade prostatic intraepithelial neoplasia (HPIN), in population-based screening. To gain further insight into the need for diagnostic follow-up after a diagnosis of HPIN, we compared these figures to those of men with lesions in which the pathologist is uncomfortable in making a definite malignant diagnosis, i.e. a prostate biopsy suspicious for malignancy (PBSM), and to those with an benign biopsy result.

Part IV. Towards a Refining of Screening in Low PSA Ranges

A particular area under fierce debate is the low PSA-ranges (0.0 – 3.9 ng/mL). In this PSA range, digital rectal examination (DRE) is the mainstay of early detection, and it has

been described previously that its efficacy is only low. ***Chapter 7*** again addressed the efficacy of DRE as a screening test for prostate cancer in low PSA ranges with a special focus on the tumor characteristics of the cancers detected. The number of presumably clinically significant cancers was assessed and related to the number of men that came for rectal examination and the number of men biopsied. In doing this, the number of men required to undergo DRE, and the number of men needed to undergo prostate biopsy to detect one case of clinically significant disease could be assessed. ***Chapter 8*** builds on these figures, and addresses another method of cancer detection in the low PSA ranges, i.e. that of chance only (serendipity).

Part V. On the Predictive Value of Prognostic Tissue Markers

Chapter 9 deals with the prognostic value of three tissue markers ($p27^{kip1}$, MIB-1 and CD44s) in surgically treated patients with prostate cancer. We determined whether these tissue markers were of additional value to predict the outcome of disease compared to grade and stage alone. To account for sufficient follow-up, a series of cancers was examined that was surgically treated in the 1980s. ***Chapter 10*** deals with a specific problem we encountered coincidentally in our laboratory, i.e. the decay of antigenicity in stored tissue sections. ***Chapter 11*** determined whether the expression level of three tissue markers was able to predict the expression level in matched radical prostatectomy specimens, and whether tissue markers could help to predict well-established prognostic factors as grade and stage in the radical prostatectomy specimen. One of the mechanisms by which a tumor suppressor gene is silenced is the methylation of the promoter region within a gene. Furthermore, it was reported that tumor-derived DNA could be detected in the serum of cancer patients. ***Chapter 12*** questioned whether the assessment of the methylation status of the promoter region of the tumor suppressor gene *CD44* is feasible in serum of prostate cancer patients, and whether it could distinguish between cases *with* and *without* metastatic disease.

Part VI. General Discussion, Epilogue and Summary

Chapter 13 shortly outlines the important findings of this scientific report. Guided by the tumor characteristics of the cancers detected, it is hypothesized how screening efforts can be made more selective and more efficient in population based screening programs for prostate cancer. Potential directions for future studies on the clinical and pathological characteristics of prostate cancer are addressed as well.

LIST OF ABBREVIATIONS

AAH	Atypical Adenomatous Hyperplasia
ACS	American Cancer Society
AGIKO	Assistent Geneeskundige In opleiding tot Klinisch Onderzoeker
ANN	Artificial Neural Networks
AR	Androgen Receptor
AUA	American Urological Association
BPH	Benign Prostatic Hyperplasia
CGH	Comparative Genomic Hybridization
DNA	Deoxyribo Nucleic Acid
DRE	Digital Rectal Examination
ERPSC	European Randomized study of Screening for Prostate Cancer
FISH	Fluorescence In-Situ Hybridization
FHS	Fritz H Schröder
F/T	Free to Total
GSS	Gleason Sum Score
HPIN	High-grade Prostatic Intra-epithelial Neoplasia
IKR	Integraal Kankercentrum Rotterdam
LHRH	Luteinizing Hormone Releasing Hormone
LPIN	Low-grade Prostatic Intra-epithelial Neoplasia
NPV	Negative Predictive Value
PAH	Postatrophic Hyperplasia
PBSM	Prostate Biopsy Suspicious for Malignancy
PCR	Polymerase Chain Reaction
PLCO	Prostate, Lung, Colorectal, and Ovary
PPV	Positive Predictive Value
PSA	Prostate-specific Antigen
RCT	Randomized Controlled Trial
ROC	Receiver Operating Characteristics curve
RRP	Retropubic Radical Prostatectomy
SEER	Surveillance, Epidemiology, and End Results
TSA	Tyramide Signal Amplification
TURP	Transurethral Resection of the Prostate
TRUS	Transrectal Ultrasound

CHAPTER 1

PROSTATE CANCER AS A HEALTH BURDEN

By: André N. Vis

PROSTATE CANCER EPIDEMIOLOGY

Incidence

There is no doubt that prostate cancer is a major public health problem (TABLE 1.1). At present, prostate cancer is the most commonly diagnosed noncutaneous malignancy in men beyond middle age, with an expected 6,500 new cases in the Netherlands in the year 2000 (FIGURE 1.1). Malignancies that originate from the prostate gland account for almost one fifth of all newly diagnosed cases with cancer in males. It is calculated that a 50-year old man has a cumulative lifetime risk of more than one in eleven to be ever confronted with a diagnosis of prostate cancer [1]. The current position as the 'number one' cancer is expected to become even more pronounced in the oncoming years for the incidence rates of prostate cancer are still rising, while that of lung cancer shows a sharp decreasing trend [1]. Prostate cancer has often been described as a malignancy of older age, and in part, the increase in incidence may be attributed to an aging male population and an increased male life expectancy. So, as more men live to older ages, the absolute number of men with prostate cancer is likely to increase. Besides changes in the composition of the population, changes in the clinical tools applied to a disease may affect the epidemiological rates and trends as well. Over the past two decades, several sophisticated diagnostic techniques have been developed, and some of these are able to detect the abnormalities associated with a disease earlier, even before they produce clinical signs and symptoms [2]. It is obvious that as the thresholds for the detection of a disease become lower by use of these advanced diagnostics, the incidence rates of the disease may be affected. Previously, it has been described that the incidence rates of various non-malignant and malignant conditions indeed increased considerably just by close diagnostic scrutiny [2]. With respect to prostate cancer, there have been little changes in either the incidence rates nor its mortality up until 1985. Since that time, however, several new diagnostic 'improvements' have been introduced as well as refinements in the (surgical) treatment of the disease. The availability and applicability of markers for the presence of prostate cancer in the mid 1980s, particularly that of prostate-specific antigen (PSA), have had an inconceivable effect on the way the disease was now to be looked upon. PSA is a protein exclusively produced by epithelial cells of prostatic origin, and the molecule is known to leak into the blood circulation in small proportions. In the early 1980s, the protein was demonstrated in serum of healthy males, and increased serum PSA levels were shown to be associated with prostatic diseases such as prostate cancer. The potential role of PSA as an indicator of early stage prostate cancer was quickly recognized, and a series of convenient and reproducible blood tests was developed. At present, it is acknowledged that the application of the PSA blood test

contributed most evidently to the rise in prostate cancer incidence in the United States in the late 1980s and early 1990s [3]. This increase in the number of prostate cancer diagnoses was preferably seen in younger men aged 50 to 70 years, and it is estimated that prostate cancer is now being diagnosed approximately 2.5 years earlier than it was a decade ago [4-6]. Besides changing the incidence rates of prostate cancer and the mean age at diagnosis, the PSA blood test also affected the characteristics of the cancers detected. The application of the serum-PSA test eventuated in more men being diagnosed with clinically localized disease, whereas the cancers detected were more often of intermediate histological grade of tumor differentiation, both in absolute and relative terms [7]. Also, the age-adjusted incidence rates of regional and distant stage disease declined by use of the serum-PSA test [3,8]. In conjunction with the serum-PSA test, the introduction of a safe and easy applicable technique to sample the prostate gland under ultrasound guidance in the late 1980s may have added to the rise in prostate cancer incidence as well [9]. As a result, it is now widely acknowledged that the introduction, and more frequent application of new and more advanced diagnostic tools have remarkably altered the yield for prostate cancer, the patient, clinical and tumor characteristics of those eventually diagnosed with the disease, and probably the outcome of the disease as well.

TABLE 1.1
The impact of prostate cancer as a health burden

• Cumulative lifetime risk of prostate cancer at age 50 years.....9.0%
• Proportion of prostate cancer in all newly diagnosed cases with cancer in males.....18.6%
• Cumulative lifetime risk of dying from the disease.....3.5%
• Risk of dying from the disease when diagnosed.....40.0%
• 5-year disease-specific survival.....60.0%
• Loss of lifespan due to prostate cancer.....40.0%

The increase in prostate cancer incidence was not only represented by an increase in the detection of cancers with favorable prognostic features, but also by an increase in the absolute number of poorly differentiated, potentially more aggressive, and metastatic prostate cancers in men under the age of 60 years [10]. A genuine increase in the absolute number of clinically apparent prostate cancers was also reported between the early 1970s through the late 1980s, so before the introduction of the PSA blood test [11,12]. Whether this trend has continued in the 1990s is still uncertain, and may be veiled or even prevented by the application of case-finding screening programs with the PSA blood test. At last, an increased awareness of the disease by patients, their partners, and doctors, in part thrown up by all kinds of media, may also be responsible for the reported rise in prostate cancer incidence. It is expected that this increased attention of the disease by the lay press, as well as an increased demand for diagnostic tests, and an increased medical surveillance may further lead to an increase in prostate cancer incidence in oncoming years.

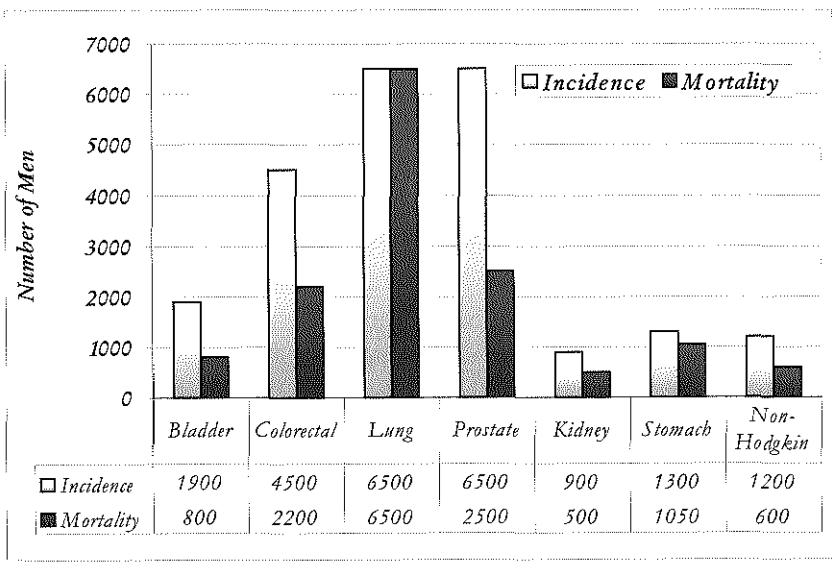


FIGURE 1.1

The expected incidence rates (number of new cases with cancer per year) and the expected mortality rates (number of cancer related deaths per year) of the seven most frequently diagnosed malignancies in males in the Netherlands in the year 2000 [1].

Because the incidence rates of prostate cancer increase with age, there is a misconception that it is a disease of the *very* elderly. Prostate cancer occurs infrequently before the age of 50 years, whereas the incidence rates rise sharply in men above the age of 60 years [1]. In the year 2000, almost 3,500 men in the age range 60 to 74 years are expected to be diagnosed with prostate cancer, while this figure is 2,500 in the age range 75 years and older. Some of the younger cases may be diagnosed as a result of the application of the serum-PSA test in case finding screening, but it is assumed that still the vast majority of cases will be diagnosed as a consequence of the evaluation of clinical signs and symptoms.

Mortality

It is calculated that the cumulative lifetime risk of a 55-year old man to succumb of prostate cancer is approximately 3.5% [13]. Of all newly diagnosed cases with prostate cancer, approximately 40% will eventually die from the disease, and of those diagnosed with prostate cancer before the age of 65 years, the mortality is far over 80% [14]. In some men, the disease kills the patient within a year after diagnosis. It is estimated that men with clinically diagnosed prostate cancer will lose an average of 40% of their life expectancy compared to an age-matched control group without prostate cancer [15]. The absolute mortality is high as well, second only after lung cancer, and will correspond to almost 2,500 deaths in the Netherlands in the year 2000 (FIGURE 1.1). Similar to the reported incidence rates, the mortality rates have increased in the Netherlands in the 1980s and early 1990s [11], and remain relatively stable thereafter [13].

Recent data from the United States of America, in which opportunistic prostate cancer screening with the PSA blood test has been vigorously applied in the mid 1980s and early 1990s, showed that the relative proportion of men dying from prostate cancer declined from 1988 through 1995 [16,17]. The greatest decline was reported among younger white men, a group that was intensely involved in early detection efforts. This proportionate decline in the prostate cancer mortality may be merely due to a rise in the 'incidence-to-mortality ratio' rather than to a genuine decrease of the number of prostate cancer deaths. Recent reports from the Surveillance, Epidemiology, and End Results (SEER), however, showed that the age-adjusted mortality rates of prostate cancer also fell by 6.5% in the United States in the mid 1990s, an observation that was also reported for other common cancers such as lung, colon and rectal cancer [18-21]. Some have already suggested that this trend provides evidence for the effectiveness of screening for prostate cancer with the serum-PSA test [19].

The significance of these data on the decline in prostate cancer mortality in SEER, however, is subject to differing interpretations [20]. It is considered by many that the application of the serum-PSA test may have been little responsible for the decline in

prostate cancer mortality given the relatively long protracted course of most prostate cancers. If at last, the PSA blood test proves an efficient screening tool, as demonstrated in randomized clinical trials, improvements in the prostate cancer death rates are not expected until halfway the present decade [21]. The reported decline in the prostate cancer mortality rate in SEER may be merely due to the interest in other screening tests for prostate cancer that began before the PSA era, such as digital rectal examination (DRE), to an increased efficacy of newly applied curative treatment options, such as radical prostatectomy, and/or to the availability of improved treatment options for advanced prostate cancer, such as LHRH-agonists. Changes in lifestyle and improvements in environmental conditions may also have been responsible for an improved outcome in recent cohorts. The observation that the death rates for prostate cancer have also declined in England and Wales in this same time period are in line with these assumptions [22]. Also, 'attribution' bias (the incorrect labeling of deaths from other causes as being death from prostate cancer) may also account for the apparent rise and decline of the prostate cancer mortality rates [23].

Clinical Presentation and Prognosis

Prostate cancer used to be diagnosed only when symptoms of metastatic, regionally advanced or locally advanced disease occurred or when patients were investigated or treated for what was presumed to be benign disease [24]. Unfortunately, in the more advanced stages of the disease, the cancer is often incurable, and consequently, only palliative treatment may be offered [25]. In the pre-PSA era, almost 30% of newly diagnosed cases with prostate cancer presented themselves with painful bony metastases (M1) [26-29]. Besides skeletal pain, men with metastatic disease may suffer from pathological fractures, spinal cord compression, and the morbidities associated with local disease progression. The morbidities associated with palliative treatment such as hormonal ablation therapy may be considerably high. In cases with metastatic prostate cancer, the median survival is in the range of 2.5 to 3.5 years despite the application of hormonal ablation therapy [30]. By 10 years, the cancer specific mortality rate will be greater than 85% [31]. Early reports have demonstrated that untreated metastatic prostate cancer is associated with an even worse prognosis [32]. Approximately 5 to 10% of men will have regionally advanced disease (e.g. N1) at the time of first presentation of the disease without evidence of distant metastases [29]. Mostly, these cases are locally advanced as well, and many will experience severe morbidity resulting from bladder outlet obstruction, urinary or rectal bleeding, and ureteral obstruction and hydronefrosis [26]. These patients also fare poorly regardless of treatment modality, and the prognosis will be only slightly better than those who are diagnosed with distant metastases initially [33]. Approximately 20 to 25% of cases will present themselves with cancers that have

already invaded adjacent organs such as the urinary bladder or the seminal vesicles. Locally advanced cases have a high tendency to metastasize to pelvic lymph nodes, or worse, to distant sites, and consequently, will have a relatively poor long-term outcome as well [34,35]. As most patients with loco-regionally advanced disease receive treatment by means of hormonal ablation therapy, only one report so far has dealt with the natural course of disease in the absence of treatment. In a highly selected group of 50 men with locally advanced prostate cancer who received no initial treatment, Adolfsson and colleagues reported a 26% prostate cancer mortality at 10 years with nearly all patients progressing locally [36]. As nearly all studied patients had well-differentiated cancer, the presented figures may not reflect the expected cancer-specific mortality rates within the whole group of patients with locally advanced prostate cancer.

The prediction of prognosis of men with clinically confined disease is even less understood. Presently, it is not clear whether aggressive treatment of men with clinically diagnosed, localized disease will eventually improve outcome compared to those who did not receive initial treatment [28,35,37,38]. Johansson showed that in a selected group of 223 men with clinically diagnosed, confined disease receiving no initial treatment, only 13% (29/223) died from prostate cancer over a 15-year period [28]. The authors claim that patients with early stage disease might thus not benefit from aggressive treatment such as radical prostatectomy. This study has been criticized for its inclusion of a relatively older male population with low grade disease, thereby substantially increasing the risk of mortality from concurrent illnesses. Chodak and associates performed a meta-analysis on six large studies of men who were treated expectantly, and showed that the risk of dying from clinically diagnosed, confined prostate cancer steadily increased with rising tumor grades, i.e. 2 to 13% for low grade disease at 5 and 10 years follow-up, 3 to 13% for cases with intermediate grade disease, and 33 to 66% for men with poorly differentiated tumors, respectively [35]. At 10 years after the initial diagnosis, the proportion of men with distant metastases was 19, 42, and 74% for grades 1, 2, and 3 prostate cancer, respectively. The authors stated that prostate cancer should be looked upon as a potentially progressive disease when managed conservatively. In line with the previous group, Albertsen and colleagues showed that men aged 65 to 75 years who were treated conservatively for low grade (Gleason scores 2 – 4) clinically diagnosed, localized disease faced only a minimal risk of death from prostate cancer even after 15 years of follow-up [38]. The cumulative mortality from prostate cancer at 15 years was 9%, whereas this figure was 28% for moderate grade (Gleason score 5 – 7) and 51% for high grade (Gleason score 8 – 10) disease, respectively. An interesting finding in this study was that patient comorbidity was nearly as potent a predictor of outcome as the histological grade of tumor differentiation. Reports from Sweden are in line with those of Albertsen *et al.* [38], and showed that as the life expectancy of cases with clinically diagnosed,

confined disease exceeded 10 years due to low competing mortality (as is true for cases undergoing radical prostatectomy), the cancer-specific mortality at 15 years may be considerably high (approximately 30%) [26,39]. This is particularly of interest, since in the Netherlands, most men in their sixties have an average life expectancy of 15 years or more, and when diagnosed with prostate cancer that is still confined to the prostate, they may be at considerable risk of developing metastatic disease, loosing substantial quality of life, and eventually, to die from prostate cancer.

Summary I: Prostate Cancer as a Burden in the Pre-PSA Era

Prostate cancer is the most prevalent type of cancer in males in most Western countries. In the absence of early detection programs, malignancies that originate from the prostate gland often (30 to 60%) present themselves in a (loco-regional) advanced stage of disease. Men who are diagnosed with prostate cancer in an advanced stage of disease may suffer from severe morbidity, and will experience a substantial loss of quality of life. No curative treatment is available in these cases, and consequently, the long-term cancer specific mortality is high. Despite the knowledge that prostate cancer has a protracted course of disease in most cases with clinically confined cancer, there is a considerable mortality in subgroups, i.e. those with poorly differentiated cancers. For now, the long-term benefits of curative treatment over surveillance and deferred treatment in clinically diagnosed cases with localized prostate cancer have yet to be established, but as the risk of competing mortality declines and the male life expectancy increases, the role of prostate cancer as a major public health problem is likely to increase even more in the future.

EARLY DETECTION OF PROSTATE CANCER

Screening Policies

Screening implies the application of a simple and relatively inexpensive test to a large number of individuals in order to classify them as likely or unlikely to have the disease that is the objective of screening [40]. Screening efforts may be applied to individuals (opportunistic screening) or to the community as a whole (population based screening). It seems intuitively logical that through the early detection of disease using specific screening tools, a disease may be more often amenable to curative treatment, and that advanced disease and fatal cases may be postponed, or preferably, prevented. However, the mere fact that a screening test is able to detect a disease early does not imply that all those subjected to screening will also benefit. First, the vast majority of individuals subjected to screening will not have the disease that is the objective of screening, and obviously, in these cases screening efforts are not likely to yield any profit. More distressing, these individuals will only suffer from the adverse effects of screening such as anxiety for the disease, the inconvenience and discomfort of diagnostic interventions, and the downstream sequelae of false-positive screening test results. On the other hand, as most solid malignancies present themselves with signs and symptoms in a stage of disease when definite cure is beyond reach, a beneficial effect of screening (and early treatment) may only be achieved in asymptomatic participants. It is assumed that when a disease occurs frequently in the population, causes a high level of suffering in those affected, and is associated with a high rate of premature deaths, screening policies directed against asymptomatic individuals may more easily be accepted as a general health care policy.

Besides the possible negative side-effects of screening to those without the disease, it is not always straightforward that the outcome of those *with* the disease will be changed. Basically, the natural course of the disease may or may not be changed by the application of a screening test. The likelihood that a screening test will alter the natural course of disease depends on the performance of the screening test and on the biological aggressiveness of disease. This may be explained by the two extremes of disease (e.g. cancer) detection by means of a screening test. At one end, the screening test may be too sensitive, and a cancer is detected that would never lead to any clinical signs or symptoms in the remaining lifetime of a screened individual. These individuals with the disease will eventually die from intercurrent illnesses rather than from the coincidentally detected cancer. The chance of diagnosing a cancer that otherwise would not have revealed itself clinically in the absence of screening will be increased in cancers that present themselves clinically at an age at which competing illnesses are highly prevalent, and in cancers that have a relatively long asymptomatic (pre-clinical) non-metastatic phase of disease. At the

other end, if a screening test is not capable of detecting a cancer early enough, so that the cancer is already advanced at the time of diagnosis, the application of methods of screening are not likely to be beneficial neither. In these cases, early detection and early treatment will not alter the natural course of disease, and patients will suffer or even die from the disease despite the implementation of screening tools. In optimal conditions, screening tests should only detect those cancers that would lead to morbidity and mortality in the absence of screening, though which are still curable when early detected. Unfortunately, as most currently applied screening tests cannot reliably distinguish between potentially aggressive, curable cancers and non-aggressive, or otherwise, non-curable cancers, many individuals need to be subjected to the screening tests to provide for an overall net benefit of screening in the population as a whole. Some criteria that enable a mass screening project for a specific disease have been developed by Hulka in 1988 (TABLE 1.2)[41].

TABLE 1.2
The five criteria necessary to justify a mass screening project for a disease [41]

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

Obviously, there is no point in screening for a disease that can be treated successfully after clinical symptoms appear, nor is there a need for screening when no reliable curative treatment options exists [40]. Furthermore, when those undergoing treatment for early

detected cancer have as good an outcome as those who do not receive treatment, the earlier detection of disease is clearly not advantageous [42]. At last, the benefits of screening may sometimes be illusory, and the evaluation of the outcome of screening programs is known to contain serious pitfalls.

Pitfalls in the Evaluation of a Screening Program

The evaluation of the outcome of screening programs is known to suffer from serious pitfalls. This implies that the application of the screening test itself may lead to an apparent improvement in outcome in those screened, while actually there is none. Several pitfalls have been described previously. First, the application of advanced diagnostic techniques prompted by abnormal screening tests may result in a seeming advantage of screening compared to the situation in which no screening is applied. One of the clearest examples that screening might be beneficial in subgroups while there is no benefit in the total cohort is caused by the performance of pelvic lymph node dissection in those planned to undergo radical prostatectomy. In all surgically treated patients with prostate cancer, both screen-detected and non-screen detected, a histopathological examination of the pelvic lymph nodes is performed to stage the disease. Contrary to clinically diagnosed cases, however, the lymph node tumor involvement is often low in screen-detected cases with prostate cancer, sometimes even limited to single cells or small cell clusters. In fact, these so-called micrometastatic lesions may only be detected with the use of immunologic or molecular markers. Due to this low tumor load in pelvic lymph nodes, it is expected that the outcome of screen-detected cases with pelvic lymph node disease is more favorable than the outcome of clinical patients who are diagnosed by routine histopathological examination only. So, as screen-detected patients with micrometastatic lymph node disease shift from N0 to N1, patients with a relatively favorable prognostic constellation are added to the group of patients with a relatively adverse prognostic constellation [43]. Conversely, screen-detected patients with an expected unfavorable outcome compared to those with clear N0 disease 'migrate' from a lower stage to a higher stage. Although the total outcome of the group will not be changed, this upward stage migration will improve the outcome in each of the constituent stages (both N0 and N1). This apparent improvement in prognosis in separate stage groups without a concurrent improvement of the total cohort has earlier been defined the 'Will Rogers' phenomenon [43]. The application of bone scans, and computer tomography (CT) in men with abnormal screening test results may also lead to an upward stage migration and an apparent improvement in outcome in subgroups.

A screening program may also cause an apparent improvement in survival because of lead-time and length-time bias. By definition, individuals with screen-detected cancer will live longer with their cancer from the time of diagnosis, whereas their actual overall

survival is not changed if screening is ineffective. Lead-time bias pertains to comparisons that are not adjusted for the timing of the diagnosis. Otherwise, in screening programs only those cases with cancer may be detected that are expected to have a long protracted course of disease. If fast growing, highly progressive cancers are not detected by the screening tests, and consequently, screening programs will only detect patients with favorable prognostic cancers, screening may appear beneficial. Length-time bias pertains to comparisons that are not adjusted for the rate of progression of the disease [2,44]. At last, screening programs are perceptive to selection bias, as it is known that health-conscious cases within the population are more willing to participate in these screening programs.

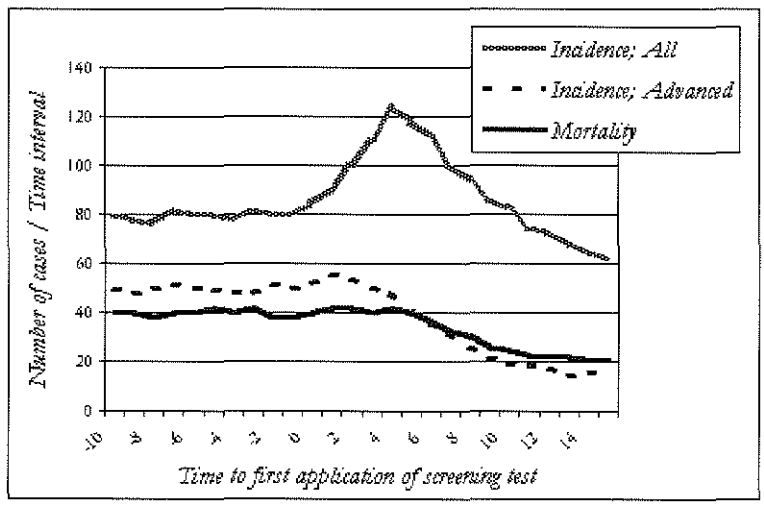
Characteristics of Successful Screening Programs

In successful screening programs, the first indication of a benefit of screening comes from the detection of a large number of prevalent cases that would have gone undetected are removed from the population (cull phenomenon) (FIGURE 1.2). The stage distribution will be shifted towards less advanced disease categories relative to those of clinically diagnosed cases. The incidence rate of advanced stages of disease will increase as well initially, but as the prevalent pool of these distant and regionally extensive cases decreases, this temporary rise in incidence will be followed by a sustained decline. In the most optimistic scenario, the survival rates, and more importantly, the mortality rates will fall below those of prior to screening.

The golden standard in evaluating the value of cancer screening is the performance of a randomized controlled trial (RCT). In such trials, invited participants are randomly assigned to a group undergoing the screening tests followed by early treatment, or to a group receiving standard medical care (and no screening). By the methodology of randomization, the screening group and control group are comparable with respect to age, descent, and other hitherto inexplicable clinico-social and prognostic variables. Differences in outcome between the group invited to screening and the group that is not can most likely be attributed to the application of the screening tests and its downstream sequelae. Mostly, the primary objective of a RCT is to prove a decrease in disease-related mortality in the group invited to screening compared to the group that is not. The final decision whether or not to conduct a nation-wide population-based screening program for cancer balances between the potential benefits of screening (e.g. a decrease in cancer-related mortality) and the disadvantages of screening (e.g. anxiety, complications, health care related costs) [45]. As was stated earlier, it is hard to estimate the value of the implementation of a population based screening program on an individual level. Some individuals will only experience the potential disadvantages of the screening efforts, while others may indeed be saved from site-specific morbidity and/or mortality. For a

participant of a population based screening program, it cannot be properly determined whether screening does more good than harm.

FIGURE 1.2
A schematic diagram of a successful screening program



Much can be learned from the earlier experiences of screening programs that were directed against two other 'high-incidence' malignancies, i.e. those of the lung and breast. Indeed, prostate cancer screening has striking analogies with lung cancer and breast cancer screening [46].

Comparison to Lung Cancer Screening

In the late 1950s until the early 1980s, lung cancer screening with annual chest X-ray and sputum cytology was endorsed by the American Cancer Society (ACS). However, the implementation of these early detection programs for lung cancer was not based on well-performed RCTs that proved a decrease in lung cancer mortality in screened participants. This is similar to the situation today in which the ACS and the American Urological Association (AUA) recommend PSA blood testing and digital rectal examination (DRE) in all men aged 50 years and older, and from the age of 45 years in men in high-risk

groups [47-50]. In most parts of Europe, however, the major local health authorities as well as the European Union discourage wide-scale opportunistic screening for prostate cancer [51]. Population based screening will only be offered to the general population if RCTs prove its efficacy with regard to an established decrease in disease-related mortality in screened participants without a substantial concomitant loss of quality of life. In the 1970s, the continuance of lung cancer screening trials was justified by the observation that the detection rate in the screened population was higher than in non-screened populations, that the cancers detected were more often resectable, and that a considerable stage shift was noted in screened lung cancer patients compared to those not subjected to the screening tests. These findings were interpreted as a clear benefit of lung cancer screening. Moreover, an improved five-year survival rate (from the time of diagnosis) was reported among populations screened. These (misleading) measures of success are also observed in the prostate cancer screening trials performed today [4,52,53]. The inexperience with the presence of all kinds of biases (such as lead-time and length-time bias) thrown up by non-randomized screening trials might have disturbed the adequate interpretation of the outcomes of lung cancer screening programs, and might even have wrongly approved the continuance of these screening programs [2]. None of the RCTs that investigated the efficacy of lung cancer screening has eventually shown that lung cancer screening was associated with a reduced mortality from lung cancer [54]. It has even been suggested that lung cancer screening might even have increased the mortality associated with the disease.

It has to be kept in mind, however, that lung cancer is known to have an eagerly more aggressive (natural) course of disease than prostate cancer, and that lung cancer patients will mostly die of the disease shortly after diagnosis (FIGURE 1.1). A successful screening program for lung cancer, therefore, should rely on very sensitive screening tests, a short screening interval, and the availability of effective treatment options for clinically localized disease. With respect to these differences, lung cancer screening and prostate cancer screening may differ substantially in their initial design.

Comparison to Breast Cancer Screening

Several RCTs performed in the 1980s proved a benefit of breast cancer screening with mammography in women aged 50 to 69 years [55,56]. With respect to these favorable outcomes, breast cancer screening is presently recommended by the Advisory Committee on Cancer Prevention of the European Union [51]. Major doubts, however, have been raised on the question whether these favorable outcomes can also be achieved in prostate cancer, and more specifically, whether prostate cancer screening is feasible and ethical. In contrast to breast cancer screening, it has often been assumed that prostate cancer screening could not be beneficial since screening with the serum-PSA test (and DRE)

would lead to a substantial overdiagnosis and overtreatment. These assumptions were largely based on observations that in autopsy and cystoprostatectomy studies the microscopical prevalence of prostate cancer was between 30 and 50% of males in the age group 50 to 70 years [57-59], i.e. substantially higher than the cumulative lifetime risk of clinically diagnosed cancer. It has long been feared that screening for prostate cancer would preferably identify only these microscopical cases, and that most men with prostate cancer were more likely to die of intercurrent illnesses instead of prostate cancer. The detection of these harmless cancers within screening programs, therefore, was expected to outweigh any potential benefit [60]. With respect to the (microscopical) prevalence of the disease, however, more and more evidence is currently available that striking analogies between the malignancies of the prostate and those of the breast are present. The prevalence of breast cancer (and ductal carcinoma in situ lesions) was reported to be remarkably similar to those of prostate cancer in males, i.e. 39% of women aged 20 to 54 years on autopsy [61]. Almost half of these cases were detected as microcalcifications on post-mortem mammography. Besides similar (microscopical) prevalence rates, the medico-social impact of breast cancer may be quite similar to that of prostate cancer. Whereas prostate cancer is often described as a silent cancer that elderly men die rather *with* (other illnesses) than *of* (metastatic disease), the reality is that prostate cancer cannot be denied as a major public health burden. In fact, the incidence rates and mortality rates of the disease, and to a lesser extent the mean age, and stage at diagnosis may be very similar to those of breast cancer, which is not often the subject of similar concerns. For instance, the incidence of breast cancer is an expected 10,000 for the year 2000 in the Netherlands, whereas approximately 3,700 women are expected to die from the disease in this year [1]. Part of the discrepancy between the incidence of prostate cancer and breast cancer can be explained by active community based screening for breast cancer, while that of prostate cancer is still discouraged by the major health care providers in the Netherlands. Besides a high incidence rate, both breast cancer and prostate cancer are associated with a tremendous morbidity in advanced cases of disease, a substantial concomitant loss of quality of life, and a considerable consumption of health care related resources. Furthermore, for both prostate cancer and breast cancer, suitable screening tests are presently available that are able to detect the disease in a pre-clinical and potentially curable stage. Remarkably, the PSA blood test in prostate cancer screening has a higher positive predictive value than mammography in breast cancer screening. Moreover, the PSA blood test is observer independent in contrast to mammography, which is subject to significant variability in interpretation. Most importantly, the screening tests in prostate cancer screening are not so sensitive to detect many of the microscopically prevalent (and clinically irrelevant) cases, which was a fear. With this respect, it is worrisome that a multiplicity of research efforts and health care resources are still preferably directed towards breast cancer studies.

Preliminary Outcome of Prostate Cancer Screening Trials

The availability of relatively valid screening tests, and the potential success of curative treatment options in patients with localized prostate cancer, some U.S. health authorities have already advocated screening for prostate cancer a recommended health care policy [47-50]. On the other hand, the U.S. Preventive Services Task Force, the Canadian Urological Association and most health authorities within the European Union discourage prostate cancer screening, while the recommendations of the American College of Physicians, and the American Academy of Family Physicians are currently under review [62-64].

Preliminary data from two institutions have suggested that prostate cancer screening might indeed lead to a reduction of prostate cancer mortality. The randomized screening study performed in Quebec, Canada, showed that PSA based screening for prostate cancer resulted in a reduction of prostate cancer mortality to up to 70% in screened participants [65]. This study has been criticized for randomizing men before they agreed to take part in the study. In fact, only 23% of the trial population were willing to participate. As claimed by the critics, a potential resulting lack of statistical power should not be solved by increasing the number of men subjected to screening with those who underwent screening in the non-invited (control) group of the trial. Vice versa, the number of men unscreened should not be obtained by adding the number of men that did not attend screening in the invited group to those who were not invited for screening at the time of randomization [66]. Second, as there was a long lag between the time of randomization and the time of first screening (i.e. on average 3 years), and taking into account that only men without a diagnosis of prostate cancer could participate into the trial, those who were unscreened at the time of analysis had been under a substantial longer risk of prostate cancer mortality than those in the screened group [66]. Therefore, the reported reduction of prostate cancer mortality could be the result of a nonrandomized comparison, and the study might have been biased. More indirect evidence for a possible beneficial effect of prostate cancer screening came from the Urological department of Innsbruck, Austria, where in contrary to other parts of Austria, the serum-PSA test had been made freely available to the population in 1993, and where the acceptance of testing was high [67]. The investigators reported 33% fewer prostate cancer deaths than expected in the Innsbruck area between 1996 and 1999 in men aged 40 to 79 years. The authors concluded that the policy of making the PSA assay universally available to the population (and at no cost) might have reduced the prostate cancer mortality rate in that population.

Despite the early (and potentially misleading) signs of success in previously performed prostate cancer screening trials, the outcome of well-performed RCTs is still awaited. The outcome of these RCTs evaluating the benefits (or disadvantages) of population-based screening for prostate cancer will not be available before the end of the present decade.

The European Randomized study of Screening for Prostate Cancer (ERSPC)

Prospective RCTs provide a means to avoid important biases and to obtain sufficient statistical power to prove or disprove a final primary end-point. The European randomized study of screening for prostate cancer (ERSPC) is a multi-institutional study that investigates the impact of screening for prostate cancer on site-specific mortality and quality of life. Originally, seven European centers participated in ERSPC, i.e. Antwerp, Belgium; Tampere and Helsinki, Finland; Florence, Italy; Rotterdam, the Netherlands; Lisbon, Portugal; Madrid, Spain, and Göteborg, Sweden. During the course of the study, two centers from France and Switzerland were added to the list of participants (i.e. Toulouse and Aarau, respectively). The final objective of ERSPC is to demonstrate a reduction of prostate cancer mortality of at least 20% (with a statistical power of 90%) in screened participants compared to non-screened participants in the control group. To achieve this, more than 200,000 men are to be invited, and are to be randomized into a screening and control group. The screening study was approved by the institutional and regional ethical and scientific committees. The ERSPC is closely associated with the Prostate, Lung, Colon, and Ovary (PLCO) screening project of the U.S. National Cancer Institute, and a combined analysis is planned.

After a series of pilot studies from 1991 to 1993, the final screening study started in 1994. In the Netherlands, participants were recruited from the general population of the city of Rotterdam and those of surrounding communities on the basis of the population registry. All men in the age range 55 to 74 years were invited to participate, and those who responded to a letter of invitation (participation rate 45%) were randomly assigned to a screening and control arm at a distribution of 1 : 1. Men with prevalent prostate cancer were excluded from randomization. Between June 1, 1994 and December 31, 1999, a total of 41,919 men were randomized, and those randomized to the screening arm were offered the screening tests (see below). The Rotterdam sceening protocol uses a screening interval of four years with intentionally the same algorithm as on prevalence screen. Men within the control group received standard medical care. Prostate cancer deaths were recorded by linkage to the database of the Comprehensive Cancer Registry, the 'integraal kankercentrum Rotterdam' (IKR).

The Rotterdam Screening Regimen

In all screened participants, PSA testing, DRE and TRUS were applied as initial screening tests for prostate cancer. Blood sampling was done before rectal examination, so that DRE and TRUS were performed without knowledge of the PSA value. Participants were informed about the PSA value and the findings on DRE and TRUS by letter, and were notified about the procedure to be followed. From June 1994 to February 1997, the screening protocol determined that screened participants with a PSA equal to

or above 4.0 ng/mL (Hybritech Tandem E Assay) were to undergo transrectal sextant prostate biopsy. In the low PSA ranges (0.0 – 3.9 ng/mL), men with a suspicious DRE (nodularity, asymmetry, induration) or TRUS (hypoechogeneity) finding were invited to undergo prostate needle biopsy on second visit. In February 1996, during the course of the study, the biopsy indication for men who presented with a PSA value below 4.0 ng/mL was changed, resulting in the omission of DRE and TRUS as a screening tool in cases where the PSA value was below 1.0 ng/mL. This was done because of the very low positive predictive value of biopsies for cancer in participants with a PSA value below 1.0 ng/mL. In February 1997, a second major change of protocol was implemented within ERSPC, when the European study group decided to exclusively take a biopsy from men with a PSA of 3.0 ng/mL or more, without performing a DRE or TRUS as screening tests at all. All biopsies were performed under ultrasound guidance using a 7 MHz end-fire ultrasound probe.

All participants diagnosed with prostate cancer on prostate needle biopsy were sent back to their General Practitioner to be referred for treatment to the University Hospital Rotterdam or to one of the regional hospitals. Surgery (retropubic radical prostatectomy), external beam radiation therapy, and brachytherapy are currently used as potentially curative treatment options for clinically localized prostate cancer. Watchful waiting is generally considered an accepted (and applied) treatment option for selected groups of patients with prostate cancer.

Summary II: Early Detection of Prostate Cancer

Screening for prostate cancer may improve the morbidity and mortality associated with the disease, but this hypothesis is unproven. Prostate cancer screening may lead to a substantial overdiagnosis and overtreatment, but the magnitude of these risks are uncertain. The effect of prostate cancer screening on the quality of life of screened participants, and on consumption of heath care related resources remain yet to be elucidated. Therefore, prostate cancer screening remains a controversial issue at this time.

TOWARDS IMPROVING THE OUTCOME OF PROSTATE CANCER PATIENTS

Which Prostate Cancers do we Wish to Detect in Screening Programs?

As already stated previously, the aggregate morbidity and mortality attributed to prostate cancer are certainly sufficient to justify a search for effective and efficient strategies for the early detection of the disease. But what factors determine whether a screening program leads to a net beneficial outcome in the screened population? And, more specifically: Which prostate cancers do we wish to detect in screening programs to reduce the mortality of the disease, and conversely, which cancers do we wish to be left untreated?

It is well known that screen-detected cancers are basically different from those that are diagnosed clinically, i.e. those that are found in patients with signs and symptoms of prostate cancer. From numerous studies it was reported that screen-detected prostate cancers differ substantially from clinically diagnosed cases in their clinical, biochemical and tumor features [6,7,15,52,53,68-74]. Although the clinical course of disease in clinically diagnosed cases is not completely comprehended in all cases with respect to their risk of progression and metastases, the expected biological behavior of screen-detected prostate cancers is even less understood. In general, the severity of prostate cancer ranges from non-fatal, asymptomatic, slow-growing tumors that do not require treatment, to fast-growing, aggressive, and quickly metastasizing tumors that definitely are responsible for mortality. As screening tests are capable of detecting prostate cancers early in their biological development, screening efforts will shift the whole of detected cancers towards the slow-growing, non-progressive extreme of the spectrum of biological aggressiveness. Basically, screen-detected prostate cancers can be divided into (at least) three different prognostic subgroups based on the expected clinical course of disease. First, there are the screen-detected cancers that would never lead to any clinical signs and symptoms, nor to any mortality in the absence of screening. These cancers have previously been referred to as 'latent', 'indolent', 'incidental', 'microscopic', or 'silent'. It is clear that these cancers are not the main objective in prostate cancer screening as the detection of these cases will only lead to unnecessary diagnostic procedures and treatment with their associated morbidities. Second, there are the screen-detected cancers that are already advanced at the time of diagnosis, and cause their hosts to die of the disease despite the implementation of screening. In these cases, it is unlikely that early detection will improve their outcome, though it cannot be excluded that some cases with advanced disease might indeed benefit (survive longer) from the early application of hormonal ablation therapy. Third, there are the cancers that have not yet led to any

clinical signs and symptoms, nor to any mortality, but are prone to do so if these were not detected by screening efforts. The major objective in screening trials is to detect these future clinically advanced or metastatic cancers, which at the time of screen-diagnosis are still confined to the prostate and amenable to curative therapy. A reduction in prostate cancer mortality is most likely achieved by the detection of these intermediately aggressive cancers. Previously, detection of these cancers was referred to as the 'window of opportunity' in screening for prostate cancer [75,76].

Recognizing that prostate cancer in most cases is a slow-growing disease, it is evident that a 70-year old male with painful metastatic prostate cancer once was a 60-year old man with clinically organ-confined disease, and that his disease burden and his potential risk of mortality might be prevented if the disease was diagnosed and treated earlier. This concept of detection of disease by screening is illustrated in FIGURE 1.3.

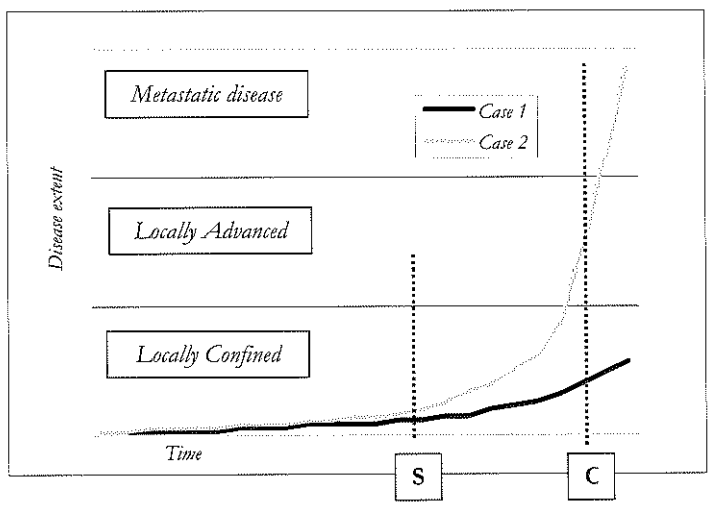


FIGURE 1.3

Schematic diagram illustrating the expected natural course of disease in two cases with prostate cancer. At time 'S' the cancers are assumed detected by screening efforts, whereas at time 'C' the cancers are assumed to appear clinically. The time between 'S' en 'C' is considered the lead time. At time 'S' case 1 and 2 have similar tumor features. At time 'C' case 2 has advanced disease and is prone to die of the disease, while case 1 will still have locally confined disease with a long protracted course. The outcome of case 1 depends on his remaining life expectancy and his risk of competing mortality

An often used classification of screen-detected cancers is the one that divides them into clinically significant and clinically insignificant disease. By this definition, clinically insignificant cancers are those that cause no symptoms and will never do so in the rest of a man's lifetime, (FIGURE 1.3, represented by black line), whereas clinically significant cancers are those that have already caused symptoms, or are expected to do so in the future (FIGURE 1.3, represented by grey line). Besides including men that are likely to benefit from screening efforts with respect to the prevention of future morbidity and mortality, this definition of clinically significant disease also includes men that are prone to die of their disease. The distinction into clinically significant and clinically insignificant disease is based on the assumption that men with clinically significant disease need some sort of treatment, while those with clinically insignificant disease should be refrained from any interventions, and preferably, even their detection. Detection of clinically insignificant cancers in screening programs is referred to as overdiagnosis, whereas the treatment of these cases is considered overtreatment. It is obvious that the features (i.e. grade and extent) of the cancers detected and more specifically, the tumor doubling times and growth rates of the cancers, determine whether a cancer will ever appear clinically. From FIGURE 1.3 it can be demonstrated that as the curve of a cancer becomes steeper (i.e. the tumor doubling time increases), the different stages (confined, locally advanced, metastatic) of the disease are passed more quickly. An important determinant in the definition of what actually constitutes clinically insignificant disease is the patient's life expectancy at the time of diagnosis. As prostate cancer is a slow-growing disease in most cases, the tumor needs time to appear clinically and cause morbidity and mortality. The expected life expectancy of a man can be extracted from lifetables and depends on the age at screen-diagnosis and the risk of death from other causes [77]. It may be expected that a 64-year old man with no comorbidities who has clinically confined prostate cancer detected in a screening program has a substantial risk of future metastatic prostate cancer and death, whereas a 74-year old man in similar conditions has a much lower risk. On the other hand, the likelihood that a 64-year old, severely cardiac compromised man will ever suffer from this prostate cancer is low. An appropriate management of the disease thus requires a scrutinized assessment of a patient's risk: How likely is a given man's screen-detected cancer to progress or metastasize over his remaining lifespan? With this in mind it is worth mentioning that curative therapy such as radical prostatectomy or radiotherapy is only assumed to be effective in men with a life expectancy of 10 years or more. Again, it must be emphasized that the remaining life expectancy at screen-detection is as much a determinant of outcome of screening as are grade, volume and extent of the disease [38].

How Can We Predict the Biological Behavior of Prostate Cancer?

To assess the usefulness of early cancer detection programs, and to define which cancers may appear clinically in the future in the absence of screening, it is necessary to examine in detail the characteristics of screen-detected prostate cancers. From FIGURE 1.3 it can be demonstrated that, with respect to patient and tumor characteristics, the differences between the cancers that are prone to present themselves clinically in the future and those that remain silent in the rest of a man's lifetime are only subtle. At present, it is not yet possible to determine with any degree of certainty, which locally confined cancers at screen-detection will progress, and which cancers will remain confined to the prostate. It may be expected that some of the biological potential for progression and metastases is already present at the time of screening, and that an examination of the histopathological tumor characteristics as well as of the molecular and genetic constitution of the tumor may identify those at risk for progression and metastases. Some evidence for the observation that a substantial proportion of screen-detected cancers may have an unfavorable prognostic constitution comes from the finding that only about one-half to two-thirds of screen-detected cancers prove organ-confined at the time of surgery [78,79]. Also, adverse prognostic genetic events have been reported in premalignant lesions of the prostate and even in the tiniest screen-detected prostate cancers [80,81]. Thus, a thorough examination of prognostic factors, both those that are well-established and those that under investigation, may eventually help to distinguish clinically significant from clinically insignificant disease in prostate cancer screening.

A potential caveat in the examination of histologic, genetic and molecular characteristics of screen-detected prostate cancers is the understanding that screen-detected tumors might not have undergone all the events necessary to produce a life-threatening disease [82]. In other words, not all of the adverse prognostic indicators are present at the time of screen-detection. So, it is likely that, besides time, these prostate cancers require additional malignant events to produce clinically aggressive tumors. It is obvious that this assumption will hamper an adequate distinction between clinically significant and clinically insignificant disease at the time of screening. Moreover, we do not yet know all the molecular and genetic events that are necessary to allow a cancer to progress or to metastasize. It is thus possible that some screen-detected cancers already have metastasized at the time of screen-detection, and did not have treatment that was adapted to their stage of disease. What is needed is markers of progression and markers of metastatic ability that can be used to discriminate those at risk for (or already have) metastases and require treatment from those that have a neglectable risk of progression and metastases and do not need treatment.

Risk Factors and Prognostic Factors

A prognostic factor may be defined as a marker of disease that increases the accuracy in predicting the outcome (prognosis) of the disease, and generally, should be distinguished from a risk factor that may be defined as a marker that increases the likelihood of a diagnosis of disease [83]. Some risk factors also have prognostic value, and vice versa. The only known risk factors for prostate cancer are (increased) age, (black) race, and a family history of prostate cancer. Other variables have only inconsistently been associated with a higher incidence of disease, such as weight, cigarette smoking, alcohol consumption, sexual activity, vasectomy, and the intake of animal fats. The most important risk factor currently known is the serum-PSA level, as are some of its derivatives (such as PSA-velocity, PSA-density, free-to-total PSA, complexed-to-total PSA). Prognostic factors are to a more or lesser degree capable in predicting the extent of disease, the likelihood of recurrence after treatment, and/or the risk of death from the disease. Several clinical (e.g. age, clinical tumor stage, radiographic images) and biochemical (e.g. serum-PSA level, alkalic phosphatase, kidney function) features are known to hold prognostic information in patients with clinically diagnosed or screen-detected prostate cancer. In screening for prostate cancer, however, the prognostic impact of clinical and biochemical parameters is limited. Adverse prognostic findings such as clinically advanced stage (i.e. cT3 or cT4), positive bone scintigrams, and PSA-levels equal to or higher than 50.0 ng/mL are relatively uncommon in prostate cancer screening programs. As a matter of fact, most screen-detected cases with prostate cancer have disease that remains clinically confined to the prostate, no abnormalities on bone scintigraphy, and a PSA level between 3.0 and 10.0 ng/mL. The prognostic factors that have proven to be of most predictive value as indicators of outcome (i.e. the extent of disease, recurrence rates, cancer-specific death) are those that are determined by the pathologist.

Pathological Prognostic Factors

The prognostic arsenal of the pathologist consists of markers that are currently well supported and useful in clinical patient management (i.e. category I prognostic factors), factors of which the prognostic value is promising though remains to be validated in well-powered studies (i.e. category II prognostic factors), and factors of which the prognostic importance remains to be established (category III prognostic factors) [84]. The first category of prognostic factors relates to the determination of the extent of the disease, the determination of the histopathological grade of tumor differentiation, and the determination of the surgical margin status. All three are easily and cost-effectively assessable at histopathological examination of routinely processed tissue specimens. PSA is a clear prognostic factor in this category I as well, but falls behind the scope of this

thesis (i.e. pathological prognostic features). Still worth mentioning is that the serum-PSA level is strongly associated with pathological tumor stage, tumor grade, and tumor volume [85,86]. The second category of prognostic factors mainly concerns tumor volume (as determined on the prostatic needle biopsy and the radical prostatectomy specimen). The third category of prognostic factors relates to a large number of factors with unclear prognostic impact or those under investigation for their prognostic value. The reporting of perineural invasion and microvessel density are examples of these category III factors, whereas the determination of the expression level of tissue markers and the assessment of genetic and molecular changes fall into this category as well.

Pathological prognostic factors may be determined on the prostatic needle biopsy and in the radical prostatectomy specimen. As the biopsy cores only sample the prostate gland, they may not always be fully representative for the entire gland. Consequently, prognostic factors determined on the biopsy specimen may not always reflect those within the cancer in the prostate, and needle biopsies are known to suffer from serious 'sampling error'. This is particularly true for tumor volume, and the histological grade of tumor differentiation [87-91]. Moreover, different study groups reported that a favorable outcome on the biopsy does not imply by any means that a cancer with favorable prognostic tumor features is to be expected. [87,92-97]. In other words, it is not yet possible to distinguish clinically insignificant disease from clinically significant disease on basis of biopy tumor features alone.

Extent of Disease

Historically, staging is used as the prominent prognostic factor in solid malignancies such as prostate cancer. Basically, the stage of disease determines the anatomical extent of disease, and does not in itself measure the biological aggressiveness of disease. The TNM-staging (T = primary tumor status, N = lymph node status, M = distant status) system is most often used and is now an established stratification means [98]. The clinical stage (cT) is based on the results of DRE and the serum-PSA level, supplemented in selected cases by bone scintigraphy and other imaging studies, whereas the pathological stage (pT) is based on the microscopical evaluation of the radical prostatectomy specimen [98,99]. As a substantial proportion of cases is misclassified by clinical staging [78,88,99], pathological staging predicts disease recurrence and patient outcome much more accurately than cT. Prostate cancers detected by PSA-based screening are more often organ-confined (pT2a-b; pTNM'97) than those in historical controls or those detected clinically [7,15,53,68,69,100,101]. In fact, up to 80% of surgically treated patients will have organ-confined disease at the time of screen-detection [7,15,53,68,69,101]. Conversely, tumors that show extraprostatic extension (pT3a) and particularly those that invade adjacent organs such as the seminal vesicles (pT3b) and bladder neck (pT4a) are seen less frequently in prostate cancer screening trials [7,53,68,101,102]. Mostly , tumors

that transgress the boundaries of the prostate are large, poorly differentiated, and have an adverse prognostic outcome. As most of these tumors are already beyond the reach of cure at the time of screen-detection, early detection programs do not particularly aim at the detection of these advanced cancers. Rather, screening efforts are directed at the detection of cancers that are still organ-confined at the time of screening, though have additional adverse prognostic signs. On the other hand, recent reports have suggested that pT3a tumors may still be cured by radical prostatectomy when the amount (volume) of tumor outside the border of the prostate is only low or when poorly differentiated tumors were excluded [103]. A distinction between focal and established extraprostatic extension, therefore, has been proposed [100].

Histological Grade of Disease

Histological grade is an indeniable prognostic factor in prostate cancer. Several grading systems have been proposed for prostate cancer, and presently, the Gleason grading system has found most widespread acceptance in clinical practice. The Gleason system has proven prognostic value in nearly all studies that in any way reported on prostate cancer outcome. In this grading system, five different growth patterns of prostate cancer are distinguished based on the degree of glandular differentiation, and the architectural arrangement of the tumor as it relates to the prostatic stroma [89,104]. The Gleason growth patterns range from well differentiated (Gleason grade 1) to poorly differentiated (Gleason grade 5) cancer. The system takes into account the tumor heterogeneity by adding the primary (dominant) growth pattern and the secondary (non-dominant) growth pattern into a Gleason sum or score. When no secondary growth pattern is present or constitutes less than 5% of the total tumor load, the primary growth patterns is simply doubled. Thus, the Gleason score has nine digits and ranges from 2 to 10. Based on similarities in predictive capacity and for statistical analyses, the Gleason score is often compressed into 2 – 4, 5 – 6, 7 and 8 – 10, or simply 2 – 6, 7, 8 – 10. In screening studies, biopsy Gleason scores of 6 or 7 are most common with approximately 50% and 35% of screen-detected cases, respectively [52,58,69,70,72]. Cases with an excellent outcome (Gleason 2 – 4) or a definite poor outcome (Gleason 8 – 10) are seen only infrequently. It its assumed that cases with high grade components (i.e. Gleason grade 4 and 5) in the tumor (biopsy or radical prostatectomy specimen) are particularly prone to appear clinically later on in the lifes of their hosts.

Surgical Margin Status

Surgical margins may be determined after radical prostatectomy only and obviously, do not exist before surgery. Positive surgical margins may result from cancer extending outside the prostate to the margins of resection or from inadvertent surgical incision into

the prostate itself [105]. So, due to the finding that part of the etiology of positive surgical margins is explained by human factors, the prediction of the surgical margin status before surgery seems pointless. Its (independent) prognostic impact is based on the assumption that tumor is residual in the body after removal of the target organ. Though, a positive surgical margin in a prostate cancer does not inexplicably imply that the tumor will also recur. It is described that one third of patients with a positive surgical margin does not have a recurrence of disease at a substantial follow-up as determined by PSA relapse after nadir [102,106-108]. The occurrence of positive surgical margins is increased in cancers with larger tumor volumes and in those with a higher stage of disease [102,108].

Tumor Volume

Tumor volume is a category II prognostic factor, which implies that its prognostic value needs further validation. Historically, tumor volume has often been shown to be of predictive value at univariate analysis, though lost its predictive capacity when associated with other conventional prognostic factors such as grade at multivariate analyses. This lack of an independent predictive value of the tumor volume was propably caused by the methodology of tumor volume measurement. First, earlier studies calculated the tumor volume by means of an estimation of the gross clinical appearance on subtotally submitted prostates or by calculating the percent of prostate involved with tumor [109]. As prostate cancer often has an irregular growth pattern and is multifocal in approximately 50% of cases [110], these studies are potentially flawed [111]. The current consensus is that the tumor volume should be determined by morphometric analysis of fully submitted prostates [111]. Second, some study groups included the volume of the transition zone cancers into that of the total cancer volume. Transition zone cancers are often large at presentation, though of low grade (Gleason scores 2-4) mostly [111]. So, despite the fact that these transition zone cancers often cause symptoms early due to their large size, they are unlikely to change overall outcome. Tumors detected within screening programs are generally smaller that those detected clinically or those in historical controls [53,73,74]. Currently, more and more evidence (and consensus) is available for the assumption that the volume of the peripheral zone cancer is of prognostic value, and adds in making a proper estimate of disease outcome. In this, cancers with a tumor volume less than 0.5 mL are thought to represent a general indicator of clinically insignificant disease [101,112]. Furthermore, it is considered that not the tumor volume or the grade of the tumor itself determine the clinical course best, but rather the constituent of the two, i.e. the volume of poorly differentiated cancer (i.e. the volume of Gleason grade 4 and 5 cancer) [113,114]. One of the determinants that refrains one to consider the tumor volume a 'category I' prognostic factor is the lack of a uniform (and relatively laborious) method of measurement and reporting [84].

The volume of a cancer may be estimated on the needle biopsy by calculating the number of cores with cancer, the biopsy tumor involvement, and the total length of tumor on the cores. Although there is a correlation between the tumor volume as determined on the needle biopsy and the tumor volume in the radical prostatectomy specimen (i.e. the golden standard), sampling error may create substantial outliners and for now, makes an individual estimate of the actual tumor volume hardly possible.

Tissue and Tumor Markers

Several proteins expressed in the cell nucleus, the cell cytoplasm, or the cell membrane are detectable by immunohistochemical staining methods, and are known to have an altered expression in malignantly transformed cells. It is assumed that the altered expression level of tissue markers in cancers (whether higher or lower) may help to predict the tumor behavior in conjunction with conventional prognosticators as grade and stage. Moreover, it is possible that tissue markers may identify those cases that have an eagerly aggressive course of disease, or conversely may have a relatively benign course of disease, within the group of cancers that otherwise would have similar outcomes if assessed by stage and grade alone. It is evident that the use of these tissue markers must add to the accuracy of prognostic prediction since it is found that new prognostic factors are often strongly interrelated with conventional prognosticators. All kind of tissue markers such as TP53, MIB-1 (Ki-67), Bcl-2, $p27^{kip1}$, $p16^{ink4A}$, CD44s, and E-cadherin have been studied extensively in laboratory settings in prostate cancer, but none so far has found its place in clinical routine. The widespread application of tissue markers in clinical practice faces problems related to proper tissue handling, standardization of methodology, quality assurance, and low reproducibility [84,115]. Moreover, different study groups show discrepant and contradictory results with respect to outcome that discourage the routine clinical use of these markers. Scrutinized research is necessary to overcome these problems and to assess which of these tissue markers might indeed have the potential to discriminate between future aggressive and non-aggressive disease.

Other and Future Prognostic Markers

There is an increasing interest in the use of new determinants of cancer outcome to assist clinical decision making. It is likely that molecular cytogenetic analyses studying genetic changes such as deletions, losses, amplifications or gains of specific chromosomal regions may enable use to establish more accurate methods of prognostication. All kind of new diagnostic techniques are currently being tested in laboratory setting. Results from new molecular biological techniques such as loss of heterozygosity (LOH) and microsatellite analyses, fluorescent *in situ* hybridization (FISH) analyses, and comparative genomic hybridization (CGH) analyses appear with increasing frequency in the medical literature and many show considerable promise. A probable future surplus value of

studies examining cellular and genetic processes may be the early identification of cases that have already metastasized or are at great risk of progression. At this time, however, most factors are still in an experimental phase and require clinical validation. As for tumor markers, most of the investigational prognostic factors lack standardization of methodology, quality control, and reproducibility. Some of the reported conflicting results on these investigational prognostic factors may in part be explained by this absence in consensus.

Combining Prognostic Factors

As was stated previously, the clinical course of screen-detected prostate cancer cannot be adequately predicted on an individual basis by the assessment of only one (or two) prognostic variables. This is mainly due to a large heterogeneity among prostate cancers, even if they are of similar grade or stage, and screen-detected. Probably, combining different independent prognostic factors into a so-called 'prognostic index' will enhance the actual predictive capacity compared to that of individual prognostic factors [116]. It is likely that, in addition to powerful prognosticators as grade and stage, the application of future prognostic parameters will provide for a more accurate outcome estimate in individual patients and thus to more reliable treatment guides. The use of nomograms that combine different prognostic factors for the determination of a specific outcome parameters might be of help for an adequate individual assessment of the extent of disease or the risk of progression and metastases. For instance, the nomograms developed by Partin and colleagues combining serum-PSA, clinical tumor stage and biopsy Gleason score are currently being used by some to predict the pathological stage of disease and thus, to more accurately determine the appropriate treatment (or the absence of treatment). [68,117]. Similar nomograms have been developed to predict the risk of positive lymph node involvement [118] and the likelihood of recurrence of disease after initial curative treatment [119]. In both settings, the incorporation of new promising variables may increase the predictive capacity [120]. At last, artificial neural network analysis (ANN) and computer and regression trees (CART) analysis are being investigated regarding their use in the prediction of prostate cancer outcome measures.

Summary III: Prostate Cancers to be Detected

Prostate cancers detected in screening programs are often organ-confined, of intermediate histological grade of tumor differentiation, and of low tumor volume. With respect to these prognostically favorable tumor features, early detection programs may indeed detect prostate cancer in its curable phase more often. Therefore, PSA based screening for prostate cancer has the potential to decrease the rate of disseminated disease and to decrease prostate cancer mortality. As of yet, however, we are not able to

distinguish clinically significant from clinically insignificant disease at the time of screening. An even more scrutinized histopathological examination of the prostate cancers detected in screening programs, combined with a continued search for prognostically independent, relevant and applicable tissue markers and molecular and genetic techniques, must eventually help to identify those cases that are particularly prone to present themselves clinically and cause disease-specific mortality if not screen-detected.

Again, it must be emphasized that the age of a screened man, and the number and severity of his comorbidities, are as much predictors of outcome as the characteristics of the tumor. The constellation of tumor characteristics, whether those well-established or under investigation, should always be related to the remaining life expectancy of a screened individual. Although there is no absolute tumor size, pathological tumor stage or histological grade associated with clinical complaints or the occurrence of metastatic disease, the chance of prostate cancer related morbidity and mortality increases steadily with increasing tumor volume, tumor stage and histopathological grade of tumor differentiation, and declines with an decreased patient's life expectancy.

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Part II

Towards Predicting the Outcome of Prostate Cancer Screening

CHAPTER 2

DEFINING THE WINDOW OF OPPORTUNITY IN SCREENING FOR PROSTATE CANCER

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SUMMARY

BACKGROUND. Subdividing cancers according to the natural course of disease, both at the time of diagnosis and after radical prostatectomy, may influence management decisions of patients with prostate cancer. We investigated whether categorization of prostate cancers into different prognostic subgroups is feasible.

METHODS. In 218 screened participants of a randomized study, conventional post-operative tumor features were assessed for their accuracy in predicting PSA-relapse after radical prostatectomy using Cox regression analysis. Independent prognostic tumor features were combined to identify subsets of cancers with similar biological potential. A cancer was defined that may be curable after its detection by screening tests, though is destined to progress to clinically manifest disease and cancer-related mortality in absence of screening.

RESULTS. After a median follow-up of 33.0 months, pathological tumor stage ($p = 0.03$), tumor volume ($p = 0.04$), and surgical margin status ($p = 0.01$) each independently predicted PSA-relapse after surgery. The proportion of poorly-differentiated cancer proved highly superior to Gleason score and most strongly predicted PSA-relapse after radical prostatectomy ($p < 0.0001$). Based on combined independent prognostic tumor features, a tumor classification model powerfully predicted PSA-relapse.

CONCLUSIONS. Based on tumor characteristics, possibly harmless, and conversely, possibly non-curable disease, may be distinguished from cancers that are likely to show clinical progression in the absence of screening and treatment. Prediction of these subclasses prior to treatment may eventually lead to proper patient management.

INTRODUCTION

In the last decade, extensive efforts have been undertaken in large randomized and case-finding screening trials, for the early detection of prostate cancer. In these trials, serum prostate-specific antigen (PSA), digital rectal examination (DRE) and transrectal ultrasound (TRUS) are used as indicators for the presence of prostate cancer. When screening tests for prostate cancer are applied to the general population, it is anticipated that a considerable proportion of screen-detected cancers may be detected and treated unnecessarily, since the cancers have seemingly innocuous tumor characteristics. In the absence of treatment, these cancers might not have caused clinical symptoms in their host ever. Conversely, it is likely that some men are treated with erroneous curative intent, since the treated tumors have features associated with poor prognosis. The high recurrence rates of these cancers after curative therapy indicate that these particular cases might better be treated with (neo-adjuvant) androgen deprivation therapy. To optimize screening-efforts and to improve patient management, it may be necessary to define a type of cancer that, if not treated, may be responsible for prostate cancer mortality, while after its early detection by screening tests, may still be curable by current treatment options. The detection and treatment of patients with this type of cancer have earlier been defined the 'window of opportunity' in screening studies [1,2].

In the current study we investigated 218 men that underwent radical prostatectomy at the University Hospital Rotterdam. All men were participants from the screening arm of a randomized screening study for prostate cancer. It is plausible that the biological potential of prostate cancer is best reflected in the radical prostatectomy specimen, since the tumor in its totality is studied [3-5]. Conventional post-operative tumor features determined in the radical prostatectomy specimen were analyzed for their accuracy in predicting PSA-relapse, as an intermediate endpoint after surgery. Independent prognostic tumor features were combined to identify subsets of cancers with similar biological potential. By considering tumor characteristics only, an attempt was made to distinguish possibly harmless cancers from those that are assumed to be in the 'window of opportunity'. Moreover, an effort was made to define the characteristics of cancers that might be responsible for progression and prostate cancer mortality even after therapy. A tumor classification model that is based on tumor characteristics only will be especially amenable for predictive analysis before treatment. By including treatment-induced variables (e.g. surgical margins) into the model, the risk of disease recurrence after radical prostatectomy may be determined as well.

MATERIALS AND METHODS

Patients

Between June 1994 and December 1998, a total of 34,930 participants, aged 55 to 74 years, were randomized to a screening and control arm within the Rotterdam section of the European Randomized Study of Screening for Prostate Cancer (ERSPC). No participant had a previous diagnosis of prostate cancer. Written informed consent was obtained from every participant prior to randomization and the study was approved by the local medical ethics committee. Up until February 1997 the screening protocol determined that screened participants with a serum PSA-level equal to or above 4.0 ng/mL (Hybritech Tandem E; Hybritech Inc., San Diego, CA) and/or a suspicious DRE and/or TRUS-finding at low PSA-values (0.0 – 3.9 ng/mL) were to undergo prostate biopsy. Additional biopsies were directed at ultrasound detectable (hypo-echogenic) lesions when present. In February 1997, a major change of protocol was implemented within ERSPC, when the study group decided to exclusively take a biopsy from men with a PSA of 3.0 ng/mL or more, without performing a DRE or TRUS as screening tests at all. Sextant transrectal biopsy was performed using a Bard (C.R. Bard, Convington, GA) spring-loaded biopsy gun and an 18-gauge biopsy needle. Ultrasound-guidance was performed using a 7 MHz end-fire ultrasound probe. Until December 31st 1998, 17,424 men were randomized to the screening arm of ERSPC, and 777 participants were diagnosed with prostate cancer after histopathological examination of the ultrasound-guided sextant biopsy. All prostate cancer patients were sent back to their General Practitioner to be referred for treatment to one of the regional hospitals. The choice of treatment (i.e. radical prostatectomy, radiotherapy, androgen deprivation therapy or watchful waiting) was determined on basis of the biopsy tumor features, patient's age, his comorbidities and his preferences, as well as on the preferences of his treating Urologist. A total of 219 consecutive patients within the screening arm of ERSPC underwent bilateral pelvic lymph-node dissection and subsequent radical prostastectomy for prostate cancer at the University Hospital Rotterdam. No patient received (hormonal) treatment prior to operation.

For all but one case, follow-up data were available, leaving 218 patients included in the study. Patients were followed at intervals of 3 months for the first year after radical prostatectomy, semiannually for the second year, and yearly thereafter for evidence of PSA-relapse. Time to biochemical progression was defined as the time from radical prostatectomy to the time of first recurrence of serum PSA (i.e. $\geq$ 0.1 ng/mL), and until last follow-up, if the patient did not experience PSA-relapse. Two sequential elevated PSA-levels were required to confirm PSA-progression. No patient received adjuvant

hormonal or radiation therapy, until eventual PSA-relapse occurred. Two patients died within one year after radical prostatectomy without evidence of recurrent prostate cancer.

Pathological Tissue Examination

All radical prostatectomy specimens were fixed, totally embedded, and processed according to well-established protocols [6,7]. For each case, a Gleason score was determined, and the tumor was staged according to the TNM '97 classification by a single pathologist (ThvdK). Considering the proportion of high-grade cancer (Gleason growth pattern 4/5) five categories were distinguished: 0, no high grade; I, < 5% high grade; II, 5-24% high grade; III, 25-49% high grade; IV, ≥ 50% high grade cancer. Presence of tumor cells at the inked margin of resection was considered a positive surgical margin. All tumor areas were traced and outlined on the slides, and subsequent morphometric analysis was performed to determine the tumor volume as described in detail by Hoedemaeker et al. [5]. Cancers were classified based on combined conventional tumor characteristics into minimal, moderate, and advanced disease, according to the arbitrary models proposed by Epstein et al. [3] and Hoedemaeker et al. [4].

Statistical Analysis

Statistical analysis was performed using the statistical package for the social sciences (SPSS 9.0; SPSS Inc., Chicago, IL). Cox proportional regression analysis was used to assess the relationship between the (combined) post-operative variables and PSA-relapse after radical prostatectomy. The Gleason score, pathological tumor stage, the proportion of high grade cancer, tumor volume, and surgical margins were categorized according to TABLE 2.1. Kaplan-Meier curves were constructed to show the probability of remaining free of PSA-relapse as a function of time after radical prostatectomy. The Logrank test was used to assess differences between baseline variables and biochemical progression. The assumption that no predictive value (H0) existed for the variable evaluated was rejected if $p < 0.05$. To identify independent prognostic factors, backward stepwise Cox regression analysis was performed by removing variables from the model that were not statistically significant at the univariate level, while controlling for other variables. Forward stepwise elimination was performed to verify that the same parameters remained of prognostic significance in the final models. We then combined independent prognostic tumor features in an attempt to improve the predictive capacity for PSA-progression after radical prostatectomy.

RESULTS

Patient Characteristics

For the cohort of 218 included patients, the median follow-up for PSA-relapse was 33.0 months (range, 5 - 63), the mean age was 64 years (SD ± 4.8), and the median PSA-level at the time of biopsy was 5.3 ng/mL (range, 0.8 - 29.5). No patient had positive lymph-nodes on fresh-frozen tissue examination intra-operatively, while just one patient experienced metastatic lymph-node disease after the evaluation of paraffin-slides (i.e. $pT_{3b}pN_1$). The median tumor volume was 0.68 mL (range, 0.002 - 13.48). PSA-relapse occurred in 24 patients (11.0%) after a median follow-up of 12.5 months (range, 1 - 41) after radical prostatectomy.

Conventional Prognostic Tumor Features and PSA-relapse (TABLE 2.1)

Gleason score significantly predicted PSA-relapse after radical prostatectomy. Of the 13 progressing cases with Gleason score 7, 9 had a dominant Gleason growth pattern 4. No statistically significant difference was found for PSA-relapse between different subsets of tumors with a low proportion of high grade cancer (0 - 49% high grade). Cases with tumors containing ≥ 50% high grade cancer, i.e. Gleason score 7 (4 + 3) or Gleason scores 8 - 10, progressed more frequently than cases with tumors containing less than 50% high grade cancer. The association of the proportion of high grade cancer and PSA-relapse free survival is depicted in FIGURE 2.1A. The pathological tumor stage significantly predicted PSA-relapse after radical prostatectomy (FIG. 2.1B). No statistically significant difference ($p = 0.108$) was found between organ-confined disease (pT_2) and tumors penetrating the prostatic capsule (pT_{3a}), indicating that statistical significance was gained only for tumors invading the seminal vesicles (pT_{3b}) and/or bladder neck (pT_4). Most recurrences were observed in the group of patients with a tumor volume of equal to or more than 1.0 mL (FIG. 2.1C). Of the 79 patients with a tumor volume less than 0.5 mL none eventually progressed. Positive margins were identified in 56 patients (25.7%), and significantly predicted PSA-relapse. Of 26 cases with capsular penetration (pT_{3a}), and that were specimen-confined after surgery, 2 (7.7%) experienced PSA-relapse. Both progressing cases had a tumor volume ≥ 1.0 mL and ≥ 50% high grade cancer. Of 17 cases in which the tumor penetrated the prostatic capsule, of the ones that were not specimen-confined after surgery, 4 (23.5%) experienced PSA-relapse. Of these 4 cases, all had a tumor volume ≥ 1.0 mL, and 3 had ≥ 50% high grade cancer.

TABLE 2.1

Distribution of post-operative tumor features of screened participants of ERSPC, section Rotterdam (n = 218). Univariate and multivariate analysis by Cox regression analysis of variables in association with PSA-relapse after radical prostatectomy.

Variable	Number of total (%)	Number relapse (%)	Univariate analysis χ^2	Univariate analysis p-value*	Multivariate analysis Hazard ratio	Multivariate analysis CI	Multivariate analysis p-value
Pathological Tumor stage							
pT_2	156 (71.6)	8 (5.1)					
pT_{3a}	43 (19.7)	6 (14.0)	31.53	< 0.001	2.60	1.08–6.25	0.03
pT_{3b-4}	19 (8.7)	10 (52.6)					
Gleason score							
2-6	120 (55.0)	6 (5.0)					
7	92 (42.2)	13 (14.1)	19.11	< 0.001	-	-	ns
8-10	6 (2.8)	5 (83.3)					
% High-grade (HG)							
< 50% HG	198 (90.8)	11 (5.6)					
≥ 50% HG	20 (9.2)	13 (65.0)	79.43	< 0.001	8.67	3.73–20.09	<0.001
Tumor volume							
< 0.5 mL	79 (36.2)	0 (0.0)					
0.5-1.0 mL	57 (26.1)	5 (8.8)	23.27	< 0.001	2.48	1.06-5.80	0.04
≥ 1.0 mL	82 (37.6)	19 (23.2)					
Surgical Margin status							
Positive	56 (25.7)	15 (26.8)					
Negative	162 (76.3)	9 (5.6)	17.34	< 0.001	2.93	1.25-6.86	0.01
Tumor categorization†							
Minimal	50 (22.9)	0 (0.0)					
Moderate	117 (53.7)	8 (6.8)	22.50	< 0.001	-	-	-
Advanced	51 (23.4)	16 (31.4)					

* Logrank test (for trend)
CI 95% Confidence intervals
ns not significant
† According to Hoedemaeker et al [5]

On multivariate analysis tumor volume, pathological tumor stage, the proportion of high-grade cancer, and surgical margin status independently predicted treatment failure after radical prostatectomy, with the proportion of high-grade cancer being the strongest predictor of PSA-relapse (TABLE 2.1).

Tumor Categorization Model and PSA-relapse (TABLE 2.2)

A previously constructed tumor categorization model that combined pathological tumor stage, tumor volume, and Gleason score, significantly predicted PSA-relapse after radical prostatectomy on a prospective basis (TABLE 2.1).

By the incorporation of independent prognostic tumor features identified in the present study a modified model was established, that strongly improved the predictive capacity for PSA-relapse ($\chi^2 = 51.27$; $p < 0.0001$, FIG. 2.1D). Since this tumor classification was based on tumor characteristics only, it is amenable to predictive analyses before treatment and is considered the 'intention-to-treat' approach. The 'treatment-received' approach also consideres surgical-margin status and lymph-node status. By assessing treatment-induced variables also, the tumor categorization model was capable of predicting disease recurrence after radical prostatectomy on an individual basis. During the follow-up period, only cases with advanced disease, i.e. seven with pT_4, three with pT_{3b}, seven (three pT_2 and four pT_{3a}) patients with a tumor volume ≥ 1.0 mL and ≥ 50% of poorly-differentiated cancer, as well as seven patients with moderate disease and positive surgical margins, experienced PSA-relapse. Of 10 progressing cases with pT_{3b} or pT_4 stage, six had a tumor volume ≥ 1.0 mL and ≥ 50% high-grade cancer, whereas four had a tumor volume ≥ 1.0 mL and less than 50% high grade cancer. Seven of 44 (15.9%) moderate cancers with positive margins had PSA-relapse after radical prostatectomy. None of the patients in the minimal group (n = 50), nor any of the patients in the moderate disease group with negative margins (n = 96) progressed.

DISCUSSION

In recent years, large randomized and case-finding screening studies, initiated in Western-Europe and North America, provided insight into the clinical characteristics and pathological tumor features of early detected prostate cancer. However, the optimal screening-regimen and proper managing of patients who have eventually been diagnosed with prostate cancer within these screening trials have yet to be elucidated.

FIGURE 2.1

Kaplan-Meier curve of the probability of PSA-relapse as a function of: ***FIG 2.1A*** Gleason score and the proportion of high-grade (HG) cancer in the radical prostatectomy specimen, divided in: 1. Gleason score 2-6 (0-5% HG), 2. Gleason score 7 (5-50% HG), and 3. Gleason score 7, 8-10 (≥ 50% HG) ($p < 0.0001$) ***FIG. 2.1B*** Pathological tumor stage, divided in: 1. pT_2 (organ-confined), 2. pT_{3a} (extraprostatic extension), and 3. pT_{3b-4} (invading adjacent organs) ($p < 0.0001$) ***FIG. 2.1C*** Tumor volume in the radical prostatectomy specimen, divided in 1. < 0.5 mL, 2. 0.5 - 1.0 mL, and 3. ≥ 1.0 mL ($p < 0.0001$) ***FIG. 2.1D*** A combined tumor feature model, including pathological tumor stage, tumor volume and proportion of high-grade cancer (TABLE 2.2), in which 1. Minimal disease, 2. Moderate disease, 3. Advanced disease ($p < 0.0001$).

Fig. 2.1 A

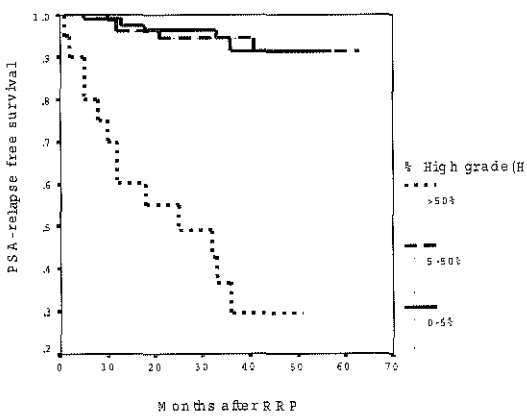


Fig. 2.1B

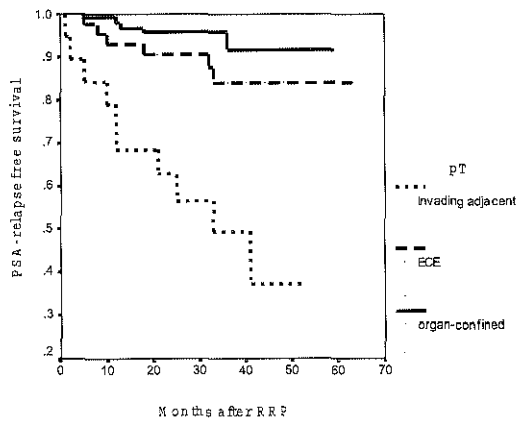


Fig 2.1 C

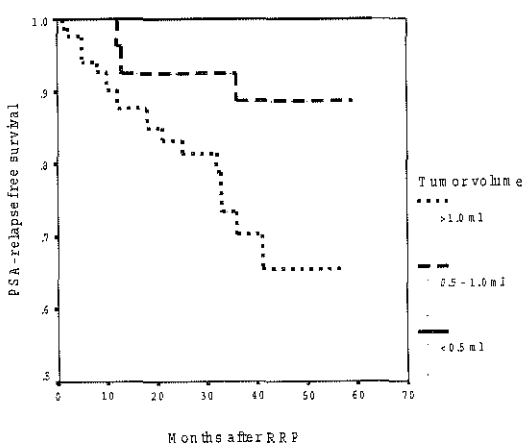
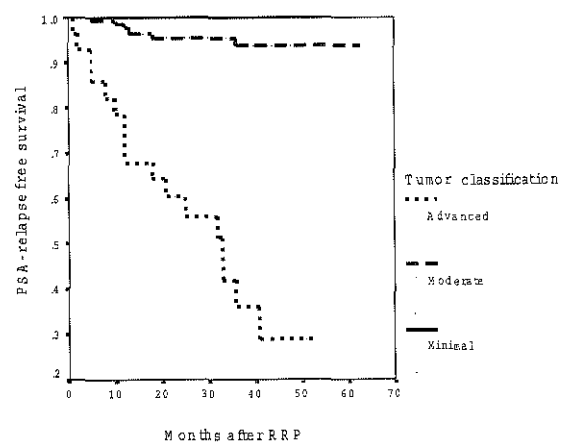


Fig. 2.1 D



These early detection programs aim at detecting cancers that are destined to progress to clinically manifest disease and cause cancer-related mortality, while they are still amenable to curative therapy. Because of the lack of sufficient follow-up, the identification of cases that are most likely to benefit from screening efforts can only be achieved using intermediate endpoints after treatment. After radical prostatectomy, PSA-relapse is the first evidence of disease recurrence and may precede clinical recurrence months or years before it can be detected by routine clinical and radiographic tests [8-15].

TABLE 2.2

Tumor categorization model, number of events (i.e. prostate-specific antigen relapse), and risk of biochemical disease recurrence after radical prostatectomy according to the intention-to-treat approach (white) and the treatment-received approach (grey). The intention-to-treat approach considers tumor characteristics only, whereas the variables of lymph-node status and surgical margin status are considered in the treatment-received approach as well.

Tumor category	Tumor volume	High-grade (HG)	Tumor extent *	Events (%)	Events (%)	Risk of Biochemical recurrence
Minimal	< 0.5 mL	No HG	pT_2	0/50 (0.0%)	0/50 (0.0%)	Low 0/146 (0.0%)
	Any	< 50% HG	pT_2			
Moderate	Any	< 50% HG	pT_{3a}	7/140 (5.0%)	0/96 (0.0%)	
	< 1.0 mL	≥ 50% HG	pT_2			
	< 1.0 mL	≥ 50% HG	pT_{3a}			
	≥ 1.0 mL	≥ 50% HG	pT_2			
Advanced	≥ 1.0 mL	≥ 50% HG	pT_{3a}	17/28 (60.7%)	8/15 (53.3%)	
	Any	Any	pT_{3b}			
	Any	Any	pT_4			High 24/72 (33.3%)
		pN1		1/1 (100.0%)	16/57 (28.1%)	
		Positive Surgical Margins		15/56 (26.8%)		

* pTNM '97

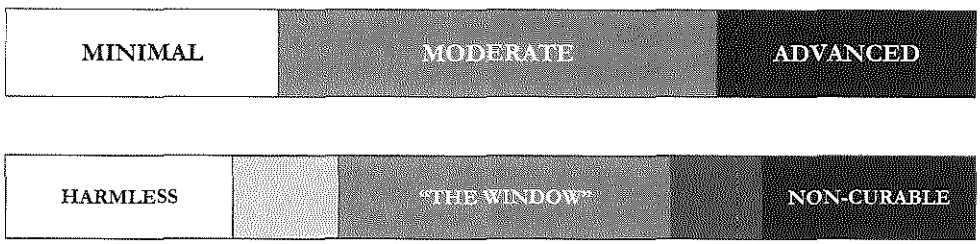
Only few reports addressed the value of an isolated PSA-elevation after radical prostatectomy with respect to prediction of prostate cancer mortality [15]. The current study investigated 218 patients that were retrieved from the screening arm of a large randomized trial and who underwent radical prostatectomy for biopsy proven prostate cancer. PSA-relapse occurred in 11.0% of cases after a median follow-up of 33.0 months. Well-established prognostic pathological variables were able to predict biochemical recurrence after radical prostatectomy (TABLE 2.1). Consistent with reports from Stanford University [16-18], our data substantiate the superior prognostic value of the proportion of poorly-differentiated cancer to the conventional Gleason score system. Cases with more than 50% high-grade cancer were at considerable risk of disease recurrence. Unexpectedly, capsular penetration did not by itself seem to confer a worse prognosis than organ-confined disease in the multivariate analysis, whereas tumor volume remained an independent predictor of disease recurrence. Our finding that patients with tumor volumes less than 0.5 mL represented a subgroup of patients that were highly unlikely to experience biochemical recurrence after radical prostatectomy is in line with other studies [2,3,17-20]. A previously proposed tumor categorization model, in which well-established prognostic tumor features were combined to reflect the intrinsic biological potential (i.e. the intention-to-treat approach), significantly predicted PSA-relapse after radical prostatectomy (χ^2 = 22.50). Taking into account other relevant prognostic pathological factors observed in the present study, the historic model could be adapted to strongly improve the predictive capacity for PSA-relapse after radical prostatectomy (χ^2 = 51.27). By also assessing treatment-induced prognosticators (i.e. the treatment-received approach), a valid stratification of patients into different risk groups was established.

During the follow-up period obtained in this analysis, only cases with advanced disease and/or those with positive surgical margins experienced PSA-relapse after radical prostatectomy. All 50 cases with minimal disease and all 96 cases assigned to the moderate disease group with negative margins remained disease free during the follow-up period (Table 2.2). It is anticipated that detection of cases with minimal disease, i.e. small (< 0.5 mL), organ-confined tumors without Gleason growth patterns 4 or 5, may be avoided, whereas patients whom are eventually diagnosed with this seemingly 'biological insignificant' disease would be suitable candidates for conservative therapy and surveillance [1-5]. Though, since all minimal cancers in this study were treated, long-term biological indolence, especially in younger men with a long life expectancy, cannot be proven with certainty. Patients assigned to the moderate disease group, i.e. tumors with only small amounts of high-grade cancer and intermediate sized (< 1.0 mL) tumors with dominant (≥ 50%) poorly differentiated components (TABLE 2.2), are particularly prone to follow an adverse prognostic course, if the screening-tests, diagnosis and subsequent

treatment had not been applied. On an intention-to treat basis, all surgically treated cases with tumor features corresponding to moderate disease remained free of disease during the follow-up period. Though, it is possible that with longer follow-up some cases with moderate disease will relapse despite treatment. On the other hand, an unknown proportion of men with features of moderate disease should rather be considered as potentially harmless (i.e. as having minimal disease) even without treatment. For now, no further refining of the moderate disease group is possible. The definite answer to the question what is the exact 'window of opportunity' in screening for prostate cancer can only be determined after the completion of randomized clinical trials that prove a reduction of prostate cancer mortality in (subsets of) screened men (FIGURE 2.2).

FIGURE 2.2

According to the tumor classification model, tumors with features corresponding to minimal disease are assessed as harmless, while tumors with features of advanced disease are beyond the reach of cure. All cancers with features in between those of minimal and advanced (i.e. moderate) disease are assumed to cause prostate cancer mortality in the absence of screening and treatment. Until screening programs prove a reduction of cancer mortality, the exact borders of the model have yet to be defined.



Seventeen out of 28 (60.7%) cases with advanced disease and 7 out of 44 (15.9%) cases with moderate disease and positive margins recurred, indicating that these patients are at considerable risk of developing (or already developed) non-curable local recurrent and/or systemic disease. By the intention-to-treat approach, presence of tumor in bladder neck (pT_4), seminal vesicle (pT_{3b}), and/or a large amount (i.e. $\geq$ 0.5 mL) of poorly-differentiated cancer lead to a high treatment failure rate after surgery with curative intent. Since the follow-up period was relatively short, and the fact that the biological behavior of disease also depended on inexplicable host factors, not all patients with

advanced disease and/or positive surgical margins experienced PSA-relapse. An unknown proportion of these cases might be cured despite their highly adverse prognostic tumor features. Moreover, recent reports suggested that positive margins solely at the prostatic apex may not confer a worse prognosis than negative margin, organ-confined disease only [21-24].

Our data demonstrate that 16 out of 17 (94.1%) cases with moderate disease, and a sole positive apical surgical margin, remained disease-free after radical prostatectomy. Therefore, a wider window of curability, i.e. a subgroup of advanced tumors and moderate cancers with only minor positive apical surgical margins, may indeed exist.

Serum PSA-value was not incorporated in the present model, since our and other studies demonstrated that a proportion of clinically significant (i.e. moderate and advanced) disease was present at low PSA-values (0.0 – 3.9 ng/mL), indicating that silent aggressive tumor growth may occur [1,25-28]. Otherwise, clinical staging was not incorporated in the model, since it cannot reliably differentiate between curable and incurable disease, nor between clinically significant and clinically insignificant disease.

CONCLUSIONS

The current study provided some arguments that a prognostic classification of prostate cancers is conceivable. The presented tumor categorization model incorporated the powerful independent outcome predictor of the proportion of poorly-differentiated cancer, as well as tumor volume, and pathological tumor stage. Application of a tumor categorization model will identify patients at increased risk of disease recurrence after surgery, thereby opting for increased surveillance and/or application of early adjuvant therapy. On the other hand, since the tumor classification model is based on tumor characteristics only, it may be especially suitable for predictive analyses before treatment, using regular statistics or artificial neural network analyses. For now, our definition of the 'window of opportunity' in screening for prostate cancer is speculative and its precise definition is an ongoing continuous process. A further prospective evaluation at multiple institutions is needed to prove its validity.

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CHAPTER 3

PRELIMINARY RESULTS FROM A POPULATION BASED RANDOMIZED CLINICAL TRIAL OF SCREENING FOR PROSTATE CANCER: SCREENING VERSUS CONTROL ARM

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Manuscript in Preparation

INTRODUCTION

Randomized controlled trials (RCT) are presently performed in Europe and the United States to assess the impact of systematic screening for prostate cancer on cancer-specific mortality and quality of life. The European randomized study of screening for prostate cancer (ERSPC) is a large multicenter RCT that seeks to demonstrate a reduction of prostate cancer mortality of at least 20% in men randomized to screening compared to men in the control arm. It has been calculated that at least 100.000 men are to be screened (with 100.000 men in the control group) to provide for sufficient statistical power [1]. ERSPC is closely associated with the Prostate, Lung, Colorectal and Ovary (PLCO) trial of the National Cancer Institute (NCI), and a combined analysis is planned.

This chapter provides the first preliminary report on the comparison between the screening arm and control arm of a large RCT that investigates the efficacy of systematic prostate cancer screening. Since the beginning of ERSPC in October 1993, more than 40.000 men have been randomized into screening and control in the Rotterdam section of ERSPC. In the present report, special attention is given to the number of men diagnosed with prostate cancer within either of the two randomization arms, as well as to the number of men with lymph node and distant metastatic disease, and the distribution of well-established prognostic tumor features determined on the biopsy and in the radical prostatectomy specimen. These data represent an important intermediate endpoint of population based screening for prostate cancer. In fact, this same comparison will take place later on this decade to compare the mortality rates.

PATIENTS AND METHODS

Between October 1993 and December 1998, a total of 35,149, aged 55 to 74 years, were randomized to the screening arm and control arm of ERSPC, section Rotterdam (17,636 in the screening arm and 17,513 in the control arm). Men in the screening arm underwent initial screening at the Department of Urology, and comprised of PSA testing, digital rectal examination (DRE) and transrectal ultrasound (TRUS). In all men blood sampling was done prior to rectal examination, so that DRE and TRUS were performed without knowledge of the PSA value. From October 1993 to February 1997, the Rotterdam screening regimen called for sextant transrectal biopsy if the PSA level was equal to or higher than 4.0 ng/mL, and if DRE and/or TRUS were suspicious for cancer at low PSA values (0.0 – 3.9 ng/mL). The biopsy procedure was performed in a second visit to our Department. From February 1997 unwards, only men who had PSA $\geq$ 3.0 ng/mL were

to return for prostate biopsy. In these men, DRE and TRUS were not anymore applied as initial screening test for prostate cancer. Again, all men with a biopsy indication were scheduled to undergo systematic sextant transrectal biopsy at our Department. Four years after initial prostate cancer screening all men in the screen group were invited to undergo repeated screening. The conditions and algorithm of the screening regimen of ERSPC are described in greater detail elsewhere [2-4].

Cases within the control arm of ERSPC received standard medical care, which meant that the evaluation of symptoms, a diagnosis of prostate cancer and subsequent treatment (or refrainment from treatment) were provided by local Urologists (or our own). To identify the cases with prostate cancer in the control arm, a linkage was performed with the database of the local Comprehensive Cancer Registry (CCR). Men diagnosed with prostate cancer, and those known to have died from whatever cause were identified, and data were returned to ERSPC. The CCR provides for a 100% cancer registration within the population. Data related to lymph node metastases and distant metastatic disease were obtained by a review of the patient's charts at the local hospitals.

For prostate cancers detected in the screen group, a Gleason score was assessed prospectively for each case by a single genito-urinary pathologist (ThvdK). After the identification of men with prostate cancer in the control group, the histological slides with prostate cancer were retrieved from the pathologic storage facilities of the local hospitals (or that of our own), and the Gleason scores were reviewed for all cases.

Various variables related to men who underwent radical prostatectomy at our Department for screen-detected prostate cancer (e.g. Gleason score, pathological tumor stage) had been stored prospectively in a comprehensive database. The pathological tumor features of the men who underwent radical prostatectomy in the control group were obtained similarly to those of the biopsy specimens, i.e. by retrieving the histologic slides from local hospitals, and subsequent reviewing of radical prostatectomy specimens. All tumors were staged according to the pTNM '97.

The Pearson χ^2 test was used to assess differences between the screen group and control group of ERSPC with respect to prognostic tumor features. The assumption that no difference existed for the variable evaluated (H0) was rejected (H1) if $p < 0.05$.

RESULTS

The important findings of this preliminary comparison between the screen and control group of ERSPC are listed in TABLE 3.1 and TABLE 3.2. The number of prostate cancers detected, the extrapolated number of cancers per 100.000 randomized men (*), and the absolute number of men with pelvic lymph node disease was higher in the screen group

than in the control group (TABLE 3.1). However, the absolute number and relative proportion of men with distant metastatic disease was higher in the control group than in the screen group. TABLE 3.2 shows that the histologic grade of tumor differentiation on the biopsy was statistically significant more favorable in the screen group than in the control group (χ^2-test: $p < 0.01$). The pathological tumor stage and Gleason in the radical prostatectomy were not statistically different between the two randomization arms, potentially because of selection bias before treatment.

TABLE 3.1

A comparison between the screen and control group of ERSPC, section Rotterdam. Men were randomized between October 1993 and December 1998

Variable	Screening Arm	Control Arm
Randomized	17,636	17,513
Biopsies	3,481	N/D
Cancers (% of biopsies)	818 (23.5%)	150
Rate *	4,638/100,000 (4.6%)	856 /100,000 (0.8%)
Distant metastases (M1)	5 (0.6)	10 (6.7)
Lymph-node metastases (pN1)	9 (1.1)	2 (1.3)

* Number of cancers divided by the number of men randomized; that is the detection rate for the screen group and the incidence rate for the control group

N/D No data available

DISCUSSION

This preliminary study shows a favorable prognostic shift in the screening arm of this population based RCT compared to the control arm. Most pronounced is the observation that the Gleason score on the biopsy was significantly lower in the screen group. Another important finding is that the number of men with distant metastatic disease was lower in the screen group compared to the control group (5 versus 10; TABLE 3.1). Men with metastatic disease are most likely to die from prostate cancer later on despite hormonal treatment, and figures on the metastatic rates may best reflect the final mortality rates. However, the absolute number of men with metastatic disease was

only low, especially when compared to the total number of men diagnosed with prostate cancer, i.e. 0.6% of men (5/818) in the screen group, and 6.7% of men (10/150) in the control group. The relative proportion of men presenting with distant metastatic disease in the control group was also remarkably lower than that reported in historical controls (i.e. 20 to 25%) [5]. This figure might be explained by the application of screening tests in the control group (contamination), though it is known that the contamination rate in the control group of ERSPC is only about 11 to 13% [unpublished data]. Lead time (the time between screen-detection and the clinical appearance of disease), which is known to be 4 to 6 years for prostate cancer, has only just been passed, and the differences between the screen and control group of ERSPC are likely to become even more pronounced in the future.

TABLE 3.2

A comparison between the pathologic prognostic features of the cancers detected in the screen and control group of ERSPC, section Rotterdam. All men were randomized between October 1993 and December 1998

Variable	Screening Arm N (%)	Control Arm N (%)	p-value *
Biopsy Gleason score			
4-6	516 (64.3)	68 (48.6)	
7	214 (26.7)	40 (28.6)	< 0.01
8-10	73 (9.1)	32 (22.9)	
Total	803	140	
Pathological Tumor Stage †			
pT2	207 (75.5)	14 (63.6)	
pT3a	47 (17.2)	5 (22.7)	ns
pT3b- pT4	20 (7.3)	3 (13.6)	
RRP Gleason score			
4-6	161 (58.8)	11 (42.3)	
7	106 (38.7)	13 (50.0)	ns
8-10	7 (2.6)	2 (7.7)	

* χ^2-test
† pTNM '97
RRP retropubic radical prostatectomy
ns not significant

A difference in the absolute number and relative proportion of men with lymph node positive disease was observed as well, though in favor of the control group. At least part of this difference may be explained by the performance of pelvic lymph node dissection in men planned to undergo radical prostatectomy for clinically localized prostate cancer. The number of men undergoing surgery was considerably higher in the screen group than in the control group, and it was reported previously that the application of advanced diagnostic tools (such as a staging lymph node dissection) may result in an upward stage migration and an eponym called the 'Will Rogers phenomenon' (See for explanation: General Introduction). Again, the relative proportion of men with metastatic lymph node disease in the control group was only low compared to that in historical controls [5].

A previously mentioned drawback in prostate cancer screening is the detection of presumably clinically insignificant disease (cancers that would never lead to any signs and symptoms). In our study, the ratio of cancer detection between the screen and control group was 5.5 (818 divided by 150), and it is assumed that at last part of these might be cancers that are overdiagnosed and overtreated. However, it can be calculated that approximately 1,575 men (17.500 randomized × 9.0%) with (clinically significant) prostate cancer reside in each of the two randomization arms, and that approximately 630 men (1,575 cancers × 40%) are expected to die from their disease some time in the future (See: TABLE 1.1). We therefore do not have stringent evidence that overdiagnosis (and overtreatment) is presently occurring in the screen group of our randomized clinical trial.

As was stated earlier, these results are only intermediate signs of success of prostate cancer screening, and do not provide by any means the evidence that prostate cancer screening reduces the mortality of the disease.

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CHAPTER 4

DETECTION OF PROSTATE CANCER: A COMPARATIVE STUDY OF THE DIAGNOSTIC EFFICACY OF SEXTANT TRANSRECTAL VERSUS SEXTANT TRANSPERINEAL BIOPSY

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SUMMARY

BACKGROUND. The optimal biopsy strategy for the detection of prostate cancer still needs to be established, as a considerable proportion of clinically significant cancers remains undiagnosed on routine sextant transrectal biopsy. To assess the efficacy of transperineal biopsy for the detection of prostate cancer, we compared this approach to systematic sextant transrectal biopsy in a simulation experiment.

METHODS. Ultrasound-guided sextant transverse (transrectal) biopsy and subsequent sextant longitudinal (transperineal) biopsy were performed on 40 radical prostatectomy specimens of patients with (transrectal) biopsy-detected prostate cancer. Conditions were simulative and may not be completely analogous to patient settings. Ultrasound-determined prostatic volume, biopsy tumor involvement, number of cores with cancer, and tumor volume were determined. Detailed mapping of radical prostatectomy specimens provided insight in the representativity of the biopsy techniques.

RESULTS. Of 40 cancers 33 (82.5%) were re-detected by the transperineal approach, while this was 29 (72.5%) by repeated transrectal biopsies. For both approaches, tumor volume of undiagnosed cancers was significantly smaller ($p < 0.01$), and prostatic volume was significantly larger ($p < 0.01$) than in re-detected ones. Between the two approaches no difference was found for either of the variables determined in re-detected cancers. Prostate-maps clarified that transperineal undiagnosed tumors were either small (≤ 0.2 mL) or notably located at the prostatic base.

CONCLUSIONS. The biopsy procedure in which the biopsy needles enter the prostate at the apex for a longitudinal direction may efficiently sample the prostatic peripheral zone. Since the experiment was artificial in design, caution should be kept in extrapolating these results to patient settings.

INTRODUCTION

Since its introduction by Hodge et al. in 1989, systematic sextant transrectal biopsy of the prostate under transrectal ultrasound-guidance (TRUS) has become an accepted, routinely performed technique for prostate cancer detection, that is to be preferred over digitally-guided or ultrasound-directed transrectal biopsy [1]. PSA-driven screening is accepted by many men, and is promoted as health care policy in some countries, especially the U.S.A. As a result, the number of men undergoing routine sextant biopsy has increased exponentially, and correspondingly the incidence rate of prostate cancer increased. However, sextant transrectal biopsy may represent an inadequate sampling of the prostate, since 20 - 35% of cancers, detectable by repeat biopsies, remain undiagnosed in a single sextant biopsy session [2-6]. With the intent to improve the diagnostic yield of prostate biopsy for the detection of prostate cancer, various biopsy schemes and biopsy needle trajectories, that seek to represent a more thorough sampling of the prostatic peripheral zone, have recently been evaluated [4,7-9]. Still, the optimal biopsy strategy for prostate cancer detection needs to be defined.

We determined, *ex vivo*, the sensitivity of sextant transperineal biopsy for the detection of prostate cancer, compared to systematic sextant transrectal biopsy. Despite artificial conditions, performing biopsies on radical prostatectomy specimens has the advantage of knowing true prevalence of disease (100% prevalence by definition) in the selected population of men. Features of prostate cancer determined on both biopsy specimens and radical prostatectomy specimens were assessed in comparative analyses.

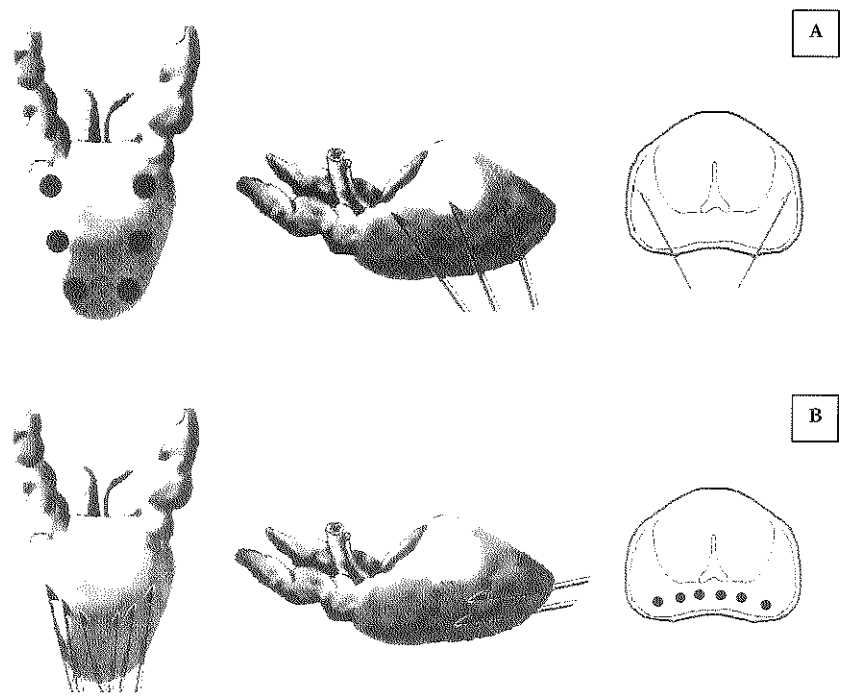
MATERIAL AND METHODS

A total of 40 consecutively obtained radical prostatectomy specimens from participants of the European Randomized Study of Screening for Prostate Cancer (ERSPC) was studied. All evaluated cases had prior diagnosis of prostate cancer, prompted by an elevated PSA ($\geq$ 3.0 ng/mL) and confirmed by TRUS-guided sextant transrectal biopsy. After retropubic radical prostatectomy (RRP), sextant (bilaterally, base, mid-gland, and apex) transverse and sextant (bilaterally, paramedian, median, and lateral) longitudinal biopsies were performed on the specimen by one of the investigators (ANV) using a Bard (C.R. Bard, Convington, GA) spring-loaded biopsy-gun and 18-gauge biopsy needle (FIGURE 4.1). Ultrasound-guidance was established by an experienced urological resident (MOB), using a 7-MHz end-fire ultrasound-probe positioned at the dorsal aspect of the radical prostatectomy specimen. Both investigators were blinded with respect to location

of cancer on pre-operative biopsies. No additional biopsies were directed towards ultrasound-detectable (i.e. hypo-echogenic) lesions. Conditions in the experiment were simulative and may not be completely analogous to patient settings. For consistenty in terminology it was decided that needles entering the prostate for a longitudinal and transverse direction were further arbitrarily referred to as transperineal and transrectal, respectively, although no perineum or rectum were actually present.

FIGURE 4.1

(A) Schematic systematic sextant transversal (transrectal) biopsy, and *(B)* Schematic sextant longitudinal (transperineal) biopsy. *Left. Dorsal view; Middle. Sagittal view; Right. Transverse view.* In the transperineal approach the biopsy needle enters the prostatic peripheral zone at the apex, transverses the gland towards the prostatic base, following a trajectory parallel to the rectum wall



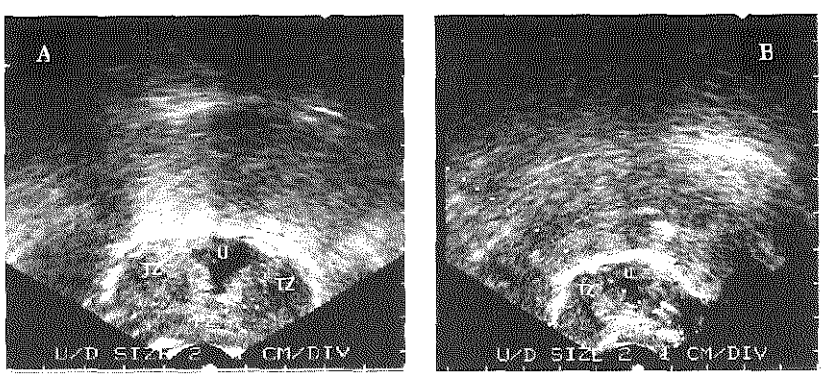
All biopsy cores were separately labeled, fixed and processed according to standardized and established protocols [10,11]. Presence of tumor in both sets of biopsy cores was

assessed by a specialized genito-urinary pathologist (THvdK), who was unaware of the method and blinded with respect to the location of the biopsy cores, as well as to outcome of pre-operatively performed diagnostic biopsies. The number of cores involved with cancer (1-6) and the biopsy tumor involvement (%), i.e. cumulative length of cancer divided by the cumulative length of biopsy cores, were assessed for each case. The tumor was staged according to the pTNM '97 classification, the Gleason-score and the proportion of high-grade cancer were assessed and morphometric analysis was performed to determine tumor volume [12]. All tumors were classified according to a previously developed predictive model (TABLE 4.1; Vis et al., unpublished data) [12]. Detailed prostate-maps were developed to illustrate the size, extent and tumor location. In this, range and trajectory of various biopsy needles could be reconstructed for both approaches. Apically located tumors were arbitrarily defined as any tumor presence in the first two 4-mm transverse slices of the radical prostatectomy specimen [11]. Of 38 cases ultrasound-determined prostatic volume could be obtained from the ERSPC-database.

Various parameters determined on biopsy specimen and corresponding radical prostatectomy specimen were compared using the Mann-Whitney U test. The assumption that no difference (H0) existed for variables evaluated was rejected if $p < 0.05$.

FIGURE 4.2

Ultrasound of the prostate simulating transrectal ultrasound (TRUS), using the 7-MHz ultrasound probe positioned at the rectal site of the radical prostatectomy specimen. (A) Transverse view, demonstrating the transition zone (TZ), urethra prostatica (U) and peripheral zone (PZ), and (B) longitudinal view of mid-base prostate, demonstrating the trajectory of the transrectal needle biopsy



RESULTS

Of the 40 evaluated radical prostatectomy specimens the median tumor volume was 0.860 mL (range 0.012 - 4.166), whereas for 38 available cases, the median prostatic volume was 43.0 mL (range 17.6 - 174.8). Thirty-four (85.0%) tumors were staged pT_2, 5 (12.5%) were staged pT_{3a}, while 1 (2.5%) tumor showed seminal vesicle invasion (pT_{3b}). Twelve (30.0%) cases were classified as having minimal disease, whereas 23 (57.5%) and 5 (12.5%) cases were classified as having moderate and advanced disease, respectively (TABLE 4.1).

TABLE 4.1
Predictive model of tumors; Sensitivity of sextant transperineal and transrectal biopsy in identifying prostate cancer in a selected group of patients undergoing retropubic radical prostatectomy

Category	Tumor volume	High-grade (HG)	Tumor Extent†	Transperineal Sensitivity (%)	Transrectal Sensitivity (%)	Total
Minimal	< 0.5 mL	No HG	pT_2	6 (54.5)	5 (45.5)	11
Moderate	Any	<50% HG	pT_2	23 (95.8)	19 (79.2)	24
	<1.0 mL	≥ 50% HG	pT_2			
	Any	No HG	pT_{3a}			
Advanced	≥ 1.0 mL	≥ 50% HG	pT_2	4 (80.0)	5 (100.0)	5
	Any	Any HG	pT_{3a}			
	Any	Any	pT_{3b}			
	Any	Any	pT_4			

† pTNM '97

With the ultrasound probe applied to the dorsal aspect of the radical prostatectomy specimen adequate ultrasound-visualization of the prostate was obtained for each case (FIGURE 4.2). Of 40 cases, 33 cancers (82.5%) were re-detected by transperineal biopsy, while repeat transrectal biopsy detected only 29 out of 40 (72.5%). No statistically

significant difference in number of cores involved with cancer, biopsy tumor involvement, tumor volume, or prostatic volume was found between tumors detected with either of the two repeat biopsy procedures. TABLE 4.1 depicts the tumor characteristics of (un)diagnosed cancers on repeat biopsy. For both approaches, the majority of undiagnosed cancers was small (i.e. < 0.5 mL), organ-confined, and without Gleason grade 4/5, i.e. minimal disease (TABLE 4.1). For both approaches, the tumor volume of undiagnosed cancers was significantly smaller ($p < 0.01$), and the prostatic volume was significantly larger ($p < 0.01$) than their re-detected counterparts. Examination of prostatic tumor mappings clarified that 25 out of 27 apically (92.6%) located tumors were re-detected on transperineal biopsy, while this was 22 out of 27 (81.5%) for the transrectal approach. Cancers that remained undiagnosed on transperineal biopsy were either small (i.e. five cases with a tumor volume ≤ 0.2 mL) or notably located at the prostatic base (i.e. two cases). The one advanced tumor undiagnosed on transperineal biopsy comprised a pT_{3a} tumor of 1.10 mL with a Gleason score 7 (< 50% high-grade), located exclusively at the base of a 45.6 mL large prostate. For the 11 undiagnosed cancers on repeated transrectal biopsy, 9 had a tumor volume less than 0.20 mL, whereas 2 (moderate) cancers had a tumor volume 1.70 mL and 2.57 mL, respectively. Besides small tumor size, no particular pattern of tumor extension or tumor location in the prostate-maps could be determined for cancers that remained undiagnosed on repeated transrectal biopsy.

DISCUSSION

Sextant transrectal ultrasound-guided biopsy of the prostate, though considered the standard modality for prostate cancer detection, has been criticized for its limited capacity to provide an adequate sampling of the prostate, since a significant proportion of cancers remains undiagnosed [2,3]. On the other hand, in early-detection programmes, men are frequently diagnosed and treated for prostate cancer that, because of small size and low-grade, may intuitively be considered clinically insignificant and therefore would have been better treated with an observational intent only. Some of the limited sensitivity of the biopsy procedure may be explained by the fact that, on basis of random chance, cancers are too small (e.g. ≤ 0.2 mL) to be detected. Furthermore, some cancers remain undiagnosed, because of their localization in areas that are not systematically sampled, e.g. those anteriorly or those in the transition zone. Until now, no randomized prospective trials have been conducted that clarify the clinical insignificance of these undiagnosed cancers. Since clinically significant disease cannot yet be predicted on basis of clinical variables and/or biopsy tumor features, the aim in prostate cancer detection,

for the time being, should be to detect and treat as many cancers as possible with highest efficacy and least patient morbidity.

Previous studies have sought to increase the accuracy of the diagnostic transrectal procedure by increasing the number of biopsy cores [4-6,13,14], or by modifying the biopsy needle trajectory [7-9]. For all these studies the biopsy needle enters the prostate at the rectal surface, and transverses the gland for an anteriorly directed angle. In transperineal biopsies, the prostatic gland is approached from an angle perpendicular to that of the transrectal approach, i.e. entering the peripheral zone at the apex and transversing the prostate parallel to the rectum wall. Outcome of digitally-guided or TRUS-guided, lesion-directed, transperineal biopsies has been grossly described in early studies [15,16]. Other study-groups investigated functionality of the transperineal approach in patients after abdominoperineal resection of the rectum, i.e. without knowing true prevalence of disease [17-19]. After its first description and popularization in the late 1980's, however, the transperineal biopsy approach was abandoned and replaced by randomly performed systematic sextant transrectal biopsies. In subsequent years, no proper evaluation has been performed on the sensitivity of systematic sampling of the prostate by transperineal biopsies, as has been done for transrectal biopsies. Only recently, Shingal and Terris [20] that sensitivity of sextant transperineal biopsy in re-detecting prostate cancer was low (i.e. 10%), in 20 patients scheduled for RRP [20]. In their study, both sextant transrectal and sextant transperineal biopsy were performed in the same set of patients. The authors imitated the biopsy-setting applicable to patients who had their rectum excised, because of colorectal cancer or inflammatory disease. In the absence of a rectum, prostate biopsy was performed under transperineal ultrasound-guidance (TPUS) instead of TRUS. As was already suggested by the authors, TPUS may have pronounced limitations in visualizing abnormalities of the prostate, hypo-echogenic areas in particular.

We report a prostate cancer detection rate for sextant transperineal biopsy of 82.5% compared to 72.5% for routine sextant transrectal biopsy in a simulation experiment performed on a selected group of patients undergoing RRP for biopsy proven prostate cancer. The sensitivity of each of the biopsy procedures in prostate cancer detection for a general population, and with this the features of cancers missed by the original biopsy, cannot be calculated, since the underlying prevalence of disease remains unknown. The proportion of cancers that remained undiagnosed on repeated transrectal biopsy was similar to that reported in similarly perfomed studies [2,6]. Under artificial and optimized conditions, i.e. without a rectum or perineum, and by properly positioning the biopsy needle, transperineal biopsies may prove at least as effective for prostate cancer detection as routinely performed sextant transrectal biopsy. Sensitivity of TRUS-guided sextant transperineal biopsy might be improved by performing additional biopsies of hypo-echogenic or digitally suspect laesions, or by performing additional biopsies of the

transition zone. In long prostates, use of elongated needles with an increased stroke or advancement of the biopsy needle through the prostatic gland may improve adequate sampling of the prostatic base. Moreover, in this study, sensitivity of the transperineal procedure may be underestimated by the potential bias that all cancers were previously detected by the transrectal approach.

TABLE 4.2

A comparison between the minor and major complications after sextant transperineal (ERSPC, section Florence, Italy) and sextant transrectal biopsy (ERSPC, section Rotterdam, the Netherlands).

	Transrectal approach†	Transperineal approach‡
	Number of patients (%)	Number of patients (%)
Minor Complications		
Haematuria > 3 days	398 (23.6)	30%
Haematospermia	765 (45.6)	50%
Major Complications		
Fever >38.5° C	71 (4.2)	ND
Antibiotic therapy	52 (3.1)	3 (0.70)
Admittance to hospital	7 (0.4)	3 (0.70)
Sepsis	3 (0.18)	2 (0.46)
Total of men biopsied	1.687 (100.0)	431 (100.0)

† After: Rietbergen *et al.* Complications of transrectal ultrasound (trus) guided systematic sextant biopsies of the prostate: Evaluation of complication rates and risk factors within a population based screening program [24]

‡ Figures obtained from the Department of Diagnostic Medical Imaging, Centro per lo Studio e la Prevenzione Oncologica, Florence, Italy.

The issue, of course, is whether our reported high efficacy of transperineal biopsy for prostate cancer detection, *ex vivo*, should be further evaluated *in vivo*, i.e. in patient settings. For proper decision-making about which technique to prefer, gain of sensitivity needs to be weighted against patient tolerance and the frequency of adverse effects and

procedure-related complications. As has been suggested, transperineal biopsies, with the patient in lithotomy position, may cause considerably more discomfort (and pain), compared to routine transrectal biopsy [15]. Chart-data from our ERSPC-partner in Florence, Italy, who performs sextant transperineal biopsy on a routine basis, clarify that patient acceptance to the procedure can be achieved in the majority of patients by giving proper information about the biopsy prior to examination. Further acceptance to the procedure might be obtained by a TRUS-guided transperineal biopsy technique, with the patient in the left lateral decubitus position, in which the needle is properly positioned and guided by a puncture attachment [21]. Procedure-related pain can be adequately reduced by application of local perineal infiltrative anesthesia. Frequency of minor and major complications, severe infectious in particular, is reported similar to that in our clinic, although no antibiotic profylaxe is recommended (TABLE 4.2) [22-24]. Certainly, withholding patients from antibiotic prophylaxis will decrease health-related concerns as antibiotic hypersensitivity and microbiotic antibiotic resistance.

The optimal biopsy strategy for prostate cancer detection has yet to be defined. The present study has provided some arguments that the biopsy procedure in which the biopsy needles enter the prostate at the apex for a longitudinal direction, previously arbitrarily referred to as transperineal, may efficiently sample the prostatic peripheral zone. Since the experiment was artificial in design, caution should be kept in extrapolating these results to patient settings.

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PART III

THE PREDICTIVE VALUE OF PRECURSOR LESIONS OF PROSTATE CANCER

CHAPTER 5

PROSTATIC INTRA-EPITHELIAL NEOPLASIA AND PUTATIVE PRECURSOR LESIONS OF PROSTATE CANCER -- A CLINICAL PERSPECTIVE --

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INTRODUCTION

In recent years, prostate cancer has become an increasing health problem in North America and Western Europe, now being the most commonly diagnosed noncutaneous malignancy in men beyond middle age, and the second cause of cancer related death after lung cancer [1]. The causes of prostate cancer, the target cells of prostatic carcinogenesis, and the histological changes preceding and leading to the initiation and progression of prostate cancer have yet to be elucidated. Many research groups are trying to solve the puzzle of prostatic carcinogenesis, with their attention focused within the morphological continuum between benign glands at one end, to premalignant lesions and invasive disease at the other. Also clinicians are sometimes confronted with morphological features on the diagnostic prostatic needle biopsy that although negative for cancer raise suspicion of concomitant malignancy. These findings present a particular diagnostic challenge (TABLE 5.1).

This review highlights the current understanding and knowledge of the main putative premalignant lesions of the prostate and of lesions that raise particular suspicion of concomitant malignancy. Their association with clinical variables and incidence rates were assessed in different study groups and in ours, as were the predictive values for prostate cancer on follow-up biopsy. The consequences of finding these distinct morphological entities on the diagnostic needle biopsy are set in a wider clinical perspective.

POSSIBLE TARGET LESIONS OF PROSTATIC CARCINOGENESIS

Carcinogenesis is a complex multistep process, involving molecular, cellular, and histological changes. It describes the conversion of benign epithelial glands, through premalignant lesions, to invasive carcinoma. Several requirements should be met to consider a lesion premalignant (TABLE 5.2). An epidemiological relationship must be shown, especially when the development of a premalignant lesion to early stromal invasion and full blown malignant disease takes months or years The precursor lesion presents itself at an earlier age than its malignant equivalent, and the age-adjusted prevalence is expected to rise synchronously with that of histologically proven and/or clinically manifest malignant disease. Typically, the age-adjusted prevalence of the precursor lesion decreases at a partucular time, while that of cancer continues to increase (FIGURE 5.1). As an epidemiological association does not rule out that premalignant and malignant conditions simply coexist with each other, clear morphological (cellular, histological, architectural) similarities should be present as well. In addition, organs

harbouring invasive cancer should have a greater frequency, severity and extent of the premalignant lesion than organs that have not. In the organ, premalignant lesions should be located in close proximity with their presumed malignant equivalents, whereas sometimes micro-invasion of the stroma by the precursor lesion may be seen at the microscopical level. The definite proof of a relationship between a precursor lesion and malignancy is the clinical evidence of progression into invasive carcinoma.

Previously, several morphological lesions have been but forward that may act as potential precursor lesions of prostatic adenocarcinoma. These are the morphologically distinct entities of focal atrophy or postatrophic hyperplasia (PAH), atypical adenomatous hyperplasia (AAH) or adenosis, and prostatic intra-epithelial neoplasia (PIN). Lesions designated as 'atypical' or 'suspicious' have been associated with the presence of prostate cancer as well. As a wide diversity of morphological features is reported, a clear description in histological terms is not possible and consequencely 'suspicious for malignancy' lesions should not be looked upon as premalignant lesions of the prostate in a strict sense, but should be regarded as a separate diagnostic entity associated with concomitant prostate cancer.

FIGURE 5.1

The schematic epidemiological relationship between the age-dependent prevalence of the precursor lesion, for instance HPIN, and malignant disease (i.e. prostatic adenocarcinoma). As the true prevalence of disease is not known, the ordinate has no denominator.

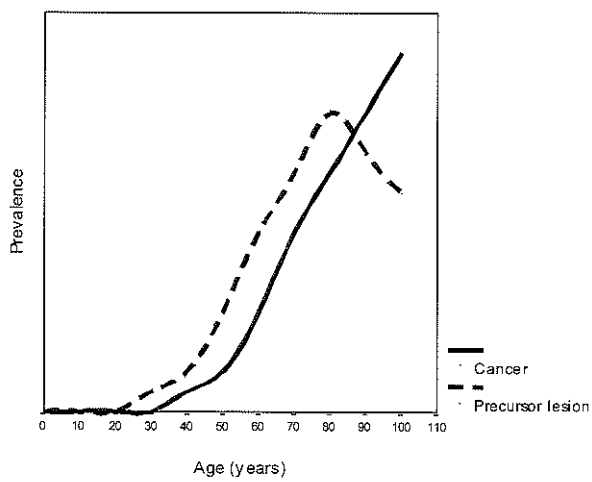
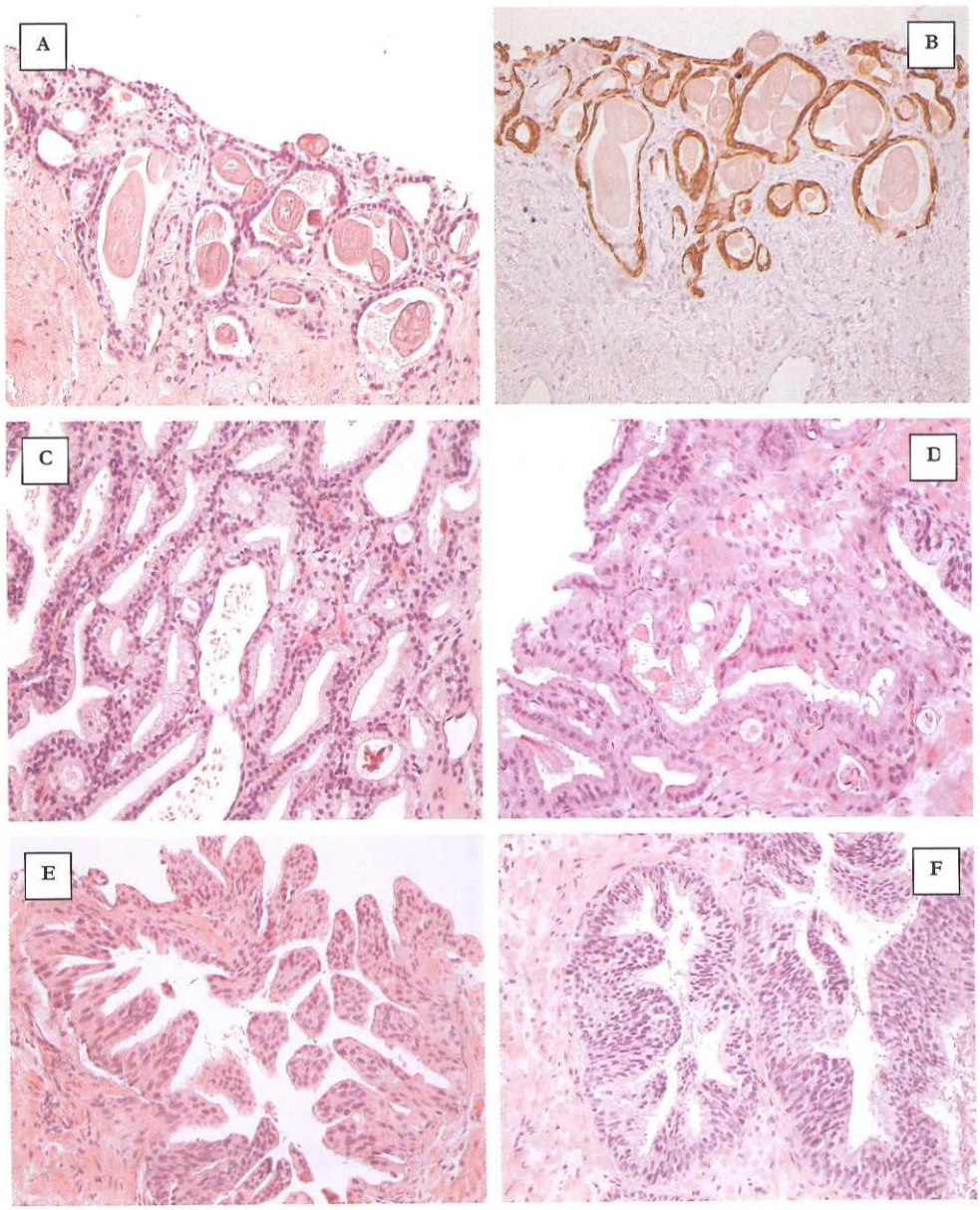


FIGURE 5.2

The histopathological features of the main putative precursor lesions of prostate cancer and of lesions that hold an increased risk for concomitant malignancy. ***A.*** Focal atrophy ***B.*** The same lesion of image A, immunostained with basal cell specific antibody 34βE12 cytokeratin. *C.* Atypical adenomatous hyperplasia (AAH) ***D.*** prostate biopsy suspicious for malignancy (PBSM). *E.* Micropapillary high-grade intra-epithelial neoplasia (HPIN) ***F.*** Tufted HPIN



Conventional histopathological examination is used to distinguish the different precursor lesions of prostatic adenocarcinoma (Figure 5.2). In addition, immunostaining with antibodies directed against basal cell specific cytokeratin (clone 34βE12) may be applied to discriminate putative premalignant lesions from benign glands and prostatic adenocarcinoma. Characteristically, benign glands show a continuous basal cell layer, while in adenocarcinomas the basal cell layer is immunhistochemically absent.

TABLE 5.1
The histopathological diagnosis rendered on the prostatic needle biopsy

1	Prostatic adenocarcinoma (PC)
2	Prostate biopsy suspicious for malignancy (PBSM)
3	High-grade prostatic intra-epithelial neoplasia (HPIN) - Micropapillary - Tufted - Flat - Intraductal (cribriform, trabecular, small-cell, comedo-carcinoma, solid)
4	'Benign' - Atypical adenomatous hyperplasia (AAH) - Focal atrophy - Postatrophic hyperplasia (PAH) - Low-grade prostatic intra-epithelial neoplasia (LPIN) - Chronic or acute prostatitis - Benign prostatic epithelial glands
5	Other malignancy - Carcinosarcoma - Squamous-cell carcinoma - Urothelial-cell carcinoma - Rectal adenocarcinoma - Metastasis from other primary
6	Other diagnosis
7	Insufficient material for histopathological diagnosis (IM)

It is plausible that the aforementioned morphologies may not account for all malignancies of the prostate, and that the human prostate gland may harbour other hitherto unrecognized premalignant lesions.

Focal atrophy, postatrophic hyperplasia (PAH), and atypical adenomatous hyperplasia (AAH)

Focal atrophy should be distinguished from diffuse atrophy, as the latter is not considered premalignant. Diffuse atrophy may be a consequence of a decrease in circulating androgens, and results in a uniform decrease in volume of pre-existing epithelial glands and prostatic stroma. Focal atrophy, including simple atrophy, sclerotic atrophy, and PAH, reportedly occur in up to 85% of prostates at autopsy and in a considerable proportion of biopsies [2,3]. A role in the genesis of PIN and/or carcinogenesis was proposed by Frank as early as 1954 [4]. The recent observation that focal atrophic lesions showed an increased proliferative activity of luminal cells and a decreased frequency of apoptosis added to this assumption [5]. The hyperplastic form of atrophy, PAH, may closely mimic the histology of prostatic adenocarcinoma and represents a diagnostic pitfall [3,6,7]. Recent studies reported that a spatial relationship between PAH and prostate cancer could not be shown, and that the frequency of PAH in radical prostatectomy specimens was remarkably similar to that in cystoprostatectomy specimens [3,7]. This implies that the simultanous finding of PAH together with prostate cancer is coincidental. Despite the observation that focal atrophic lesions and PAH consist of flattened and dispersed acini, immunostaining with 34βE12 cytokeratin is almost always positive and continuous, i.e. similar to that of benign epithelial glands [6,7]. To date, there have been few genetic and molecular analyses.

AAH can be found throughout the prostate, but is most often located in the transition zone of the prostate in intimate association with benign nodular hyperplasia [8,9]. This distinct morphological entity was formerly thought to be associated with the well-differentiated carcinomas that originate in the transition zone of the prostate [8]. Indeed, several epidemiological and histological findings have caused some to suggest that AAH may be related to prostate cancer [9]. For instance, the basal cell layer is discontinuous and fragmented on 34βE12 cytokeratin immunostaining, although recent studies noted that only few genetic alterations are present [10-12].

Prostatic intra-epithelial neoplasia (PIN)

Lesions with the morphology of PIN are regarded as the most likely precursor lesion of (peripheral zone) prostatic adenocarcinoma [12,13]. It is now widely accepted that low-grade PIN (LPIN) should be distinguished from high-grade PIN (HPIN), as the former lesion is only infrequently associated with coexistent cancer [14,15]. Because of this finding and the high interobserver variation among pathologists for the recognition of

LPIN, it is now the consensus that LPIN should no longer be reported as a separate diagnostic entity [16].

Epidemiological evidence for the hypothesis that PIN precedes its possible malignant equivalent is provided by the presence of PIN in men as early as in their 4th and 5th decade of life, whereas the incidence and extent of PIN tend to increase with age [17]. Therefore some have suggested that PIN lesions pre-date the onset of cancer by at least 5 to more than 10 years [17]. Further evidence for the suggested relationship was given by the finding that in autopsy and surgical series PIN was identified in 60 to 90% of prostates harbouring carcinoma, and PIN was often close (< 2 mm) to its presumed invasive equivalent [14,17-20]. The anatomic distribution within the gland shows that PIN is predominantly located in the prostatic peripheral zone, the area in which most clinically important prostatic adenocarcinomas (> 70%) are found [14,18]. Very similar to prostate cancer, PIN is often multifocal [18,21]. In addition, multiple phenotypical and genotypical studies indicate that there are remarkable morphological, molecular, and biochemical similarities between PIN and prostatic adenocarcinoma [13,21-24]. The observed molecular abnormalities in PIN are mostly intermediate between benign prostatic epithelium and prostate cancer, reflecting an impairment of cell-differentiation and regulatory control (TABLE 5.2).

The morphological entity referred to as HPIN consists of architecturally benign prostatic acini and ducts, lined by cytological atypical cells. Unlike prostate cancer, an incomplete disruption of the basal cell layer can be shown by 34βE12 cytokeratin immunostaining. The cytological changes are characterised by prominent nucleoli in a substantial proportion (≥ 5%) of cells, nuclear enlargement, nuclear crowding, an increased density of the cytoplasm, and anisonucleosis [13]. HPIN lesions can be subdivided into at least four different architectural patterns, based on the arrangement of the cells within pre-existing ducts or glands, i.e. tufted, micropapillary, flat and intraductal HPIN (TABLE 5.1). Tufted and micropapillary HPIN are most common, whereas flat and intraductal variants are less frequent [25]. At present, this distinction of different architectural patterns appears to be of diagnostic utility only, for no substantial differences have been detected in the development of prostate cancer and overall prognosis. Some argued that those HPIN lesions that span the glandular lumen may not be a premalignant lesion of the prostate, but may represent intraductal spread of concurrent carcinoma [26,27]. It was recently reported that these lesions might indeed have their own clinical and prognostic significance [26,28,29]. Intraductal HPIN in prostates with established carcinoma was associated with high tumour volumes, the presence of poorly differentiated tumour components, and a higher progression rate after radical prostatectomy than prostate cancers without these coexisting proliferations.

Therefore, a separate histological entity was proposed that should be distinguished from HPIN, i.e. intraductal carcinoma of the prostate [27,28].

Despite the remarkable morphological and genetic similarities between HPIN and invasive prostatic adenocarcinoma, it is not yet clear which proportion of HPIN remains stable, regresses or progresses to invasive cancer or simply coexists with its presumed malignant equivalent.

TABLE 5.2

Evidence of the precursor lesion relationship of high-grade prostatic intra-epithelial neoplasia (HPIN) to invasive cancer

Relationship	Evidence
Epidemiological	- HPIN occurs in the 4th and 5th decade of life - The incidence and extent of HPIN increase with age
Morphological	HPIN has similar cytological and histological features to invasive carcinoma - Prominent nucleoli - Nuclear enlargement - Nuclear crowding - Increased cytoplasmatic staining - Anisonucleosis - Fragmented and disrupted basal cell layer
Zonal and Spatial	- HPIN is most often located in the prostatic peripheral zone - HPIN is most often multifocal - HPIN is more frequent in prostates containing invasive carcinoma than in those that do not - HPIN is found in close proximity to invasive carcinoma
Genetical	HPIN has similarities to invasive carcinoma - Downregulation of markers of secretory differentiation (i.e. PSA, neuroendocrine cells, cytoskeletal proteins, cell-adhesion proteins) - Increased microvessel density - Increased markers of proliferation and apoptosis suppression - Altered expression of growth factors (receptors) - Loss of heterozygosity and chromosomal gains - Hypermethylation of DNA
Clinical	HPIN is associated with increased yield of cancer on repeated biopsy (?)

Prostate biopsy suspicious for malignancy (PBSM)

As a consequence of programmes for the early detection of prostate cancer, the number of biopsies taken, and the number of biopsy specimens evaluated have increased substantially. In more than 95% of cases the diagnosis of these biopsies will be equivocal 'benign' or 'prostatic adenocarcinoma'. However, as a result of the limited quantity of tissue that is sampled, the probability of finding a lesion that raises particular diagnostic confusion has increased. Besides lesions that mimic the histology of prostatic adenocarcinoma (such as PAH, AAH, or HPIN), architectural anomalies may be present that lack sufficient cytological or histologic criteria to convince the pathologist that the lesion represents an overt carcinoma. In other words, the constellation of cytological and histological changes of these abnormalities fall below the diagnostic threshold of carcinoma. Mostly, these 'suspicious for, but not conclusive for malignancy' lesions are small and have a wide diversity of architectural and morphological features. As the histology of these 'suspicious for malignancy' lesions is so distinct, pathologists should refrain from terms that imply a confined morphological entity, such as 'atypical small acinar proliferations (ASAP)' [30-33]. As, by definition, these lesions are only present within the needle biopsy, we recently proposed the terminology 'prostate biopsy suspicious for malignancy' (PBSM) to classify these lesions (TABLE 5.1) [34].

There may be interobserver variability among pathologists in the classification of these lesions, and it is likely that pathologists who are not 'experts' may inappropriately designate some cases as focal atrophy, PAH, AAH, HPIN or even prostatic adenocarcinoma. The lesion in PBSM generally fails to stain with the basal cell specific cytokeratin (34βE12) antibody, creating greater confusion with other lesions such as AAH or PIN. Biopsy samples may be obtained from immunohistochemically negative areas within lesions that are known to have a discontinous basal cell layer; a small focus of negative immunostaining may thus produce false-negative results. Results of immunohistochemical tests are only used to support the histopathological diagnosis of prostatic adenocarcinoma, given the presence of cytological and architectural features diagnostic of carcinoma [30]. Pathologists are becoming increasingly aware that false-positive results may strongly influence a man's quality of life through unnecessary psychological stress, unnecessary treatment and treatment associated morbidities. For medicolegal reasons, it is obvious that false-positive biopsy results are to be avoided. On the other hand, reporting of the histopathology should be as unequivocal and concise as possible and vague diagnoses should not lead to unnecessary biopsy with its associated morbidities [35]. Urologists should be aware that PBSM is not a clear morphological entity or a premalignant lesion of the prostate in a strict sense, but rather a diagnostic entity raised by pathologists who are uncomfortable in establishing a definite malignant diagnosis. As was stated earlier, adopting a term such as 'ASAP' will possibly lead to an

underestimation of the risk of coexistent malignancy by Urologists and with this, a potential for a delay in diagnosis [36]. Indeed, it is likely that PBSM (or ASAP) often represents a marginally sampled, tangentially sectioned, or outpouching prostatic adenocarcinoma [32].

THE ASSOCIATION WITH CLINICAL PARAMETERS

As PIN is frequently associated with a disruption and fragmentation of the basal cell layer, it was previously assumed that proteins like PSA could easily gain access to the systemic circulation. Men with PIN lesions were expected to have serum-PSA levels between those of benign epithelial glands and carcinoma. Nowadays, it is the opinion that PIN lesions do not contribute to an elevation of serum-PSA, PSA-density, or to a decrease in free-to-total PSA ratio [14,37,38]. This view is substantiated by the observation that PIN lesions show less expression of PSA in luminal cells, as determined by immunohistochemistry, than do benign epithelial glands [39]. An elevation of PSA should be attributed to the presence of associated prostate cancer, gland volume, and/or concurrent prostatic inflammations rather than the presence of PIN.

The incidence of HPIN does not differ substantially between men with or without abnormalities on DRE or TRUS. Moreover, most studies do not report a predictive value of current screening tools for prostate cancer on follow-up biopsy for cases diagnosed with isolated HPIN [14,15,40-44]. As there is a considerable overlap for cases with benign, premalignant, and malignant diagnoses for age, this clinical variable is not a valuable discriminative factor [14,43,44]. Also for cases that were initially diagnosed with PBSM (or ASAP), patient's age, PSA levels, and findings on DRE and TRUS could not assist in predicting those patients who were later diagnosed with prostate cancer [30-32,45].

INCIDENCE AND YIELD ON REPEATED BIOPSY

A remarkable variation in the incidence of HPIN and 'suspicious for malignancy' lesions has been reported in different institutions. Published data reporting on HPIN in the absence of identifiable carcinoma have shown incidence rates of 0.15 to 16.5% of needle biopsies [14,30, 35,42,43,46-48], and this figure was 1.5 to 6.3% of biopsies in case of a 'suspicious for cancer' diagnosis or 'ASAP' [30,33,35,43-48]. If the criteria for establishing a diagnosis are better defined, and more cases are included, the two diagnoses are rendered less frequently [35]. In studies reporting on series of over a

thousand biopsies, the reported incidence of HPIN were lower at 0.15 to 3.7% of biopsies [14,30,33,35,46,47], while that of a 'suspicious' lesion varied between 2.5 and 4.8% of biopsies [30,45,47]. Apparently, referral and consultation bias may have occurred in some of the smaller series.

The variations from one institution to another can be explained by the method of patient selection in these hospitals, the recommendations for biopsy, and the biopsy compliance rates. Also the side of the prostate gland that is biopsied, the number of biopsies taken, and the quality and processing technique of the biopsy cores greatly influence the incidence rates of prostate cancer, and those of lesions with an assumed increased risk of concomitant prostate cancer. The experience and ease of establishing a diagnosis by the pathologist will determine the reported rates as well, especially for the threshold of making the diagnosis of PBSM.

The frequency of cancer on repeated biopsy after an initial diagnosis of isolated HPIN was reported to vary between 22 and 100% of repeated biopsies [14,15,42,46-50]. This figure was between 29 and 58% of repeated biopsies after an initial diagnosis designated as 'atypical' , 'ASAP' , or 'suspicious for malignancy' [30-33,44,45-47,49]. The confounding factors mentioned earlier also influence the predictive value for prostate cancer on follow-up biopsy. Furthermore, the yield for prostate cancer on repeated biopsy will depend on the effectiveness of the initial biopsy procedure to detect prostate cancer. For instance, when the initial biopsy procedure fails to detect some of the prevalent cases of prostate cancer, the yield for prostate cancer on repeated biopsy will be increased. Also, the more reluctant a pathologist is to render a diagnosis of malignancy on initial biopsy, the more frequent the cancer will appear in subsequent biopsies. Finally, when more extensive repeated biopsy is undertaken in men with an assumed increased risk of prostate cancer, the likelihood of detecting prostate cancer on follow-up biopsy is increased, and vice versa.

When follow-up biopsies are taken in men with foci of isolated HPIN, the site of prostate cancer may not always be similar to the site that raised the suspicion of concurrent carcinoma [33,41,42,50]. The detection of prostate cancer may then be considered coincidental. In contrast, the re-biopsy strategy after an initial diagnosis of PBSM mostly concentrates on the site of the prostate where the initial 'suspicious for cancer' lesion was found, and therefore, the frequency of (coincidental) cancer detection has not been evaluated.

CLINICAL MANAGEMENT

Clinical management of men with lesions that raise suspicion of coexisting malignancy depends on the risk of concurrent prostate cancer, on the way in which the lesions were initially diagnosed, and on patient factors. It is clear that men in whom the finding of a 'non-cancerous' lesion is associated with a negligible risk of prostate cancer, or with a risk that is not substantially higher than that of men with no such lesion, should be saved from further diagnostic follow-up and/or therapeutical interventions. A proportion of prevalent (and clinically significant) prostate cancers will remain undetected on routine transrectal sextant biopsy. As a result, cancers will be diagnosed on repeated biopsy irrespective of the outcome of the initial biopsy, even if the initial biopsy was designated as 'benign epithelial glands'. Accordingly, we designated morphological lesions with a risk of prostate cancer that was not substantially different from that of benign epithelial glands as 'benign' (TABLE 5.1).

Since unnecessary diagnostic procedures and unnecessary therapeutical interventions are to be avoided, it is likely to assume that only men with a risk of prostate cancer which is substantially higher than baseline should be offered diagnostic follow-up. However, the decision to take a repeated biopsy should also be related to the expected benefits of diagnosing prostate cancer overall, i.e. the detection (and treatment) of prostate cancer should be beneficial to those subjected to diagnostic procedures, especially within early detection programmes. Large RCTs currently being undertaken investigate the impact of systematic screening for prostate cancer followed by early treatment on cancer-specific mortality and quality of life. Until these early detection programmes for prostate cancer prove beneficial, the need for and the gain of undertaking diagnostic follow-up in men with screen-detected premalignant lesions of the prostate remains a controversial issue. Furthermore, of the currently available curative treatment options for clinically localised prostate cancer, none has definitely proven to reduce prostate cancer mortality. So, for now, both repeated biopsy and curative treatment may at best be considered as controversial clinical interventions.

Clinical decisions should be restricted to the morphological components that are expected to be associated with the worst prognosis. This implies that when premalignant lesions are diagnosed together with prostatic adenocarcinoma, therapeutical decisions are determined by the invasive component only, and should be taken irrespective of the presence, severity and extent of precursor lesions. For instance, prostate cancer in the presence of HPIN should be treated no differently (i.e. no more aggressively) than prostate cancers that are not accompanied by these preneoplastic proliferations. This may be further strengthened by the observation that the volume of HPIN was reported to be

inversely correlated with pathological tumour stage, overall tumour volume and volume of high-grade cancer [15]. The presence of HPIN on the needle biopsy (in the absence of cancer) might therefore be interpreted as a favourable prognostic indicator. On the other hand, as some HPIN lesions were associated with small, high-grade cancers, the occurence of HPIN on diagnostic biopsies may selectively identify those malignancies that are particularly prone to cause prostate cancer mortality [18,20].

The decision to undertake a diagnostic follow-up in men with putative premalignant lesions of the prostate should also relate to patient's age, general physical condition and co-morbidities. Obviously, men who might not potentially benefit from curative treatment or early hormonal therapy should not be subjected to follow-up biopsy.

TABLE 5.3
The clinical implications of different diagnosis rendered on the prostatic needle biopsy

Diagnosis	Clinical Implications
Focal atrophy	No
PAH	No
AAH	No
LPIN	No
HPIN	Controversial
PBSM	Yes

PAH post-atrophic hyperplasia
AAH atypical adenomatous hyperplasia
LPIN low-grade prostatic intra-epithelial neoplasia
HPIN high-grade prostatic intra-epithelial neoplasia
PBSM prostate biopsy suspicious for malignancy

Diagnostic intervention

Data from others and those of ERSPC showed that focal atrophy (including PAH) is present in a substantial proportion of needle biopsies (up to 90%) [3], whereas AAH is a relatively rare event (< 1%) [52]. Moreover, atrophic lesions are encountered in most radical prostatectomy specimens, whereas AAH is not an unusual finding in TURP material. For this high reported prevalence of focal atrophy and PAH, the presence of these putative precursor lesions on the needle biopsy is not a valuable discriminator for

the coexistence of malignant conditions within the prostate. Any future confirmed relationship of AAH with low-grade transition zone cancer is not expected to have clinical consequences either. Besides a minor effect on patient prognosis as a consequence of detecting these cancers, diagnostic and/or follow-up biopsies would have to be re-directed at the transition zone of the prostate, the area in which these tumors mostly reside. Currently the consensus is that the finding of focal atrophy, PAH, AAH, or LPIN on the needle biopsy or in TURP material for BPH should not lead to any diagnostic follow-up (TABLE 5.3). The clinical management of men with these 'benign' conditions should be determined from variables other than morphological features.

HPIN is a generally accepted premalignant lesion of the prostate (TABLE 5.2). The need for and the extensiveness of diagnostic follow-up first depend on the way this premalignant lesion was diagnosed. Obviously, the isolated finding of HPIN in the cystoprostatectomy specimen holds no clinical implications since the target organ was removed together with the urinary bladder, and clinical management decisions and the prognosis of the patient are determined by the initial indication for surgery (i.e. urothelial cell carcinoma of the bladder). The presence of HPIN in TURP material for BPH is uncommon and has a low predictive value for cancer [53,54]. In our opinion, the finding of HPIN in these men needs no further action.

From a RCT, we reported that the additional predictive value for prostate cancer of an isolated finding of HPIN on initial biopsy is limited, and that the high predictive values for cancer reported earlier may be prejudiced by referral and consultation bias, and by variable biopsy techniques [34]. In our opinion, asymptomatic men within screening settings who are eventually diagnosed with isolated HPIN do not need to be subjected to an *early* diagnostic follow-up. Men with these lesions should be followed at regular timing, including PSA testing, assessment of clinical symptoms, and if clinical suspicion persists, repeated sextant biopsy. The precise interval of these assessments remains as yet unclear. The impact of a finding of intraductal HPIN on initial biopsy needs further investigation. Despite the observation that only a minority of HPIN lesions were classified as 'intraductal' in ERSPC, and that no unequivocal cancers were detected on repeated biopsy, it cannot be excluded that this putative precursor lesion represents an intraductal spread of carcinoma. Particularly because of the earlier finding that this lesion is possibly related to potentially aggressive cancer and a poor prognosis, we recommend follow-up biopsy in all cases that are initially diagnosed with intraductal HPIN [26-29].

The appropriate management of patients with bladder outlet obstructive symptoms, an increased serum-PSA level, a decrease in free-to-total PSA ratio, a large prostate, or with clear abnormalities on DRE or TRUS, with or without isolated (intraductal) HPIN, remains unclear. At present, repeated biopsy in these cases is recommended since the overall risk of being diagnosed with prostate cancer regardless of finding of HPIN is increased.

By definition, 'suspicious for cancer' lesions are diagnosed within biopsy cores only, and will not be a separate finding in surgically obtained material. In a recent study [34] we noted that this diagnostic entity was associated with a considerable risk of prostate cancer. We therefore advocate an early diagnostic follow-up and repeated biopsy in all men diagnosed with PBSM on initial biopsy (TABLE 5.3).

Therapeutical intervention

Despite the finding that isolated HPIN may or may not be associated with an increased risk of prostate cancer on repeated biopsy, it is the consensus that the (repeated) finding of HPIN (and no cancer) on the biopsy will have no therapeutic implications. The same is true for an initial diagnosis of PBSM, i.e. radical prostatectomy and radiotherapy should only be used in men with histopathologically confirmed prostatic adenocarcinoma.

Chemoprevention

Chemoprevention implies the administration of drugs or other agents aimed at preventing the initiation of prostate cancer (primary prevention), or the inhibition of progression of prostate cancer to clinically manifest disease and advanced disease (secondary and tertiary prevention, respectively). Recently, it was suggested that anti-androgen therapy may be offered to men with HPIN (and no cancer) on the biopsy for this might halt or reverse the process of carcinogenesis, and prevents the transition of PIN to overt prostate cancer [55]. As HPIN precedes the development of prostate cancer by 5 to 10 years, and is easily identifiable, some considered the application of anti-androgen therapy a unique opportunity to decrease the incidence of prostate cancer and its morbidity and mortality [55]. Androgens are required for the normal development, differentiation, and functioning of the human prostate gland. Similar to benign prostatic glands, PIN is androgen dependent and after hormonal deprivation therapy, the prevalence and extent of HPIN decrease [56]. This observation has been attributed to an actual volume decrease of PIN glands, as well as to a diminished ability of the pathologist to identify PIN [56]. On a molecular level, anti-androgen therapy induces the regression of epithelium by the enhancing apoptosis, suppressing proliferative activity, and inhibiting angiogenesis in benign prostatic epithelium, PIN and prostatic adenocarcinoma [57,58]. Importantly, the observed morphological changes caused by anti-androgen therapy are reversible and HPIN lesions seem to recover rapidly and will even further expand after the cessation of androgen deprivation therapy [58]. To have a possible protective effect against prostate cancer, chemopreventive agents should be administered for life. Moreover, it was reported that androgen-receptor gene-amplication occurs in cases that were eventually diagnosed with prostate cancer during the treatment of BPH with finasteride. This finding is worrisome as the amplification of the androgen-receptor gene in androgen-deficient conditions had been observed to occur exclusively in

hormone-refractory metastasised prostate cancer [59-61]. Cote and associates showed that men with no evidence of carcinoma on initial biopsy who were pretreated with finasteride had a significantly greater prostate cancer detection rate at 1 year than had men in the observation-only group (30 versus 4%)[62].

Besides anti-androgens, other drugs (e.g. anti-angiogenic) and nutritional supplements (e.g. vitamin D, selenium) may be applied in chemoprevention trials [63]. It is likely that global differences in prostate cancer incidence may be attributed to differences in dietary habits and that changes in nutrition might lower the incidence rates of prostate cancer. Several nutritional supplements have been shown to have an anti-tumorigenic effect in animals, although there is little human-based evidence.

In our opinion, the natural biological behaviour of HPIN is poorly understood, whereas its clinical impact is limited, especially within early detection programmes. Moreover, beneficial effects of 'promising' chemoprevention agents in reducing prostate cancer incidence have not been confirmed in well-conducted RCTs. It has even been reported that some chemopreventive agents may have serious and harmful side-effects, that HPIN recurs quickly and is more pronounced after the cessation of therapy, and that some agents might even enhance the outgrowth of unfavourably prognostic cancers. Until certain proof of progression to invasive prostatic carcinoma is established, and until population-based screening for prostate cancer proves beneficial, there should be reluctance to offer chemopreventive agents to men with isolated HPIN on the diagnostic biopsy.

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CHAPTER 6

THE PREDICTIVE VALUE FOR PROSTATE CANCER OF LESIONS THAT RAISE SUSPICION OF CONCOMITANT CARCINOMA

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SUMMARY

BACKGROUND. Suspicion of prostate cancer may persist after an initial negative biopsy result and repeated biopsy is suggested. We assessed whether diagnostic follow-up of men with an initial diagnosis of isolated high-grade prostatic intraepithelial neoplasia (HPIN) and a prostate biopsy suspicious for malignancy (PBSM) is needed.

METHODS. The frequency of isolated HPIN and PBSM was determined in 4,057 participants of a population-based screening study, who underwent systematic sextant transrectal biopsy. The predictive value for prostate cancer of HPIN and PBSM was determined by performing repeated biopsy at 6-weeks interval. The additional predictive value for cancer within a screened population was assessed by performing repeated biopsy at 1-year interval in consecutively recruited men with an initial benign biopsy result. Participants were subjected to 2nd screen at 4-year interval. The biopsy and radical prostatectomy tumor features were determined.

RESULTS. Isolated HPIN and PBSM were diagnosed in 0.8% and 2.6% of biopsied men, respectively. Cancer detection rates on repeated biopsy were 10.0% (3/30) for isolated HPIN, 38.7% (36/93) for PBSM, and 11.0% (51/462) for men with initial benign biopsy results. Except for two cases (one PBSM and one HPIN), all others remained free of prostate cancer on 2nd screen. Tumor features of cancers detected after PBSM were comparable to those detected on initial biopsy, whereas the few cancers diagnosed after HPIN had highly favorable tumor features.

CONCLUSIONS. In contrast to men with PBSM, men with isolated HPIN on initial biopsy are at no greater risk of being diagnosed with prostate cancer than if their initial biopsies were assessed as benign only. Moreover, tumor features of cancers diagnosed after an evaluation of HPIN warrant no early, extensive diagnostic follow-up.

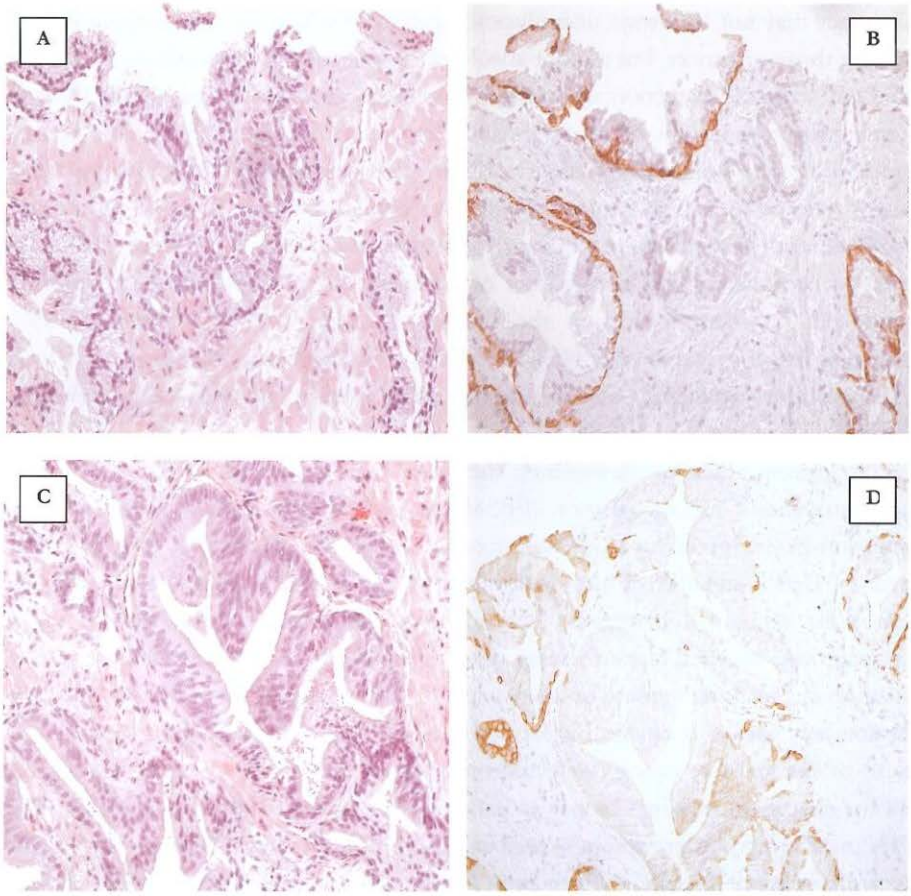
INTRODUCTION

Serum prostate-specific antigen (PSA) testing, and to a lesser extent, digital rectal examination (DRE) and transrectal ultrasound (TRUS) are used in early detection programs for their ability to indicate the presence of prostate cancer. However, the histopathological examination of the prostate biopsy specimen remains the only tool to establish a definitive diagnosis of prostate cancer. Due to the limited quantity of tissue that is sampled and/or insufficient cytological or architectural atypia, the diagnosis of malignancy may not be always unequivocal. Lesions of which the morphological features resemble those of cancer, but are not absolutely diagnostic for cancer, have recently been associated with the detection of prostate cancer on repeated biopsy [1-3]. Otherwise, putative precursor lesions of prostate cancer represent a different diagnostic entity and these lesions may closely mimic their early invasive counterparts. The histopathological changes referred to as high-grade prostatic intra-epithelial neoplasia (HPIN) are generally considered the most likely precursor of invasive carcinoma [4,5]. Since HPIN has a presumed predictive value as a marker of concomitant prostatic adenocarcinoma, it is argued that its identification in the biopsy specimen warrants further search for concurrent invasive carcinoma [4,5]. Recently, it is suggested that men with isolated HPIN may be candidates for chemoprevention therapy for this may decrease the incidence of prostate cancer [6,7].

In the current study we determined the frequency of isolated HPIN and a prostate biopsy suspicious for malignancy (PBSM) in participants from a large randomized population-based screening study for prostate cancer. The predictive value for prostate cancer of HPIN and PBSM was determined by performing repeated biopsy within six weeks after the initial diagnosis. The additional predictive value within a screened population was assessed by performing repeated biopsy at 1-year interval in an unselected consecutively recruited group of men with an initial benign biopsy result [8]. Special attention was focussed on the biopsy and radical prostatectomy tumor features (grade, extent) of the prostate cancers detected on repeated biopsy. By assessing the diagnostic yield for cancer on repeated biopsy in patients with an initial diagnosis of either PBSM, HPIN, or a benign biopsy result, as well as by studying the tumor features of the cancers detected, particular insight is given into the need for and the frequency of diagnostic follow-up of these two diagnostic entities.

FIGURE 6.1

A. Prostate biopsy suspicious for malignancy (PBSM). *B*. 34ßE12 cytokeratin immunostaining of this same suspicious for cancer lesion. The immunohistochemical expression is mostly absent, while it is present in the surrounding benign glands in the basal cell layer. This particular patient had prostatic adenocarcinoma on repeated biopsy *C*. Tufted architectural pattern of isolated high-grade prostatic intra-epithelial neoplasia (HPIN) on the initial biopsy *D*. Scattered 34ßE12 cytokeratin immunostaining expression within this same HPIN lesion at the basal cell layer. This patient did not have prostatic adenocarcinoma on repeated biopsy.



PATIENTS AND METHODS

The Screening Regimen, Participants and Cancers Detected

Between June 1st 1994 and March 31st 2000, 41,919 men, aged 55 - 74 years, were randomized to the screening and control arm of the Rotterdam section of the European randomized study of screening for prostate cancer (ERSPC). The ERSPC investigates the impact of systematic population-based screening for prostate cancer on cancer specific mortality and quality of life. ERSPC is closely associated with the Prostate, Lung, Colon, and Ovary (PLCO) screening project of the National Cancer Institute, and a combined analysis is planned. The Rotterdam protocol provides for re-screening after four years, but since participants continue to be enrolled into this second screening round (2nd screen), this report will concentrate on the first screening round (prevalence screen) notably. The conditions and algorithm of the screening regimen are described in detail elsewhere [9-11]. According to the screening protocol, participants in the screening arm with a PSA equal to or above 4.0 ng/mL (Hybritech Tandem E Assay; Hybritech Inc., San Diego, CA) and/or a suspicious DRE or TRUS finding at low PSA-values (0.0 – 3.9 ng/mL) were to undergo prostate needle biopsy. In all cases in which a biopsy was prompted, sextant transrectal biopsy was performed using a Bard (C.R. Bard, Convington, GA) spring-loaded biopsy gun and an 18-gauge biopsy needle. Ultrasound-guidance was performed using a 7 MHz end-fire ultrasound probe. Additional biopsies were directed to palpable and/or ultrasound detectable (hypo-echogenic) lesions when present. In February 1997, a major change of protocol was implemented in ERSPC, when the European study group decided to exclusively take a biopsy from men with a PSA of 3.0 ng/mL or more, without performing a DRE or TRUS as screening tests at all. The Rotterdam screening protocol calls for repeated biopsy within six weeks in men in whom the initial biopsy specimen was inconclusive for malignancy (i.e. PBSM) and/or showed isolated foci of HPIN. In participants with PBSM, four new biopsies were obtained from the area of suspicion, while in participants with isolated HPIN repeated systematic sextant biopsy was performed. No repeated biopsy was prompted in participants with an initial histopathological diagnosis of isolated atypical adenomatous hyperplasia (AAH) or adenosis, low-grade prostatic intra-epithelial neoplasia (LPIN), or atrophy. Participants that were eventually diagnosed with prostate cancer were sent back to their General Practitioner to be referred for treatment to the University Hospital Rotterdam or to one of the regional hospitals. The choice of treatment (i.e. radical prostatectomy, radiotherapy, androgen deprivation therapy, deferred treatment) was determined on basis of patient's age, his comorbidities and his preferences, as well as on the preferences of his treating Urologist.

Between June 1st 1995 and July 31st 1999, an unselected consecutively recruited group of men in whom the initial (or repeated biopsy results at 6-weeks interval) were negative for prostate cancer were offered repeated screening one year after the initial screening application. The screening team was blinded to the results of the initial screening tests. The screening algorithm and management of patients were similar to those on initial screen. The inclusion criteria of participants on 1-year repeated screening, the clinical and pathological parameters responsible for not diagnosing cancers on initial screening have been outlined in detail in a previous report from our department [8].

Histopathological Processing, Examination and Diagnosis

All sextant biopsy cores were labeled and processed separately. The biopsy cores were routinely fixed in 10% buffered formalin at pH = 7.5, embedded in paraffin, freshly cut into 4 μm thick tissue sections and mounted on glass slides. Haematoxylin & eosin (H&E) slides of three subsequent levels of the needle biopsy were histologically examined by one of the regular pathologists of the University Hospital Rotterdam. A specialized genito-urinary pathologist (ThvdK) was consulted on cases of doubt or when putative precursor lesions and/or otherwise suspect lesions were observed. The Uro-pathological 'reference' pathologist reviewed all cases with cancer and the number of cores with cancer (1-6), a biopsy Gleason score [12] and a three-tiered biopsy MD Anderson score [13] were determined for each case.

The histopathological diagnoses on the biopsy sextant were categorized according to TABLE 6.1 of the previous chapter. A PBSM was characterized by the presence of an architectural anomaly in a prostatic needle biopsy that lacks sufficient cytological or histologic criteria to convince the pathologist that the lesion represents an overt carcinoma (FIGURE 6.1). In general, immunostaining for basal cell specific cytokeratin, if performed, is negative in these lesions. A PBSM should be distinguished from HPIN since in the latter lesion, the normal glandular architecture is maintained (FIGURE 6.1). Cases assessed as HPIN were further classified into four architectural patterns, i.e. tufted, micropapillary, flat and intraductal [14,15]. The latter variant included cribriform, trabecular, small-cell, solid and comedo-carcinoma like morphologies. When more than one histological entity was observed in the biopsy sextant, the diagnostic classification concerned the prognostically worst entity only. Foci of prostate cancer and HPIN together on the biopsy sextant were classified as prostatic adenocarcinoma, while for instance HPIN and LPIN together on the biopsy were categorized as isolated HPIN. Since the true predictive value of PBSM and HPIN for the presence of cancer was unknown, the presence of both PBSM and HPIN on the diagnostic biopsy sextant was assessed as a separate diagnostic category, i.e. (*4*).

Radical prostatectomy specimens were fixed, totally embedded and processed according to well-established protocols [16,17]. For each cancer, a Gleason score was determined, and the tumor was staged by a single pathologist (ThvdK) according to the TNM '97 classification. Morphometric analysis was performed to determine overall tumor volume as described in detail by Hoedemaeker *et al* [18]. Using the criteria of Epstein *et al.* [19], Ohori *et al.* [20], and Vis *et al.* [21], small (< 0.5 mL) organ-confined tumors without Gleason growth patterns 4 or 5 were classified as minimal disease (i.e. possibly harmless), while cancers with tumor features other than those of minimal disease were assumed clinically relevant.

Statistical Analysis

Statistical analysis was performed using the statistical package for the social sciences (SPSS 9.0; SPSS Incorporated, Chicago, IL). The chi-square (χ^2) test was used to assess differences for ordinal variables (e.g. Gleason score) and the Mann Whitney U (MWU) test was used to assess differences for continuous variables (e.g. the serum-PSA level). The assumption that no difference existed for the variable evaluated (H0) was rejected (H1) if $p < 0.05$.

RESULTS

Initial and Repeated Biopsy at 6-weeks Interval on Prevalence Screen

Until March 31st 2000, 20,979 men were randomized to the screening arm of ERSPC and 19,475 (92.8%) behaved in compliance with the screening tests. Of these, 4,057 (20.8%) eventually underwent sextant transrectal biopsy, and 959 (23.6%) participants were diagnosed with prostate cancer in the first biopsy session. PBSM and isolated HPIN were diagnosed in 106 (2.6%) and 33 (0.8%) biopsied men, respectively (TABLE 6.1). Of 33 cases with isolated HPIN, 27 were tufted and/or micropapillary, 2 were flat, 2 showed intraductal components, while 2 sets of biopsies could not be retrieved. Despite the fact that the screening protocol called for repeated biopsy after 6 weeks, 12 (11.3%) and 3 (9.1%) men with PBSM and isolated HPIN on initial biopsy, respectively, refused diagnostic follow-up or have not yet undergone repeated biopsy. In one case (0.9%) with PBSM, the repeated biopsy cores were inadequate for histopathological diagnosis. For participants that underwent repeated biopsy for PBSM and in whom a histopathological diagnosis was available, 38.7% (36/93) were diagnosed with prostate cancer (TABLE 6.1). For those who underwent repeated biopsy for isolated HPIN and had their biopsies

histopathologically evaluated, 10.0% (3/30) had prostate cancer. All three cases had tufted and/or micropapillary components on initial biopsy. In the two men with PBSM and isolated HPIN together on the initial biopsy sextant, the repeated sextant biopsy showed benign prostatic epithelial glands only.

TABLE 6.1

The absolute number and the relative proportion of men that underwent repeated biopsy within six weeks after the initial biopsy for the different diagnostic entities. The histopathological diagnosis after repeated biopsy (Prevalence screen)

			Histopathological diagnosis after repeated biopsy (6 weeks)				
Diagnosis after initial biopsy	N (% of total)	Repeated Biopsy N (%)	PC N (%)	PBSM N (%)	HPIN N (%)	HPIN/ PBSM N (%)	Benign N (%)
PC	959 (23.6)	*	*	*	*	*	*
PBSM	106 (2.6)	93 (87.7)	36 38.7)	3 (3.2)	0 (0.0)	1 (1.1)	53 (57.0)
HPIN	33 (0.8)	30 (90.1)	3 (10.0)	2 (6.7)	4 (13.3)	0 (0.0)	21 (70.0)
Both	2 (0.1)	2 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100.0)
Benign	2,950 (72.7)	18 (0.1)	3 (16.7)	0 (0.0)	1 (5.6)	0 (0.0)	14 (77.8)
Other	7 (0.2)	*	*	*	*	*	*
Total	4,057	143 (3.5)	42 (29.4)	5 (3.5)	5 (3.5)	1 (0.7)	90 (62.9)

PC prostate cancer
PBSM prostate biopsy suspicious for malignancy
* no repeated biopsy
HPIN high-grade prostatic intra-epithelial neoplasia

For 106 cases with PBSM, the median (mean ± SD) PSA-level was 4.4 ng/mL (6.3 ng/mL ± 7.3), and the median time to repeated biopsy was 3.9 weeks (range, 2 - 19). For the 36 men with PBSM on initial biopsy and prostate cancer on repeated biopsy, the

median PSA (mean ± SD) was 5.5 ng/mL (8.3 ng/mL ± 11.0). For the 33 cases with isolated HPIN, the median (mean ± SD) PSA was 3.7 ng/mL (5.0 ng/mL ± 3.4) and the median time to repeated biopsy was 3.4 weeks (range 1 - 35). The median PSA (mean ± SD) for the three cases with isolated HPIN and prostate cancer on repeated biopsy was 3.2 ng/mL (3.5 ng/mL ± 0.6). No statistically significant difference was found for PSA-level between men with an initial diagnosis of PBSM and those with an initial diagnosis of isolated HPIN (MWU; $p = 0.18$). Also the proportion of men in different PSA-ranges was not different between those who had an initial diagnosis of PBSM and those who had an initial diagnosis of isolated HPIN, i.e. 18.9% (20/106) and 18.2% (6/33) for low PSA-ranges (0.0 – 2.9 ng/mL) and 11.3% (12/106) and 12.1% (4/33) for high PSA-ranges (≥ 10.0 ng/mL), respectively.

The biopsy tumor characteristics of the cancers detected in the first biopsy session and of those on repeated biopsy are listed in TABLE 6.2. The biopsy Gleason score for cancers diagnosed on repeated biopsy after PBSM was not statistically different from that of prostate cancers diagnosed on the first biopsy sextant (χ^2; $p = 0.15$). A trend was observed for more favorable MD Anderson scores in cases diagnosed after PBSM and repeated biopsy compared to those diagnosed with prostate cancer on initial biopsy (χ^2; $p = 0.08$). The number of cores with cancer was significantly lower (χ^2; $p < 0.01$). Due to small numbers ($n = 3$), no statistical comparison was made between the biopsy tumor characteristics of cancers diagnosed on repeated biopsy after isolated HPIN and those with prostate cancer on initial biopsy.

Six of 36 men with an initial diagnosis of PBSM, who were diagnosed with prostate cancer on repeated biopsy, underwent radical prostatectomy at our department. All but one had organ-confined disease, and a radical prostatectomy Gleason score of 6. Using previously developed criteria [19-21], half of the surgically treated cases were assessed as clinically significant, and half as minimal (i.e. potentially harmless). Two of 3 men with isolated HPIN on initial biopsy, and who were diagnosed with prostate cancer on repeated biopsy, were surgically treated at our department. An examination of tumor characteristics explained that both cancers were classified as minimal.

In 18 participants, in whom the initial biopsy was benign (i.e. no prostate cancer, no PBSM, no HPIN), repeated sextant biopsy was performed. Although the screening protocol did not recommend so, repeated biopsy was performed because of a persistently elevated serum PSA-level, mostly. In three cases (16.7%) prostate cancer was diagnosed (TABLE 6.1). Two of these had clinical signs of BPH, the PSA-levels were 30.8 and 16.9 ng/mL, and the biopsy Gleason scores were 8 (3 + 5) and 7 (3 + 4), respectively. The third case had LPIN on initial biopsy, a PSA of 1.6 ng/mL, and a biopsy Gleason score of 6 (3 + 3).

Repeated Screening and Repeated Biopsy at 1-year Interval

A total of 1,839 consecutive cases were offered repeated screening one year after the application of the prevalence screening tests, and 1,403 (76.3%) eventually underwent the screening tests. In 510 men (36.4%) a biopsy was prompted and 485 (95.1%) men in fact underwent repeated sextant transrectal biopsy. Of these, 470 men already had a sextant biopsy one year earlier. In five men that were previously biopsied and came for 1-year repeated screening, an earlier evaluation of PBSM on the initial biopsy was done and all five were benign on repeated biopsy at 6-weeks interval and on 1-year repeated screening. Three previously biopsied men had an initial diagnosis of isolated HPIN and two of three proved benign on repeated biopsy at 6-weeks interval. One case had a diagnosis of PBSM on repeated biopsy and prostate cancer on 1-year repeated screening. This surgically treated case had a 2.8 mL large, organ-confined tumor with a Gleason score 6 (3 + 3) and no high-grade components.

A total of 462 men underwent repeated biopsy one year after an initial benign biopsy result. The median (mean ± SD) PSA was 4.8 ng/mL (5.3 ng/mL ± 3.4) and the median time to repeated biopsy was 58.4 weeks (range, 43 – 123). No statistically significant difference for PSA was found between men on 1-year repeated screening and those that underwent repeated biopsy after an initial PBSM (MWU; $p = 0.19$) or isolated HPIN (MWU; $p = 0.64$). The proportion of men in high PSA-ranges was lower, i.e. 6.5% (30/462), than that of men with an initial diagnosis of PBSM or isolated HPIN, whereas the proportion of men in low PSA-ranges was similar, i.e. 19.9% (92/462). In 11.0% (51/462) of the men with an initial benign biopsy result prostate cancer was diagnosed and their median PSA-level (mean ± SD) was 5.2 ng/mL (6.3 ng/mL ± 4.3).

The biopsy tumor features of cancers detected on 1-year repeated screening are listed in TABLE 6.2. The MD Anderson score in cases diagnosed after PBSM was significantly lower than that in cases with a diagnosis of cancer on 1-year repeated screening (χ^2; $p = 0.02$). No difference was found for biopsy Gleason score (χ^2; $p = 0.29$) and the number of cores with cancer (χ^2; $p = 0.45$).

Initial and Repeated Biopsy on 2nd Screen at 4-year Interval

Until April 30th 2000, approximately one fourth of the men in the screening arm of ERSPC (5,101 participants) were to undergo 2nd screen at a 4-year interval. Of these, 3,266 (64.0%) eventually underwent the screening tests, 620 biopsies were performed and 121 (19.5%) prostate cancers were diagnosed. PBSM and isolated HPIN were diagnosed in 2.9% (18/620) and 2.3% (14/620) of biopsied cases, respectively. The cancer detection rate on repeated biopsy at 6-weeks interval was considerably lower than that on prevalence screen. These figures on 2nd screen were 6.7% (1/15) in biopsied men with an

initial diagnosis of PBSM and 0.0% (0/10) for men that had a diagnosis of isolated HPIN on initial biopsy.

TABLE 6.2

Features of cancers detected on the initial biopsy and of those detected on repeated biopsy after six weeks and on repeated biopsy after one year, respectively

Biopsy tumor features	No repeated biopsy	Repeated biopsy after six weeks		Repeated biopsy after one year
	Initial biopsy Prostate cancer N (%)	Initial PBSM N (%)	Initial HPIN N (%)	Initial Benign N (%)
Gleason score				
2-4	42 (4.4)	2 (5.6)	0 (0.0)	3 (5.9)
5-6	565 (58.9)	27 (75.0)	3 (100.0)	35 (68.6)
7	272 (28.4)	4 (11.1)	0 (0.0)	12 (23.5)
8-10	80 (8.3)	3 (8.3)	0 (0.0)	1 (2.0)
MD Anderson score				
1	631 (65.8)	30 (83.3)	3 (100.0)	31 (60.8)
2	257 (26.8)	4 (11.1)	0 (0.0)	19 (37.3)
3	71 (7.4)	2 (8.3)	0 (0.0)	1 (2.0)
Number of cores with cancer				
1	237 (24.7)	15 (41.7)	1 (33.3)	22 (43.1)
2	230 (24.0)	17 (47.2)	2 (66.7)	18 (35.3)
3	189 (19.7)	2 (5.6)	0 (0.0)	8 (15.7)
4-6	303 (31.6)	2 (5.6)	0 (0.0)	3 (5.9)
Total cancers	959	36	3	51

Of the 121 cases that were diagnosed with prostate cancer on 2nd screen, one (0.8%) had an earlier diagnosis of PBSM on prevalence screen, 74.4% (90/121) did not have sextant transrectal biopsy earlier and 24.8% (30/121) underwent a previous biopsy that was classified as benign. For cases with a diagnosis of PBSM on prevalence screen and no cancer on repeated biopsy, all but one remained free of prostate cancer after 4 years. None of the participants with HPIN on initial biopsy was diagnosed with cancer on 2nd screen.

DISCUSSION

In the last decade, extensive efforts have been made for the early detection of prostate cancer using serum prostate-specific antigen (PSA), digital rectal examination (DRE) and transrectal ultrasound (TRUS) as markers for prostate cancer. Early detection programs aim at the detection of cancers that are likely to reveal themselves clinically and cause prostate cancer mortality. Since missing potentially harmful cancers may possibly interfere with the outcome of randomized clinical trials, i.e. the reduction of prostate cancer related mortality, diagnostic follow-up of men for whom clinical, biochemical or pathological suspicion of prostate cancer persists after a non-cancerous biopsy result is pursued. In particular, the presence of putative precursor lesions of prostate cancer or otherwise suspicious lesions on the diagnostic prostatic needle biopsy have been the topic of major concerns for these may indicate the presence of concomitant malignancy. Using data from a large randomized population-based screening trial, in which screened participants were subjected to standardized screening regimens, the current study assessed whether diagnostic follow-up of men who have been diagnosed with pathological findings associated with an increased risk of prostate cancer is needed.

The diagnostic entity referred to as prostatic intra-epithelial neoplasia consists of architecturally benign prostatic acini and ducts, lined by cytological atypical cells and an incomplete disruption of the basal cell layer [14-22]. Lesions with the morphology of PIN are regarded as the most likely precursor of (peripheral zone) prostatic adenocarcinoma. Based on the low predictive value for cancer and its high interobserver variation among pathologists, low-grade PIN (LPIN) should be distinguished from high-grade PIN (HPIN) lesions [23]. It is now the consensus that LPIN should no longer be reported as a separate diagnostic entity [24]. Until present, it is not yet clear whether HPIN remains stable, regresses or progresses to invasive cancer or simply co-exists with its presumed malignant equivalent. A remarkable variation in the incidence rates of HPIN has been reported in different institutions. Published data reporting on HPIN in the absence of identifiable carcinoma have shown incidence rates of 0.15% to up to 16.5% of needle biopsies [1,5,23,25-29]. If the criteria for establishing a diagnosis are better defined, and more cases are included, the diagnosis is rendered less frequently. In studies reporting on series of over a thousand biopsies, the reported incidence rates of isolated HPIN were lower at 0.15 to 3.7% of needle biopsies [1,23,27-29]. Apparently, referral and consultation bias may have occurred in some of the smaller patient series. The variations from one institution to another can further be explained by the method of patient selection in these hospitals, the recommendations for biopsy, and the biopsy compliance rates. Also the number of biopsies performed, and the biopsy sampling technique itself

may have influenced the incidence rates of this putative premalignant lesion of the prostate. Reports from opportunistic screening studies demonstrated that prostate cancer was revealed in 22 to 100% of men that underwent repeated biopsy after an initial diagnosis of isolated HPIN[25,29,30,32-38]. The confounding factors mentioned earlier may also influence the diagnostic yield for prostate cancer on repeated biopsy. Furthermore, the yield for prostate cancer on follow-up biopsy will depend on the effectiveness of the initial biopsy procedure to detect prostate cancer, and it may be well expected that when more extensive repeated biopsy is performed in men with an assumed increased risk of prostate cancer, the likelihood of detecting prostate cancer on follow-up biopsy is increased, and vice versa. Despite the low comparability of these studies, it has become a widely accepted routine to perform vigorous diagnostic follow-up in men in whom the needle biopsies fail to identify prostate cancer in the presence of HPIN lesions [4,5,22]. It has recently even been suggested to offer hormonal deprivation therapy to men with a diagnosis of isolated HPIN [6,7]. However, considerable concern has been raised about the actual predictive value of HPIN as an indicator of co-existent neoplasia [29,38]. This may imply that a renewed look on the recommendations of the diagnostic follow-up of men with isolated HPIN is warranted.

Other architectural anomalies may be present that do not truly convince the pathologist that the lesion represents an overt carcinoma. Due to insufficient cytological, architectural or histologic atypia an unequivocal diagnosis of malignancy may not be made. In other words, the constellation of cytological and histological changes of these abnormalities fall below the diagnostic threshold of carcinoma. The incidence rates of suspicious lesions or 'atypical small acinar proliferations' (ASAP) varied between 1.5% and 6.5% of biopsies [1,5,26-29,39,40], and between 2.5% and 4.8% when studies reporting on over a thousand needle biopsies were considered [1,26,29]. Repeated biopsy is often recommended in these cases [1,2,41]. The frequency of cancer on repeated biopsy after a suspicious for cancer diagnosis is reported to vary between 29% and 60% of biopsies [1,2,26,29,33,39,40-43]. Besides differences in study design, patient characteristics and biopsy technique, this variation may also be explained by the definition of what actually constitutes a suspicious lesion. It is likely that 'non-expert' pathologists may inappropriately have designated some cases as HPIN or prostatic adenocarcinoma. Some guidelines for the histopathological diagnosis of 'suspicious for malignancy' lesions have been given by Cheville *et al.* [1] and Iczkowski *et al.* [2,41]. The possibility cannot be excluded that these lesions often represent a marginally sampled, tangentially sectioned, or outpouching of prostatic adenocarcinoma.

The current study reported on 20,979 participants derived from the screening arm of a randomized screening trial for prostate cancer. All screened participants were subjected to a standardized screening protocol every four years using serum-PSA testing, DRE and TRUS as screening tools. An abnormal screening test prompted diagnostic sextant

transrectal biopsy. When the histopathological examination of the biopsy specimen remained inconclusive for prostate cancer (i.e. PBSM) or showed HPIN in the absence of prostate cancer, repeated biopsy was recommended at 6-weeks interval. Our data demonstrated that the incidence rate of isolated HPIN was 0.8% (33 of 4,057 cases) with a cancer detection rate on repeated biopsy of only 10.0% (3/30). PBSM occurred in 2.6% of biopsied cases, whereas the yield on repeated biopsy was 38.7% (36/93). It is likely that the cancer detection rate on repeated biopsy after PBSM may have been higher if repeated sytematic sextant biopsy was performed instead of four biopsies directed at the area of suspicion [43]. Our data further indicate that the yield on repeated biopsy does not substantially increase with longer follow-up. One additional cancer was detected on 1-year repeated screening in a case with isolated HPIN, whereas one cancer was diagnosed on 2nd screen in a case with PBSM on prevalence screen. In our opinion, the reported incidence rates of isolated HPIN and PBSM, as well as their yield for cancer on repeated biopsy are of interest since our study was population-based, and participants underwent screening (and re-screening) according to well-established and well-standardized screening protocols.

An unselected cohort of 462 consecutively recruited men was subjected to repeated biopsy one year after an initial benign biopsy result. In these men, 51 (11.0%) cancers were detected, implying that this cancer detection rate was similar to that of men with an initial diagnosis of HPIN. No statistically significant difference was found for serum-PSA between the three different indications for repeated biopsy (i.e. PBSM, HPIN or benign), indicating that this may not have largely influenced the diagnostic yield for cancer. Men who underwent repeated biopsy after an initial benign biopsy result had a PSA-level of 10.0 ng/mL or higher less often. This discrepancy may be explained by the fact that a large proportion of men in high PSA-values underwent systematic sextant biopsy on prevalence screen, were subsequently diagnosed with prostate cancer, and did not undergo subsequent repeated biopsy.

The grade (Gleason score, MD Anderson score) of cancers detected on repeated biopsy after an initial diagnosis of PBSM was comparable to that of cancers detected in the first biopsy session (TABLE 6.2). The number of cores with cancer was significantly lower for men diagnosed with cancer on repeated biopsy after an initial PBSM diagnosis than that of men diagnosed with cancer on initial biopsy. This may be explained by the fact that only four biopsies were taken from the area that raised suspicion of cancer, though also that these tumors had littler chance of being detected due to small tumor size. The tumor volume, determined in the radical prostatectomy specimen, indeed was lower in men diagnosed with cancer after PBSM (data not shown). However, it may not be excluded that some treatment selection bias may have occurred for not an even proportion of men diagnosed within the different indications of repeated biopsy underwent radical prostatectomy. Although the absolute number and the proportion of men undergoing

radical prostatectomy at our department was small, the proportion of clinically significant disease indicated that repeated biopsy after PBSM is still warranted.

The few tumors detected on repeated biopsy after an initial diagnosis of HPIN all showed highly favorable tumor features, both on the biopsy specimen and the radical prostatectomy specimen. Two of three men with cancer underwent radical prostatectomy and both were considered as having possibly harmless disease.

CONCLUSIONS

Clinicians are sometimes confronted with biopsy results that although negative for cancer, raise suspicion of concurrent carcinoma. The diagnosis of high-grade prostatic intra-epithelial neoplasia (HPIN) and a prostate biopsy suspicious for malignancy (PBSM) present a particular diagnostic challenge. In 4,057 biopsied participants of a large population-based screening trial, the frequency of HPIN on initial biopsy was 0.8%, while this was 2.6% for PBSM. Participants that were diagnosed with PBSM on initial biopsy are at considerable risk of being diagnosed with prostate cancer on repeated lesion directed biopsy (i.e. 38.7%). The tumor features of the cancers detected indicate that these resemble those of the cancers detected on initial biopsy. Therefore, men that are diagnosed with PBSM are candidates for close diagnostic follow-up.

Despite the fact that HPIN is an assumed premalignant lesion of the prostate, screened men with an isolated focus of HPIN on the needle biopsy are at no greater risk of having prostate cancer than if their initial biopsies were assessed as benign prostatic tissue only. Our data indicate that the risk of concomitant prostate cancer is similar (i.e. 10.0%) to that reported in an unselected cohort of men that underwent 1-year repeated biopsy after an initial benign biopsy result (i.e. 11.0%). Moreover, the highly favorable tumor features of the cancers detected on repeated sextant biopsy indicate that many of the cases with isolated HPIN on initial biopsy may have been subjected to unnecessary early diagnostic follow-up. In contrast to the outcome of earlier studies we cast doubt on the actual predictive value for cancer of repeated sextant biopsy in men who are initially diagnosed with isolated HPIN in population screening for prostate cancer.

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PART IV

TOWARDS A REFINING OF SCREENING IN LOW PSA RANGES

CHAPTER 7

TUMOR CHARACTERISTICS IN SCREENING FOR PROSTATE CANCER WITH AND WITHOUT RECTAL EXAMINATION AS AN INITIAL SCREENING TEST AT LOW PSA (0.0 – 3.9 NG/ML)

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SUMMARY

BACKGROUND. The value of rectal examination as initial screening test for prostate cancer at low PSA values (0.0 – 3.9 ng/mL) was determined by evaluating the number and tumor characteristics of the cancers detected.

METHODS. Two separate study populations were subjected to screening with (n = 10,226) and without (n = 10,753) rectal examination as initial screening test. The number of cancers detected at low PSA values for both screening regimens, the corresponding biopsy and radical prostatectomy tumor characteristics were assessed. Possibly harmless cancers were defined as small (< 0.5 mL) organ-confined tumors without Gleason growth-patterns 4/5.

RESULTS. At low PSA, 26.6% (117/440) of screen-detected cancers were detected after the evaluation of a suspicious rectal examination. The number of cancers and tumor aggressiveness features were highly associated with serum-PSA level. The proportion of possibly harmless disease steadily declined from 100% (PSA 0.0 – 0.9 ng/mL) to 15.4% (PSA 3.0 – 3.9 ng/mL). Rectal examinations were performed unnecessarily in 94.7% to 100% of cases, when detection of clinically significant disease was aimed at. Using PSA (and a cut-off of 3.0 ng/mL) as the only screening tool, 24.3% (121/498) of screen-detected cancers were in the PSA range 3.0 - 3.9 ng/mL, and 60.0% were assessed as clinically significant.

CONCLUSIONS. Rectal examination as initial screening test for prostate cancer at low PSA values may be replaced by screening using serum-PSA only. At PSA levels below 3.0 ng/mL, 289 rectal examinations are required to find one case of clinically significant disease, and 96 rectal examinations are needed to diagnose prostate cancer of any size, grade or stage.

INTRODUCTION

Prostate cancer is the most commonly diagnosed non-skin malignancy in elderly males in the Netherlands and in the United States, and the second cause of cancer-related death, only surpassed by lung cancer [1,2]. In recent years, extensive efforts have been made for the early detection of prostate cancer, using serum prostate-specific antigen (PSA), digital rectal examination (DRE) and transrectal ultrasound (TRUS) in case-finding and randomized screening studies. So far, screening for prostate cancer remains a controversial issue and has not yet proved to reduce disease specific mortality.

The ERSPC is a multicenter study, which seeks to demonstrate a reduction of prostate cancer mortality of at least 20% (with a statistical power of 90%) in screened participants aged 55 to 74 years, compared to non-screened participants in the control group. Within ERSPC, evaluation of the applied screening regimen is part of the study protocol in an effort to optimize the validity (i.e. sensitivity and specificity) of the screening tools [3-7]. An evaluation by logistic regression analysis led to a major change of screening regimen in February 1997, at which time the original screening protocol, i.e. a prostate biopsy for all men with a serum PSA ≥ 4.0 ng/mL or a suspicious DRE/TRUS at low PSA values (0.0 – 3.9 ng/mL), was replaced by a new protocol. The new screening regimen called for prostate biopsy in all men with a serum PSA ≥ 3.0 ng/mL, irrespective of DRE/TRUS-findings [4]. Validation of this major change in screening protocol with respect to detection rates, positive predictive value (PPV), and the number of cancers found per biopsy in different PSA ranges is described in detail by Schröder *et al.* [6].

By examining tumor characteristics, the current study assessed whether the major change in screening regimen for the early detection of prostate cancer, which meant that rectal examination (DRE/TRUS) was omitted as an initial screening tool, is justified. The number of cancers detected within two separate populations subjected to screening with or without rectal examination was compared, as were the biopsy tumor features and the characteristics of the tumor in the radical prostatectomy specimen. Special focus was paid to the proportion of assumed clinically significant disease detected within either of the two screening regimens and to the yield of rectal examination and transrectal sextant biopsy for the detection of clinically significant disease. Based on these results, rational recommendations may be given concerning the value of rectal examination as a tool for the early detection of prostate cancer at low PSA values.

MATERIALS AND METHODS

Patients

Between June 1, 1994 and December 31, 1999, 41,919 men, aged 55 to 74 years, were randomized to a screening and control arm within the Rotterdam section of ERSPC. The Rotterdam protocol provides for re-screening after four years, but this report concentrates on the first screening round only (prevalence screen). The conditions and algorithm of screening are described in detail elsewhere [3-7]. Until February 1997, the screening protocol determined that screened participants in the Rotterdam area with a PSA equal to or above 4.0 ng/mL (Hybritech Tandem E Assay; Hybritech Incorporated, San Diego, CA) and/or a suspicious DRE/TRUS-finding at low PSA (0.0 – 3.9 ng/mL) were to undergo prostate biopsy. Sextant transrectal biopsy was performed using a Bard (C.R. Bard, Convington, GA) spring-loaded biopsy gun and an 18-gauge biopsy needle. Ultrasound-guidance was performed using a 7 MHz end-fire ultrasound probe. Additional biopsies were taken from any suspicious areas within the prostate gland. Within the original protocol, 10,226 men were randomized to screening, resulting in 440 cases diagnosed with prostate cancer on initial biopsy. All prostate cancer patients were sent back to their General Practitioner to be referred for treatment to the University Hospital Rotterdam or to one of the regional hospitals.

In February 1996, during the course of this study, the biopsy indication for men who presented with a PSA value below 4.0 ng/mL was changed, resulting in the omission of DRE and TRUS as a screening tool in cases where the PSA value was below 1.0 ng/mL [3]. This was done because of the very low positive predictive value (PPV) of biopsies for cancer in participants with a PSA value below 1.0 ng/mL. To simplify presentation, it was decided to extrapolate the number of biopsies and cancers which would have been found if this policy change had not occurred [6].

In February 1997, a second major change of protocol was implemented within ERSPC, when the European study group decided to exclusively take a biopsy from men with a PSA of 3.0 ng/mL or more, without performing a DRE or TRUS as screening tests at all. Until December 31, 1999, 10,753 men living in the direct surroundings of Rotterdam were randomized according to this new screening protocol and 498 patients were diagnosed with prostate cancer after the histopathological examination of the biopsy sextant.

Pathological Tissue Examination

All sextant biopsy cores were labeled and processed separately. The biopsy cores were routinely fixed in 10% buffered formalin at pH = 7.5, embedded in paraffin, freshly cut into 4 μm thick tissue sections and mounted on glass slides. Haematoxylin & eosin (H & E) slides of three subsequent levels of the needle biopsy were histologically examined and the number of cores with cancer (1 – 6), a biopsy Gleason score, and the biopsy tumor involvement (i.e. the cumulative length of cancer divided by the cumulative length of biopsy cores) were determined for each case by a specialized genito-urinary pathologist (ThvdK).

All radical prostatectomy specimens were fixed, totally embedded and processed according to well-established protocols [8,9]. For each cancer, a Gleason score was determined and the tumor was staged by a single pathologist (ThvdK) according to the TNM '97 classification. All cancers detected in the radical prostatectomy specimen were examined for the relative proportion of high-grade (HG) cancer (i.e. Gleason growth pattern 4 or 5) and subsequent morphometric analysis was performed to determine the tumor volume as described in detail by Hoedemaeker *et al.* [10]. Tumors were categorized according to a previously developed predictive model, including pathological stage, tumor volume and the proportion of high-grade cancer [11]. In this model, minimal tumors were defined as small (< 0.5 mL), organ-confined tumors without Gleason-pattern 4 and 5, whereas advanced cancers were tumors invading adjacent organs (i.e. seminal vesicle, bladder neck), cancers of ≥ 1.0 mL in tumor volume extending the prostatic capsule and/or tumors containing high amounts (≥ 0.5 mL) of poorly differentiated cancer. All cancers with tumor characteristics in between those of minimal and advanced disease were classified as moderate (i.e. potentially aggressive and curable) disease. Since the prognostic significance of the model is to be further established, we considered moderate and advanced tumor characteristics as 'clinically significant'. Minimal tumors, on the other hand, were assumed "possibly harmless" on basis of their low biological aggressiveness features.

The Efficacy of the Screening Tools

The efficacy of the screening tests (rectal examination and sextant transrectal biopsy) for the detection of clinically significant prostate cancer was determined by comparing the number of men with clinically significant disease to the number of men that came for rectal examination (DRE/TRUS), and to the number of men that eventually underwent transrectal sextant biopsy. The yield of a screening test in this context is defined as the proportion of cases accurately identified by this screening test. The yield of rectal examination for the detection of clinically significant disease was defined as the expected

number of clinically significant prostate cancers within a particular PSA range divided by the total number of rectal examinations performed within this PSA range. This figure explains how many rectal examinations are needed to detect one case of clinically significant disease. The yield of transrectal sextant biopsy for the detection of clinically significant disease was defined as the expected number of clinically significant disease within a particular PSA range divided by the number of sextant transrectal biopsies performed within this PSA range. This figure demonstrates how many clinically significant cancers are found for any biopsy taken. The number of men expected to have clinically significant disease within a particular PSA range was calculated by multiplying the total number of cancers detected in the PSA range with the observed proportion of men with assumed clinically significant disease after radical prostatectomy. Despite the fact that the true number (i.e. prevalence) of men with clinically significant disease remains unknown in the population, and that the tumor features of clinically significant disease were arbitrarily defined in our study, we assumed that the tumor features of those that underwent one of the other treatment modalities (radiotherapy or deferred treatment) was similar to those of men that underwent retropubic radical prostatectomy.

Statistical Analysis

Statistical analysis was performed using the statistical package for the social sciences (SPSS 9.0; SPSS Incorporated, Chicago, IL). Baseline variables and pathological tumor features determined on the biopsy specimen have been listed in TABLE 7.1. The χ^2–test was used to assess differences between the original protocol and the PSA driven protocol. The assumption that no difference existed between the original protocol and the PSA-driven protocol (H0) for the variable evaluated was rejected (H1) if $p < 0.05$.

RESULTS

Biopsy Tumor Characteristics of Men Diagnosed With Prostate Cancer

Of the 440 patients who were biopsied and diagnosed with prostate cancer according to the original protocol (PSA ≥ 4.0 ng/mL and/or DRE/TRUS+), 117 (26.6%) were diagnosed at a low PSA-value (0.0 – 3.9 ng/mL). Of the cancers detected at low PSA values, 43 (36.8%) were detected in the PSA-range 3.0 – 3.9 ng/mL, 27 (23.1%) were in the PSA range 2.0 – 2.9 ng/mL, and 47 (40.2%) were in the PSA range 0.0 – 1.9 ng/mL (TABLE 7.2). Forty (34.2%) cases with cancer had an abnormal DRE alone, 39 (33.3%) had an abnormal TRUS alone, and 38 (32.5%) had abnormal findings on both DRE and

TRUS. For the PSA driven protocol (PSA ≥ 3.0 ng/mL), 121 (24.3%) of the 498 cases diagnosed with prostate cancer came from the PSA range 3.0 – 3.9 ng/mL.

Baseline variables and the biopsy tumor features for cases diagnosed with prostate cancer in the PSA range 0.0 – 3.9 ng/mL are given in TABLE 7.1. Between the two screening regimens, no statistically significant difference was found for age, number of cores with cancer, or biopsy tumor involvement. The Gleason score on the other hand was statistically more favorable in the PSA driven protocol (χ^2 - test: p = 0.027).

TABLE 7.1

Comparison of baseline variables and biopsy tumor features in the low PSA ranges for cases diagnosed with prostate cancer within the original protocol (biopsy indication if PSA 0.0 – 3.9 ng/mL together with a suspicious DRE/TRUS) and the PSA driven protocol (biopsy indication if PSA 3.0 – 3.9 ng/mL) within ERSPC, section Rotterdam

	Original protocol n (%)	PSA driven protocol n (%)	p-value*
Age (years)			
55-59	30 (25.6)	28 (23.1)	
60-64	29 (24.8)	28 (23.1)	
65-69	33 (28.2)	35 (28.9)	ns
70-74	25 (21.4)	30 (24.8)	
Number of positive cores			
1	43 (36.8)	52 (43.0)	
2	43 (36.8)	33 (27.3)	
3	21 (17.9)	22 (18.2)	ns
4-6	10 (8.5)	14 (11.6)	
Gleason score			
2-4	8 (6.8)	12 (9.9)	
5-6	72 (61.5)	87 (71.9)	
7	26 (22.2)	20 (16.5)	0.027
8-10	11 (9.4)	2 (1.7)	
Biopsy tumor involvement			
0-10%	58 (49.6)	72 (59.5)	
10-30%	43 (36.8)	34 (28.1)	ns
≥ 30%	16 (13.7)	15 (12.4)	
Total number of cancers	117	121	

* χ^2 - test
ns not significant

TABLE 7.2 shows the number and distribution of cancers within different PSA ranges when classified according to Gleason score. For the original screening regimen, the proportion of moderately (i.e. Gleason score 7) and poorly (i.e. Gleason scores 8 - 10) differentiated cancers increased with rising PSA values, although some poorly differentiated cancers were also found in the very low PSA ranges.

TABLE 7.2

The number of prostate cancers detected, and the distribution of tumors according to biopsy Gleason score in different PSA ranges of cases detected within the original protocol (biopsy indication if PSA 0.0 – 3.9 ng/mL together with a suspicious DRE/TRUS) and the PSA driven protocol (biopsy indication if PSA 3.0 – 3.9 ng/mL) within ERSPC, section Rotterdam

	Original protocol (n = 117)					PSA driven protocol (n = 121)				
		Gleason score					Gleason score			
PSA (ng/mL)	N	2-4 n (%)	5-6 n (%)	7 n (%)	8-10 n (%)	N	2-4 n (%)	5-6 n (%)	7 n (%)	8-10 n (%)
0.0 – 0.9	4	1(25.0)	3 (75.0)	0 (0.0)	0 (0.0)	-	-	-	-	-
1.0 – 1.9	43	3 (7.0)	32 (74.4)	6 (14.0)	2 (4.6)	-	-	-	-	-
2.0 – 2.9	27	0 (0.0)	17 (63.0)	8 (29.6)	2 (7.4)	-	-	-	-	-
3.0 – 3.9	43	4 (9.3)	20 (46.5)	12 (27.9)	7 (16.3)	121	12 (9.9)	87 (71.9)	20 (16.5)	2 (1.7)
Total	117	8 (6.8)	72 (61.5)	26 (22.2)	11 (9.4)	121	12 (9.9)	87 (71.9)	20 (16.5)	2 (1.7)

Characteristics of Surgically Treated Patients

From the original protocol 49 out of 117 (41.9%) cases detected within the PSA range 0.0 – 3.9 ng/mL were treated with radical prostatectomy at the University Hospital Rotterdam, whereas 25 out of 121 (20.7%) cases underwent radical prostatectomy at the University Hospital Rotterdam in the PSA driven protocol. For PSA values below 4.0 ng/mL there was no statistically significant difference between the two screening

regimens for those who were surgically treated as far as baseline variables and tumor features of the diagnostic biopsy were concerned (data not shown).

Out of the 49 cases detected after the evaluation of a suspicious rectal examination in the PSA range 0.0 – 3.9 ng/mL, 24 (49.0%) had evidence of possibly harmless (minimal) disease as determined in the radical prostatectomy specimen, whereas 2 (4.1%) might be beyond cure because of advanced disease (TABLE 7.3). The proportion of minimal disease decreased from 100% for PSA values below 1.0 ng/mL, 73.7% in the PSA range 1.0 – 1.9 ng/mL, 40.0% in the PSA-range 2.0 – 2.9 ng/mL, to 15.4% for PSA values between 3.0 and 3.9 ng/mL. The relative number of cases with assumed clinically significant disease showed an increasing trend with rising PSA values (TABLE 7.3). For cases detected by PSA based screening in the PSA range 3.0 – 3.9 ng/mL, 13 (52.0%) cases were classified as having moderate disease, whereas 10 (40.0%) and 2 (8.0%) cases showed evidence of minimal and advanced disease, respectively.

The Efficacy of Screening Tests to Detect Clinically Significant Disease

TABLE 7.4 shows the yield of rectal examination and sextant transrectal biopsy for the detection of clinically significant disease in different PSA ranges. Since in the PSA range 0.0 – 0.9 ng/mL no clinically significant cancers were diagnosed, rectal examination and biopsy were of no (predictive) value when the detection of clinically relevant disease was aimed at. In the PSA range 1.0 – 1.9 ng/mL, 0.4% of rectal examinations led to the detection of cancers with clinically significant tumor features (i.e. 11 in 3,051 screened men). This figure was 2.2% (11/511) for men that eventually underwent a sextant biopsy. So, only one in every 277 rectal examinations and only one in every 46 biopsies eventuated in the detection of clinically significant disease. Conversely, 99.6% (i.e. 3,040 men) and 97.8% (i.e. 500 men) of men in the PSA range 1.0 – 1.9 ng/mL underwent a rectal examination or a sextant transrectal biopsy, respectively, that in the end may turn out to be unnecessary. The yield of rectal examination and transrectal sextant biopsy for the detection of clinically significant disease increased steadily with rising PSA values (TABLE 7.4). In the PSA range 2.0 – 2.9 ng/mL, one case of clinically relevant cancer was detected for every 77 rectal examinations (yield = 1.3%), and one for every 14 biopsies (yield = 7.2%). In the PSA range 3.0 – 3.9 ng/mL, 5.3% (one in every 19) of rectal examinations and 21.3% (approximately one in every 5) of biopsies resulted in the detection of clinically significant disease. Again, 94.7% and 78.7% of rectal examinations and biopsies, respectively, may be assessed as being performed unnecessarily in this PSA range. In the PSA range 3.0 – 3.9 ng/mL, within PSA driven screening, the yield of sextant transrectal biopsy for the detection of clinically significant disease was 12.5% (73 in 585 biopsied men) or 8 biopsies needed to detect one cancer with clinically significant tumor features.

TABLE 7.3

The distribution of tumors according to a prognostic tumor classification model in different PSA ranges of cases detected (and surgically treated) within the original protocol (biopsy indication if PSA 0.0 – 3.9 ng/mL together with a suspicious DRE/TRUS) and the PSA driven protocol (biopsy indication if PSA 3.0 – 3.9 ng/mL)

	Original protocol			PSA driven protocol		
	Possibly harmless*	Clinically significant†		Possibly harmless*	Clinically significant†	
PSA (ng/mL)	Minimal n (%)	Moderate n (%)	Advanced n (%)	Minimal N (%)	Moderate N (%)	Advanced n (%)
0.0 - 0.9	2 (100.0)	0 (0.0)	0 (0.0)	-	-	-
1.0 - 1.9	14 (73.7)	4 (21.1)	1 (5.3)	-	-	-
2.0 - 2.9	6 (40.0)	8 (53.3)	1 (6.7)	-	-	-
3.0 - 3.9	2 (15.4)	11 (84.6)	0 (0.0)	10 (40.0)	13 (52.0)	2 (8.0)
Total	24 (49.0)	23 (46.9)	2 (4.1)	10 (40.0)	13 (52.0)	2 (8.0)

* Possibly harmless: Organ-confined prostate cancer with a tumor volume less than 0.5 mL, without Gleason growth pattern 4 or 5

† Clinically significant: Prostate cancer with tumor features other than those of possibly harmless (i.e. minimal) disease

DISCUSSION

Population-based screening for prostate cancer remains a controversial issue. Large randomized screening trials performed today in Western Europe and North America, will provide a final answer to the question whether screening for prostate cancer is beneficial or not, at the end of this decade. Irrespective of the outcome of these screening trials, the validity of different screening tests with respect to detection rates, and the characteristics of the cancers detected, may give the clinician (and his patient) insight into the proper management decisions for the early detection of prostate cancer.

In general, a screening test should have a high probability of diagnosing disease when the test is abnormal, while a minimum of cancers should be present when the test is normal [12]. To determine the sensitivity and specificity of a screening test, one would need to know what proportion of participants tested positive and negative among those

who truly do or do not have the disease. For the negative predictive value of a screening test, one would need to know the proportion of participants that truly remain free of disease among those who tested negative. Unfortunately, these figures can only be calculated if all participants (even those who tested negative) would be subjected to screening and its diagnostic sequelae (e.g. sextant transrectal biopsy). This, of course, would be hard to approve ethically. So far, no screening test exists that can reliably differentiate between the presence and absence of prostate cancer, nor is there a screening test that can distinguish between aggressive and non-aggressive features in the tumor [13]. The main goal in the application of a screening test for prostate cancer, whether in randomized screening trials or in opportunistic screening, is the detection of cancers that can be cured by current treatment policies, while avoiding unnecessary testing, and avoiding the detection of cancers that are not life threatening and remain so in the patients' lifetime. Recent studies provided arguments that different prognostic subgroups of tumors, combining well-established prognosticators such as pathological tumor stage, tumor volume and the proportion of high-grade cancer could be identified, each having its own intrinsic behavior with respect to recurrence rates after radical prostatectomy [10,11,14-17]. According to this predictive model, patients with minimal disease would be suitable candidates for conservative treatment and surveillance, while many of those with advanced disease are presumed to be beyond cure. All cases with tumor features in between those of minimal and advanced disease are especially amenable to curative treatment and therefore, the detection and the treatment of these cases is considered the 'window of opportunity' in large-scale screening studies [6,11,18].

While annual PSA testing and DRE are recommended by the American Cancer Society and the American Urological Association for all men from the age of 50, validated guidelines for rational and selective screening for prostate cancer are lacking [19-21]. Different study groups have addressed the value of PSA testing and rectal examination (DRE/TRUS) as screening tests at low PSA values (0.0 – 3.9 ng/mL), but no appropriate guidelines have yet been established [7,22-26]. The present study focussed on the number and the characteristics of prostate cancers diagnosed within a large population-based screening trial when rectal examination is or is not used as an initial screening test at low PSA values.

Our data indicate that the tumor aggressiveness features are associated with serum-PSA level, even at low PSA values. When DRE and TRUS are used as initial screening tests for prostate cancer, the absolute number and the proportion of men with any poorly differentiated components (i.e. Gleason scores 7 to 10) in the biopsy specimen increased from 0% (0 cases) in the PSA range 0.0 – 0.9 ng/mL, to 44.2% (19 cases) in the PSA range 3.0 – 3.9 ng/mL (TABLE 7.2).

TABLE 7.4

The number of men compliant to screening (serum-PSA testing, rectal examination), the number of men with an abnormal screening test, the number of biopsies performed, the number of prostate cancers detected, the relative proportion and the absolute number of men with clinically significant disease to be expected in different PSA ranges. The yield of rectal examination and transrectal sextant biopsy for the detection of clinically significant disease for different PSA ranges within the original protocol (biopsy indication if PSA 0.0 – 3.9 ng/mL together with a suspicious DRE/TRUS) and the PSA driven protocol (biopsy indication if PSA 3.0 – 3.9 ng/mL).

					Clinically significant disease *					
PSA (ng/mL) Rectal	Screened N	Abnormal Testing	Biopsies	Cancers	Expected		Yield of Rectal exam	Rectal exams needed	Yield of Biopsy	Biopsies needed
					%†	No. ‡				
With										
0.0 – 0.9	3,556	509	376	4	0 %	0	0.0 %	∞	0.0 %	∞
1.0 – 1.9	3,051	556	511	43	25 %	11	0.4 %	277	2.2 %	46
1.0 – 2.9	1,199	238	221	27	60 %	16	1.3 %	77	7.2 %	14
3.0 – 3.9	701	182	174	43	85 %	37	5.3 %	19	21.3 %	5
Total	8,507	1,485	1,282	117	55 %	64	0.8 %	133	5.0 %	20
Without										
3.0 – 3.9	688	688	585	121	60 %	73	N/A	N/A	12.5 %	8

† The expected proportion of clinically significant disease as described in TABLE 7.3
‡ The number of cancers multiplied by the expected proportion of clinically significant disease
* Cancer with characteristics other than those of minimal disease (organ-confined cancer with tumor volume < 0.5 mL, no Gleason growth pattern 4 or 5)
∞ Infinite number
N/A Not applicable

The proportion of men with minimal disease (i.e. small, organ-confined tumors without Gleason growth patterns 4 and 5) declined steadily from 100% in the PSA range 0.0 – 0.9 ng/mL, to 15.4% in the PSA range 3.0 – 3.9 ng/mL. It may be well assumed that the detection of tumors with these highly favorable tumor characteristics may at the end turn out to be unnecessary. With a rising tumor volume and a PSA value rising correspondingly, the detection of these cancers is likely in successive screening rounds.

The PSA range 3.0 – 3.9 ng/mL, within PSA driven screening, included 24.3% of all screen-detected cancers. This is comparable to other studies with a similar design [19,23,27]. An examination of tumor characteristics indicated that 60% of cancers in this PSA range were assessed as clinically significant, and that 8 biopsies were required to detect one cancer with clinically significant tumor features (TABLE 7.4). Our data further indicate that in the PSA range 0.0 – 3.9 ng/mL overall more clinically significant tumors were detected (and treated) by screening using PSA ≥ 3.0 ng/mL as a trigger point for biopsy than by screening using a suspicious rectal examination as the trigger point for biopsy (73 versus 64 cases; TABLE 7.4). From this observation one might conclude that in the PSA range 0.0 – 3.9 ng/mL prostate cancer screening using serum-PSA alone could replace the screening regimen in which serum-PSA testing is followed by DRE and TRUS.

The decision to use a specific trigger point for biopsy (e.g. a PSA cut-off level, a suspicious rectal examination) in mass screening programs will in part depend on the trade-off between the gain of detecting clinically significant (and curable) disease above the trigger point for biopsy, and the risk of missing potentially aggressive tumors below the trigger point for biopsy. Missing potentially aggressive tumors may interfere with the final outcome of a randomized screening trial, namely with proving or disproving a significant difference in prostate cancer mortality. The number of missed cases with potentially aggressive tumor characteristics depends on the prevalence of clinically significant disease below the trigger point for biopsy, as well as on the (in)effectiveness of the screening tests to detect these cases. If DRE and TRUS would have been omitted as screening tools for prostate cancer in the PSA range 0.0 – 2.9 ng/mL, and were replaced by screening using serum-PSA alone, overall 27 clinically significant cancers would have been missed, and 81 cancers of any volume, grade or stage (TABLE 7.4). Though, our data also indicate that when a suspicious rectal examination was used as a trigger point for biopsy in the PSA range 0.0 – 2.9 ng/mL, the efficacy to detect potentially aggressive tumors was extremely low. A total of 289 rectal examinations were required to detect one case of clinically significant disease (27 in 7,806 screened men), and 96 rectal examinations (81 in 7,806 screened men) were needed to diagnose one case of prostate cancer of any size, grade or stage (TABLE 7.4). The number of biopsies needed to detect one potentially aggressive cancer was 41 in this PSA range, and this figure was 14 for

prostate cancer of any extent or grade. This unnecessary testing will occur even more frequently at PSA values below 2.0 ng/mL, i.e. 11 clinically significant cancers in 6,607 screened men and in 887 sextant biopsies. In our opinion, when mass screening for prostate cancer would be applied to the community, this extremely low yield of rectal examination and sextant transrectal biopsy for the detection of clinically significant disease would be unethical. The efficacy of the screening tests individually would be even worse, since in the PSA range 0.0 – 3.9 ng/mL 33.3% and 34.2% of cases with cancer had no abnormalities on DRE and TRUS, respectively. Moreover, the small proportion of interval cancers seen in this study and the favorable distribution of prognostic factors at re-screening after four years (unpublished data from our department) suggest that most of the missed cancers in the PSA range 0.0 – 2.9 ng/mL are likely to be detected at second screen.

Some restrictions should be kept in the interpretation of these results. At first, this study does not report on the yield for prostate cancer in men randomized to screening with rectal examination versus men randomized to PSA based screening only. In fact, this is an observational study in which two different study populations were subjected to two different screening regimens at two different points of time. Thus, biases may have occurred due to subtle differences in study design. Second, the efficacy of rectal examination and sextant transrectal biopsy for the detection of clinically significant disease may be underestimated in this study, since the compliance to biopsy was only 86% (1,282 out of 1,485) of men with a suspicious rectal examination in the original protocol, and 85% (585 out of 688) of men with a PSA level ≥ 3.0 ng/mL in the PSA driven protocol. If all men with a biopsy indication were in fact biopsied, the number of (clinically significant) cancers would have been higher. The yield of rectal examination and transrectal sextant biopsy may be different in populations for whom the prevalence of disease is higher (e.g. African-American men) or lower (e.g. Asian men) than that of our target population (mostly Caucasian males). Furthermore, our data indicate that rectal examination may pick up aggressive cancers more selectively than screening using serum-PSA as a tool alone. The evaluation of a suspicious rectal examination led to the detection of significantly more moderately and poorly differentiated cancers (as determined on the biopsy) than PSA based screening (TABLE 7.1). Also in the PSA range 3.0 – 3.9 ng/mL proportionally more men had a biopsy Gleason score 7 to 10 (44.2% versus 18.2% of cancers) in the original protocol. On the other hand, the absolute number of men with these high Gleason scores on the biopsy was similar in both screening protocols in this PSA range (TABLE 7.2; 22 versus 19 men). This may indicate that screening using PSA ≥ 3.0 ng/mL as the only trigger point for biopsy does also pick up these assumed aggressive cancers. Despite this relative efficacy of rectal examination for the detection of tumors with poorer grades, it should be stated that only a small

proportion of men with a suspicious finding on rectal examination and who underwent transrectal sextant biopsy were eventually diagnosed with a prostate cancer of Gleason score 7 to 10, i.e. 18 out of 1,108 biopsies (TABLE 7.4). In the PSA range 0.0 – 2.9 ng/mL and 3.0 –3.9 ng/mL, 72.2% (26 out of 36) and 38.5% (5 out of 13) of cancers found as a result of a suspicious rectal examination showed tumor volumes of less than 0.5 mL after radical prostatectomy. It is plausible that a substantial proportion of these small tumors are not detected as a consequence of the screening tests itself, but should be considered as false-positives on DRE and/or TRUS, or as detected by serendipity (chance) only [28]. Our findings that suspicious findings on rectal examination only weakly correlate or not correlate at all to the presence of cancer in low PSA ranges are in line with those of others [5,22,23]. Since only a proportion of men (i.e. 41.9%) underwent radical prostatectomy, a potential for selection bias is created. On the other hand, it is not likely that the tumor features of those that underwent prostatic surgery would differ substantially from those that underwent one of the other treatment modalities (i.e. radiotherapy, deferred treatment), particularly in low PSA ranges.

Besides the fact that PSA based screening detects clinically significant disease more frequently in the PSA range 0.0 – 3.9 ng/mL, PSA testing has also major practical advantages. The screening test is limited to the collection of one blood sample, rather than a sometimes hazardous (and more expensive) rectal examination by DRE and/or TRUS. For a population based screening program to reach its objectives (i.e. reduction of mortality within a screened population) compliance to the screening tests and avoidance of unnecessary testing are prerequisites. Serum-PSA measurement as an initial screening test offers a simple, readily accepted and relative costless tool for an effective detection of prostate cancer. Unlike rectal examination, PSA sampling proves highly reproducible, for which inter-observer variability plays no role [3,5,7]. The PSA range 2.0 – 2.9 ng/mL within PSA based screening is subject to an ongoing study within ERSPC, section Rotterdam, and figures on the cancer detection rates, the yield of sextant transrectal biopsy and the characteristics of the corresponding cancers detected within this PSA range will be presented in a future report.

CONCLUSIONS

Rectal examination (DRE/TRUS) as initial screening test for prostate cancer at low PSA values (0.0 – 3.9 ng/mL) may be replaced by screening using serum-PSA only. Due to the fact that clinically significant disease is rare at low PSA levels, the omission of rectal examination as a screening test may avoid a large amount of unnecessary testing, a

high false-positive rate, and an extremely low yield of biopsy detectable clinically significant disease.

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CHAPTER 8

SERENDIPITY SRIKES AGAIN IN LOW PROSTATE-SPECIFIC ANTIGEN RANGES

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SUMMARY

BACKGROUND. Serendipity is defined as the coincidental detection of disease (prostate cancer) during the evaluation of an abnormal screening test result. This study was performed to assess the magnitude of prostate cancer detection by serendipity when digital rectal examination (DRE) and transrectal ultrasound (TRUS) are used as initial screening tests for prostate cancer at low prostate-specific antigen (PSA) values (0.0 – 3.9 ng/mL).

METHODS. 117 participants of a population-based screening study were diagnosed with prostate cancer after the standardised evaluation of an abnormal screening test result. 49 of these underwent radical prostatectomy. Serendipity was defined threefold: (1) the presence of prostate cancer opposite to the side that raised suspicion for cancer on DRE and/or TRUS (2) a negative lesion-directed biopsy, while cancer is present in the biopsy sextant, (3) a tumour volume less than 0.5 mL.

RESULTS. Depending on the definition, 27% to 63% of prostate cancers detected at low PSA values were detected coincidentally and not as a result of a true positive test result. The proportion of cancers detected by serendipity was inversely correlated to serum-PSA level.

CONCLUSIONS. A relatively high proportion of prostate cancers diagnosed at low PSA, and in which a biopsy was prompted by a suspicious DRE and/or TRUS, are considered detected by chance only. Since these cancers are mostly small (i.e. less than 0.5 mL), with potentially low biological aggressiveness, relying on serendipity seems disadvantageous in prostate cancer screening. With regard to serendipity in prostate cancer detection, the poor performance of the screening test, and high inter-observer variability, we cast further doubt on the usefulness of DRE (and TRUS) as initial screening test for prostate cancer in population-based screening.

INTRODUCTION

Population-based screening for prostate cancer by digital rectal examination (DRE) and prostate-specific antigen (PSA) testing remains a controversial issue. Until present, screening for prostate cancer has not yet proven to reduce prostate cancer mortality in randomised clinical trials (RCTs), and the validity and usefulness of the screening tools are not completely comprehended. Despite these concerns, the American Cancer Society and the American Urological Association believe that, as preventive health care policy, annual PSA testing and DRE should be offered to all men beginning at age 50 years, and from the age of 45 years in men belonging to high risk groups [1,2]. On the other hand, the U.S. Preventive Services Task Force, the Canadian Urological Association and most health authorities within the European Union discourage prostate cancer screening, while the recommendations of the American College of Physicians, and the American Academy of Family Physicians are currently under review [3-5]. In the PSA range 0.0 – 3.9 ng/mL, the use of DRE as a screening test for prostate cancer has recently become the topic of serious debates. Adversaries of screening with DRE as a screening test for prostate cancer point to the poor performance of the screening test at low PSA values, and to its high inter-observer variability [6-9]. Advocates merely refer to the independent predictive value of DRE for prostate cancer, complementary to PSA testing, and indicate that some potentially aggressive cancers may remain undetected if DRE would be omitted as a screening test [1,10,11]. In North America, the current dispute has even reached a judicial level (Schröder FH, personal communication).

A screening test may be positive due to the presence of the disease that is the primary objective of the screening test (true positives), or it may be positive due to non-disease related morbidities (false positives). The targeted disease may also be detected coincidentally during the evaluation of a false positive screening test result. Then, the detection of the disease cannot be attributed to the screening test itself. This mechanism of the coincidental detection of disease has earlier been defined 'serendipity' [12]. Since in prostate cancer early detection programs systematic sextant biopsy and additional lesion-directed biopsies from suspicious areas of the prostate gland is prompted in cases with abnormal screening test results, prostate cancer detection by serendipity is likely. In addition, the small tumour size of a cancer and with this, its low likelihood of being palpable on DRE and/or visible on transrectal ultrasound (TRUS), indicate that the abnormal screening test itself might not have been responsible for the detection of the disease.

In the current report, the magnitude of prostate cancer detection by serendipity (chance) was determined when DRE alone and in combination with TRUS was used as

an initial screening test for prostate cancer at low PSA values (0.0 – 3.9 ng/mL). Data were obtained from the screening arm of a large population-based RCT, the European Randomized Study of Screening for Prostate Cancer (ERSPC). In our opinion, these figures will give additional insight into the performance of DRE and TRUS as screening tests for prostate cancer in this highly debated PSA range.

PATIENTS AND METHODS

Patients and Screening Regimen

The present data are derived from the prevalence screen of a multi-institutional population-based RCT (ERSPC, Rotterdam section) that investigates the impact of systematic screening for prostate cancer on cancer-specific mortality and quality of life. The ERSPC is closely associated with the Prostate, Lung, Colon, and Ovary (PLCO) screening project of the U.S. National Cancer Institute, and a combined analysis is planned. The conditions and algorithm of the screening regimen of ERSPC are described in detail elsewhere [13-16].

Between June 1994 and February 1997, 10,226 men, aged 55 to 74 years, were randomised to the screening arm of the ERSPC. In all screened participants, PSA testing, DRE and TRUS were applied as initial screening tests for prostate cancer. Blood sampling was done before rectal examination, so that DRE and TRUS were performed without knowledge of the PSA value. Participants were informed about the PSA value and the findings on DRE and TRUS by letter, and were notified about the procedure to be followed. In low PSA ranges (0.0 - 3.9 ng/mL), men with a suspicious DRE (nodularity, asymmetry, induration) or TRUS (hypoechogeneity) finding were invited to undergo prostate needle biopsy on second visit. Systematic transrectal sextant biopsy was performed using a spring-loaded biopsy gun and an 18-gauge biopsy needle as described by Rietbergen *et al.* [16] Additional biopsies were taken from any suspicious areas within the prostate gland. Ultrasound-guidance was performed using a 7-MHz end-fire ultrasound probe. Figures with respect to cancer detection rates, the positive predictive value (PPV) of the screening test and the number of biopsies needed to detect one cancer are outlined by Schröder *et al.* [15].

All separate biopsy cores were labelled and processed for routine histopathological examination and patients with prostate cancer were offered treatment guided by their Urologist. Radical prostatectomy specimens were routinely fixed and processed according to well-established protocols [17,18], and morphometric analysis was performed to determine the tumour volume as described in detail by and Hoedemaeker *et al.* [19].

Definition of Prostate Cancer Detection by Serendipity

Prostate cancer detection by serendipity may be defined as the presence of prostate cancer opposite to the side that raised suspicion for cancer on DRE or TRUS and prompted the biopsy. To assess how often prostate cancer detection by serendipity might occur, the side of the abnormal screening test (left/right/bilateral) was compared to the side of the tumour on needle biopsy (left/right/bilateral). Inconsistencies were considered serendipity-detected. The contribution of DRE and TRUS to serendipity alone was determined by excluding cases in which the other screening test was abnormal as well. A more precise indication of the magnitude of serendipity may be given by defining serendipity-detected cancers as those in which the diagnosis of cancer was made in one of the cores of the biopsy sextant, while the biopsy that was specifically directed at the suspicious area of the prostate gland and that prompted the biopsy remained free of disease. Third, we defined serendipity-detected cancers as those in which the tumour volume as determined in the radical prostatectomy specimen was not likely too cause the screening tests (DRE or TRUS) to be suspicious for cancer. A perfect sphere of 0.5 mL has a diameter of almost 1 cm ($^4/_3 \Pi r^3 = 0.5$ mL), and we assumed that this is the borderline of palpation on DRE and visualisation on TRUS. As a consequence, prostate cancers with a tumour volume of less than 0.5 mL were assumed to be detected by serendipity as well. The dependency of the percentage of serendipity findings on the 0.5 mL volume threshold was assessed by repeating the calculations for threshold volumes of 0.4 mL, 0.25 mL and 0.1 mL (sensitivity analysis).

Statistical Analysis

The Pearson χ^2-test was used to assess the trend between the serum-PSA level and the frequency of serendipity-detected cancers. The assumption that no difference existed for the variable evaluated (H0) was rejected (H1) if $p < 0.05$.

RESULTS

At low PSA, 117 cases were diagnosed with prostate cancer after the evaluation of a suspicious screening test, 40 (34.2%) after an abnormal DRE alone, and 39 (33.3%) after an abnormal TRUS alone. Using the outcome of both screening tests (DRE and TRUS), 31 of 117 (26.5%) cancers happened to be detected at the side of the prostate gland other than the palpable or visible suspicious area that prompted the biopsy (TABLE 8.1). On the basis of a suspicious DRE alone, serendipity accounted for 15 of 40 (37.5%) cases, and this figure was 13 of 39 (33.3%) for cancers diagnosed after a suspicious TRUS alone. No

association was found for the number of these serendipity-detected cancers and serum-PSA level.

TABLE 8.1

The frequency of prostate cancer detection by serendipity (*) in patients diagnosed with prostate cancer in low PSA ranges (0.0 – 3.9 ng/mL). The use of TRUS and DRE as initial screening test for prostate cancer is compared to the use of DRE and TRUS alone

	DRE and TRUS † (n = 117)		DRE alone ‡ (n = 40)		TRUS alone § (n = 39)	
PSA (ng/mL)	Serendipity n (% of total)	total n (% of Total)	Serendipity N (% of total)	total n (% of Total)	Serendipity n (% of total)	total n (% Total)
0.0 – 0.9	1 (25.0)	4 (3.4)	1 (50.0)	2 (5.0)	0	0 (0.0)
1.0 – 1.9	14 (32.6)	43 (36.8)	6 (46.2)	13 (32.5)	7 (46.7)	15 (38.5)
2.0 –2.9	6 (22.3)	27 (23.1)	3 (27.3)	11 (27.5)	3 (30.0)	14 (35.9)
3.0 - 3.9	10 (23.3)	43 (36.8)	5 (35.7)	14 (35.0)	3 (30.0)	10 (25.6)
Total	31 (26.5)	117	15 (37.5)	40	13 (33.3)	39

* The presence of prostate cancer opposite to the side of the prostate gland that raised suspicion for prostate cancer and prompted the biopsy

† χ^2–test for trend p = 0.72

‡ χ^2–test for trend p = 0.79

§ χ^2–test for trend p = 0.34

Overall, 75 of 117 (64.1%) men that were later diagnosed with prostate cancer underwent additional lesion-directed biopsy. In men in whom no additional lesion-directed biopsy was performed, the original suspicious lesion that prompted the biopsy could not be retrieved at second visit or was found to be at the opposite side compared to the first visit. In these cases, it was decided to perform sextant biopsy only. In 24 (32.0%) men who underwent additional lesion-directed biopsy, prostate cancer was present in one or more of the cores of the biopsy sextant only (TABLE 8.2). The number of prostate cancers detected coincidentally showed an inverse trend with rising PSA

values (p = 0.08), and the proportion of serendipity-detected cancers declined from 100% in the PSA range 0.0 – 0.9 ng/mL, to 20.0% in the PSA range 3.0 – 3.9 ng/mL.

TABLE 8.2

The frequency of prostate cancer detection by serendipity (*) in patients diagnosed with prostate cancer in low PSA ranges (0.0 – 3.9 ng/mL) and who underwent an additional lesion-directed biopsy

	Additional lesion-directed biopsy † (n = 75)	
PSA (ng/mL)	Serendipity N (% of total)	total n (% of Total)
0.0 – 0.9	2 (100.0)	2 (2.7)
1.0 – 1.9	10 (35.7)	28 (37.3)
2.0 – 2.9	6 (40.0)	15 (20.0)
3.0 – 3.9	6 (20.0)	30 (40.0)
Total	24 (32.0)	75

* The presence of prostate cancer in one of the cores of the sextant biopsy, while the biopsy that was specifically irected at the suspicious area of the prostate gland remains negative for cancer.

† χ^2–test for trend: p = 0.08

The absolute number and the relative proportion of 49 men who were surgically treated and who had a tumour volume of less than 0.5 mL is given in TABLE 8.3. The frequency of these serendipity-detected cancers was inversely correlated to serum-PSA level (p = 0.03), and the proportion of cancers found coincidentally steadily declined from 100% in the PSA range 0.0–0.9 ng/mL to 38.5% in the PSA range 3.0 – 3.9 ng/mL (TABLE 8.3). Serendipity still accounted for 55%, 37%, and 24% of detected cancers if a tumour volume of less than 0.4 mL, 0.25 mL, or 0.1 mL, respectively, was used as a cut-off .

TABLE 8.3

The frequency of prostate cancer detection by serendipity (*) in patients diagnosed with prostate cancer in low PSA ranges (0.0 – 3.9 ng/mL) and who subsequently underwent radical prostatectomy.

	Radical prostatectomy † (n = 49)	
PSA (ng/mL)	Serendipity N (% of total)	Total N (% of Total)
0.0 – 0.9	2 (100.0)	2 (4.1)
1.0 – 1.9	16 (84.2)	19 (38.8)
2.0 -2.9	8 (53.3)	15 (30.6)
3.0 – 3.9	5 (38.5)	13 (26.5)
Total	31 (63.3)	49

* Cancers with a tumour volume of less than 0.5 mL

† χ^2–test for trend: p = 0.03

DISCUSSION

At present, both the serum-PSA test and DRE are used as tools for the early detection of prostate cancer. It is widely acknowledged that the application of the serological PSA test has substantially improved the ability to detect prostate cancer. In the early 1990s, its introduction led to a major increase in the incidence of prostate cancer, and corresponded to an increase of organ-confined, potentially curable disease mostly [20]. In the low PSA ranges (0.0 - 3.9 ng/mL), DRE is the mainstay of early detection. The screening test has often been considered complementary to the PSA test, while its performance is PSA-dependent, and its application requires skilled examiners [2]. Recently, the use of DRE as a screening test for prostate cancer has been criticised for its subjectivity with high inter-observer variability [9], and its poor performance relative to the serum PSA test [21]. It has even been suggested that DRE as an initial screening test for prostate cancer might be discarded in the PSA area that is considered the primary domain of DRE, i.e. the low PSA ranges [8,15].

In the United States, prostate cancer screening is performed in individuals who seek screening and who are interested in the assessment of their risk of the disease

(opportunistic or case-finding screening). The American Cancer Society and American Urological Association recommend that all men from the age of 50 years should undergo PSA testing and DRE on a yearly basis, and from the age of 45 years in men in high risk groups [1,2]. However, a beneficial effect of screening has not yet been established, and with regard to this observation, the major health authorities in a number of European countries discourage opportunistic prostate cancer screening. In their considerations, a reference was made to the early signs of success in the lung cancer screening trials performed in the 1970s [1]. Despite a substantial stage shift due to screening, RCTs did not demonstrate a difference in lung cancer mortality between those screened and those who were not. Also for prostate cancer, only well-performed RCTs will eventually provide a final answer to the question whether screening does more good than harm [24,25]. Until the outcome of these RCTs, efforts should be made in the optimalisation of the applied screening approach. This implies an extensive study of the validity of the screening tests, its effects on the quality of life of screenees, and an evaluation of health care related costs.

At low PSA values, the application of DRE (and TRUS) as initial screening test for prostate cancer has been the topic of debate [6-11,13]. In these low PSA ranges, the reported cancer detection rates are low and the positive predictive values (PPV) of the screening test 'less than desirable' [6,7]. In the ERSPC, the PPV of DRE was between 4% and 33% of men with PSA levels within the 'normal' range (0.0 – 3.9 ng/mL), and this figure averaged 8.8% in men with a PSA level below 3.0 ng/mL [6]. The PPV of TRUS was low as well, i.e. 0% in the PSA range 0.0 – 0.9 ng/mL, and 11% at PSA values between 1.0 – 3.9 ng/mL. The relatively poor performance of DRE has also been encountered in other reports [6,25-28]. A recent report from our department clarified that the yield of rectal examination (both DRE and TRUS) for the detection of prostate cancer was extremely low in low PSA ranges. From a population based study, we calculated that at PSA levels below 3.0 ng/mL, 96 rectal examinations were required to find one case of prostate cancer of any size, grade or stage, and that 289 rectal examinations were needed to find a cancer with assumable clinically significant tumour features [8]. These figures were substantially higher in even lower PSA ranges. How to react on these figures ethically is a question that has to be deliberated by primary health care providers and those who will eventually finance the nation wide screening program.

The above mentioned studies all had in common that positive screening test results were considered true positives, i.e. that the observed abnormalities on DRE or TRUS were caused by the cancer in question. However, the true informative value of a screening test may be overestimated for the finding that cancers may be found after the evaluation of a false positive screening test result. In prostate cancer screening, these false positives are mainly caused by benign prostatic hyperplasia (BPH) or prostatitis. The method of tissue sampling in prostate cancer screening (i.e. sextant biopsy and lesion

directed biopsies from suspicious areas) and the recognition of favourable prognostic indicators (i.e. low tumour volumes) enable this mechanism of the coincidental detection of disease, called serendipity [8]. We defined serendipity-detected cancers as those in which a diagnosis was made at the side of the prostate gland that was opposite to the side that raised suspicion for cancer on DRE or TRUS, or those that had a negative lesion directed biopsy while cancer was present in one of the cores of the biopsy sextant. Furthermore, since it is highly unlikely that cancers with low tumour volumes are palpable on DRE or visible on TRUS, these cases were assumed to be detected by serendipity as well.

Our data from a population-based screening study (ERSPC) indicate that, depending on the definition of serendipity, between 27% and 63% of cases with prostate cancer that were detected in the PSA range 0.0 – 3.9 ng/mL, and in which a biopsy was prompted by a suspicious DRE or TRUS, were detected coincidentally. These were therefore not detected as a result of a true positive test result. The frequency of serendipity-detected cancers was inversely correlated to the serum-PSA level and serendipity accounted for proportionally more cases if DRE was used independently from TRUS (TABLE 8.1). Considering the fact that the majority of serendipity-detected cancers in the PSA-range 0.0 – 3.9 ng/mL had tumour volumes of less than 0.5 mL (TABLE 8.3), acceptance of serendipity might not be advantageous in prostate cancer early detection programs. Small prostate tumours are not considered the primary target cancers in RCTs, i.e. those that are responsible for future prostate cancer mortality, and it may be assumed that patients whom are eventually diagnosed with these seemingly 'biologically insignificant' cancers might have been suitable candidates for conservative therapy and close surveillance if not treated [29-31]. The assumption that these small cancers are biologically insignificant may be strengthened by the observation that most prostate cancers with tumour volumes of less than 0.5 mL are organ-confined and lack poorly differentiated components [29-31]. With a rising tumour volume and a PSA value rising correspondingly, it is likely that these cancers will be detected in a curable stage in successive screening rounds. Even if these small prostate cancers are prone to present themselves clinically in the future and are destined to cause future morbidity and/or mortality, relying on chance to detect these cases may not be a desirable screening objective.

The estimation of prostate cancer detection by serendipity was based on arbitrary assumptions in our study, and the actual magnitude of this coincidental detection of disease might be distinct from the presented figures. As more than half of the biopsy cores (the lesion-directed biopsy and three unilateral biopsies within the sextant) were directed to the side that was suspicious for cancer, the magnitude of serendipity may be underestimated. Bilateral suspicious screening tests may also cause serendipity to be underreported as these cases can only be classified as true-positives using this definition of serendipity. Conversely, it is likely that an investigators finding of a particular

suspicious side on DRE or TRUS may sometimes be erroneously reported (i.e. left versus right, or vice versa) due to the fact that patients are in the left lateral decubitus position. As a consequence, any inconsistencies in DRE and/or TRUS findings between first (screening tests) and second (biopsy) screening visits were not followed by additional lesion-directed biopsies. This low compliance rate to additional biopsy may thus have overestimated the magnitude of serendipity. Also, a palpable or hypoechogenic lesion may still contain cancer even though the lesion-directed biopsy was negative for cancer due to sampling error. In these cases in whom cancer was coincidentally found in one of the cores of the biopsy sextant, claiming serendipity seems premature. We have already stated that a cut-off tumour volume of 0.5 mL to define serendipity was arbitrarily chosen, and that perfect spheres with these volumes might still be palpable on DRE or visible on TRUS. On the other hand, prostate cancers are often ovoid of shape and multifocal, indicating that the likelihood of DRE or TRUS being suspicious for cancer is reduced. Moreover, sensitivity analysis revealed that serendipity still accounted for 55%, 37%, and 24% of the detected prostate cancers when cut-off volumes of 0.4 mL, 0.25 mL, and 0.1 mL, respectively, were used.

With respect to the relatively high contribution of serendipity (chance) in prostate cancer detection, and with regard to earlier studies reporting on the poor performance of DRE and TRUS as screening tests for prostate cancer, and their high inter-observer variability, we cast further doubt on the usefulness of DRE and TRUS as screening test for prostate cancer in low PSA ranges. To avoid unnecessary testing, increase compliance rates to population-based screening, and to encourage cost effective screening programs, it might well be considered to omit DRE and TRUS as initial screening tests for prostate cancer within these low PSA ranges.

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PART V

THE PREDICTIVE VALUE OF TISSUE MARKERS

CHAPTER 9

PROGNOSTIC VALUE OF CELL-CYCLE PROTEINS $p27^{KIP1}$, MIB-1, AND THE CELL-ADHESION PROTEIN CD44S IN SURGICALLY TREATED PATIENTS WITH PROSTATE CANCER

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SUMMARY

BACKGROUND. Molecular tissue markers may give the clinician additional information about prostate cancer patients at risk for treatment failure after retropubic radical prostatectomy (RRP). This study substantiates the prognostic value of three tissue markers, i.e. the cell cycle proteins $p27^{kip1}$, MIB-1, and the cell-adhesion protein CD44s, in addition to more conventional pathological prognosticators, in a historic (pre-PSA) cohort of patients with prostate cancer.

METHODS. Of 92 patients, who underwent RRP, representative tumor sections were immunohistochemically stained with antibodies against $p27^{kip1}$, MIB-1 (Ki-67) and CD44s and assessed in a semiquantitative manner. Gleason score and pathological tumor stage were recorded. All variables were correlated with clinical progression and disease specific survival on univariate and multivariate analyses.

RESULTS. On univariate analysis low ($< 50\%$) $p27^{kip1}$, high ($\geq 10\%$) MIB-1, and loss of CD44s expression were significantly associated with clinical outcome parameters, though MIB-1 did not reach statistical significance for disease specific survival. All three molecules were highly correlated with Gleason score and pathological tumor stage. Multivariate analysis showed that low $p27^{kip1}$ was independent of grade and stage in predicting clinical recurrence ($p < 0.001$) and disease specific survival ($p = 0.045$), while loss of CD44s was an additional independent prognostic factor for clinical recurrence ($p = 0.02$).

CONCLUSIONS. Reduced $p27^{kip1}$ expression is an independent predictor of poor patient outcome in prostate cancer, while MIB-1 is not. Decreased expression of CD44s yields additional information in predicting clinical recurrence. These tissue markers may identify patients at risk for disease recurrence after RRP, who may benefit from adjuvant therapy.

INTRODUCTION

Prostate cancer is the most commonly diagnosed malignancy in the Netherlands in men between 55 and 74 years of age and is, after lung cancer, the second most common cause of cancer-related death within the male population [1]. Management decisions of patients with prostate cancer should ideally depend on an accurate assessment of the biological potential of the tumor. Tumors that are likely to progress and influence patient outcome have to be distinguished from those which are indolent and will not affect patient prognosis, even without treatment. While pre-operative serum prostate-specific antigen (PSA)-level, clinical stage and Gleason score of the tumor in the prostatic needle biopsies can predict pathological tumor stage and patient outcome to some extent [2,3], this prediction is hardly applicable to the individual patient, mostly because of wide ranges of confidence intervals. After retropubic radical prostatectomy (RRP), Gleason score and pathological tumor stage remain the most powerful predictors of clinical outcome, more powerful than tumor volume, surgical margins or the presence of perineural invasion [4,5]. Again, these prognostic factors cannot be applied to the individual patient. In order to search for additional prognosticators which can predict disease recurrence and patient prognosis on an individual basis, special attention has recently been paid to certain tissue markers involved in cell cycle regulation, e.g. $p27^{kip1}$ and MIB-1.

Cell cycle regulation is influenced by nuclear proteins that enhance cell division, the cyclin dependent kinases (cdks), or disrupt cell division, the cdk-inhibitors (cdki), of which $p27^{kip1}$ is one. When cdks bind to cell cycle specific cyclins, cell proliferation is stimulated by phosporylation of certain proteins, involved in DNA-replication (G1-S) or mitosis (G2-M). $p27^{kip1}$ inhibits cell proliferation by binding and inactivating the cdk-cyclin unit, thereby blocking the transition from G1 to S-phase [6]. In a great variety of solid tumors decreased expression of $p27^{kip1}$ is associated with malignant behavior and poor patient outcome, e.g. in breast [7,8] and colorectal [9,10] carcinomas. Also in prostatic carcinoma decreased levels of $p27^{kip1}$ have recently been associated with poor tumor grade, tumor progression and poor patient survival [11-16]. The nuclear Ki-67 protein, which can be visualized by the MIB-1 antibody, is expressed in all proliferating cells (G1-S-G2-M0-phase), but not in quiescent cells (G0-phase) or in the early G1-phase. The proliferative index (PI) of prostate cancers has by some authors been indicated as a predictive marker of clinical outcome [17-20]. Other study groups could not find a relation between PI and patient prognosis, indicating that this relation needs to be further clarified [21,22]. In prostate cancer loss of the transmembranous cell-adhesion protein CD44s was found to be an independent prognostic tumor marker for biochemical and clinical progression, but not for disease specific survival [23,24].

We performed this study to compare the prognostic value of three tissue markers, i.e. $p27^{kip1}$, MIB-1 and CD44s, in addition to more conventional prognostic factors, as Gleason score and pathological tumor stage, on clinical outcome in a (pre-PSA) cohort of patients with long-term follow-up, who underwent RRP for histologically proven prostate cancer. To our knowledge, this is the first study to evaluate the prognostic role of $p27^{kip1}$, combined with other important prognostic tissue markers, by multivariate analysis.

MATERIALS AND METHODS

Patients

Between 1980 and 1988 159 consecutive patients were operated at the University Hospital Rotterdam, the Netherlands, for clinically localized prostate cancer ($cT_{1-3}N_xM_0$, TNM '92). In 49 patients pelvic lymph node dissection (PLND) showed lymphogenic metastatic disease on intra-operative examination of frozen tissue sections. In these patients no subsequent retropubic radical prostatectomy (RRP) was performed, except for two patients, who had only microscopic, focal involvement. Therefore, a total of 112 patients underwent RRP for histologically proven prostatic carcinoma with curative intent. This cohort of patients was followed at regular intervals and all data concerning pathologic tumor characteristics, time to disease recurrence, subsequent treatment and patient survival have prospectively been stored in a comprehensive database.

Routine measurement of serum-PSA was not available untill the beginning of 1988 in our clinic. Therefore, exclusion of cases with incomplete PSA follow-up data, i.e. no regularly determined postoperative PSA-measurements, resulted in only 18 cases in whom this intermediate end-point could be considered. This cohort was assumed to be of too low statistical power to make comparisons. Clinical progression was defined as histologically proven recurrence of cancer near the vesico-urethral anastomosis or as proven distant metastases on radionuclide bone scintigraphy, abdominal computerized tomography (CT), X-ray image of the thorax or ultrasound image of the liver. Time to clinical recurrence was defined as the time from RRP to the time of clinical progression or to date of last follow-up, if the subject had no evidence of disease recurrence. In case of clinical tumor progression patients were offered treatment guided by their urologist. Tumor death was recorded by the urologist as death directly related to prostate cancer, whether caused by tumor load, tumor related complications or tumor related therapy and survival was calculated as the time of RRP to time of prostate cancer related death or in case patients were still alive to date of last follow-up.

Tissue Specimens

The radical prostatectomy specimens were routinely fixed in 10% buffered formalin at pH = 7.4, embedded in paraffin, freshly cut into 4 µm thick sections and mounted on amino alkylsilane (AAS)-coated glass slides. Haematoxylin & eosin slides were reviewed by a specialized genitourinary pathologist (THvdK), for all tumor sections within the prostate the Gleason growth pattern was determined, and the tumor was staged according to the pathological TNM '92 system. The tissue material of 16 radical prostatectomy specimens was unavailable for immunohistochemical staining analysis. In three radical prostatectomy specimens obtained after transurethral resection of the prostate (TURP), i.e.T_{1a-b}, the tumor could not be found. These three patients were staged $pT_0N_{0-1}M_0$. In one patient the tumor was diagnosed as a metastasis of a coloncarcinoma. Hence, 20 patients were excluded from further analysis, leaving 92 patients included in the study. Of all remaining radical prostatectomy specimens 1-3 paraffin tissue blocks, representative for the whole tumor were selected. The selection was made on presence of the poorest grade within the radical prostatectomy specimen, assuming that these growth patterns within the tumor would predict patient outcome.

Immunostaining

After deparaffinization through xylene and 100% ethanol, endogenous peroxidase activity was blocked by immersing the slides for 20 min in a 3% H_2O_2/methanol bath. The slides were placed in a 10 mmol/L citrate buffer at pH = 6.0. Antigen retrieval was performed in a microwave oven at 700W for 15 minutes. After cooling, the slides were placed in a Sequenza immunostaining system (Shandon, UK) and pre-incubated with 10% normal goat serum (DAKO, Glostrup, Denmark) in PBS/BSA 5%. Then the slides were incubated overnight at 4 ° C with the primary antibody MIB-1 (Immunotech, France) at a optimal dilution of 1: 3000 or $p27^{kip1}$ (Novocastra, UK) at 1: 40 in PBS/BSA 5%. In each batched series negative controls were included. For all immunostainings the conventional avidin-biotin complex method was applied. Briefly, a 30 min incubation with biotinylated goat-anti mouse antibody (Biogenex, San Ramon, USA) was followed by a 30 min incubation with streptavidin-peroxidase complex (Biogenex). Subsequently, the antibody-antigen binding was visualized with diaminobenzidine hydrochloride (Fluka, Neu-Ulm, Germany) with 0.08% H_2O_2 and the specimens were lightly counterstained with Mayer's Haematoxylin, dehydrated and covered.

Quantitation

All slides were assessed by two independent observers without knowledge of clinical data. Almost all selected sections contained benign prostatic glands, which could serve as internal positive controls for both $p27^{kip1}$ and MIB-1. For $p27^{kip1}$, nuclear staining was assessed on a continuous scale from 0 to 100% by estimating a positive to total ratio, thereby assessing the whole tumor area. The tumor slides were also classified using the scoring system according to Catzavelos et al [3]: 1, 0 - 25%; 2, 26 - 50%; 3, 51 - 75%; 4, 76 - 100% positive nuclear staining. It is assumed that a decreased expression of the $p27^{kip1}$ protein is associated with worse patient prognosis. Therefore, in case of tumor heterogeneity, only those parts within the tumor that showed lowest positive to total ratio in particular were assessed. This was performed only if these regions comprised at least 10% of the tumor load in the tissue section. If more than one slide of a tumor was selected, the slide with the lowest positive to total ratio was considered to be most predictive for final patient outcome and this tumor $p27^{kip1}$ ratio was taken for further statistical analysis.

For MIB-1, nuclear staining was assessed by estimating the percentage of MIB-1 positive cells, i.e. the proliferation index (PI), in a particular area in a semiquantitative manner: r, rare (occasional nuclear staining; comparable to benign prostatic glands); 1+, < 10%; 2+, 10 - 24%; 3+, ≥ 25%. In line with the $p27^{kip1}$ staining assessment, if the tumor exhibited heterogenuous MIB-1 expression, the area with the highest density of MIB-1 positive cells was selected and analysed further.

All data concerning the semiquantitative assessment of CD44s expression (categorized in: 0, < 10%; 1, 10 - 25%; 2, 25 - 50%; 3, > 50%) were available from previous studies at our institution and these unchanged data were used for comparison to the investigated prognostic variables [23].

Statistical Methods

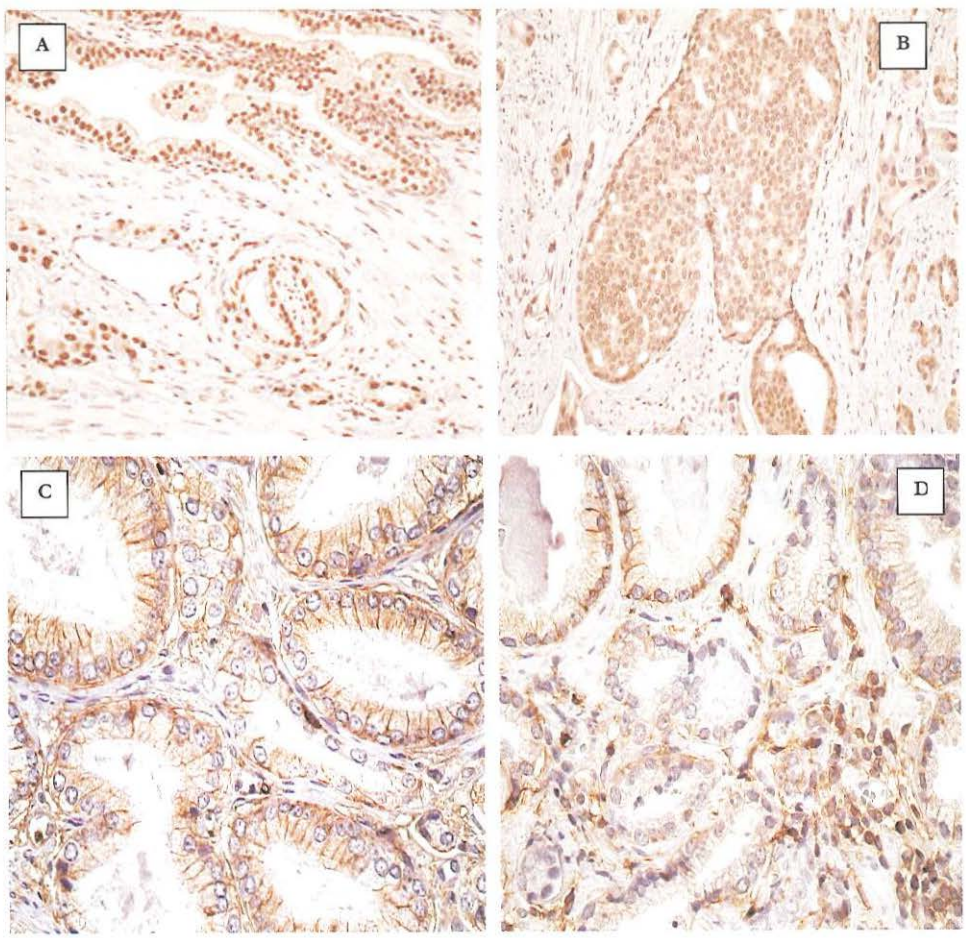
Statistical analysis was performed using the statistical package for the social sciences (SPSS 8.0). Cox proportional regression analysis was used to assess the relationship between baseline variables and clinical outcome parameters. The variables examined for their prognostic value were radical prostatectomy Gleason score (categorized in 2-6, 7, 8-10), pathological tumor stage (categorized in pT_{2a-c}, pT_{3a-b}, pT_{3c-4}; TNM '92), lymph node status (categorized in pN_0 and pN_1), expression of cell cycle proteins $p27^{kip1}$ and MIB-1, and expression of cell-adhesion protein CD44s [23].

Although we reached statistical significance for the different pathological parameters at different cut-off points of $p27^{kip1}$ expression, i.e. 25%, 40% and 50%, we choose a cut-off

point of 50% (high vs. low) $p27^{kip1}$ positive staining for final statistical analysis with respect to its ease of being determined and its reproducibility by independent observers.

FIGURE 9.1

$p27^{kip1}$ nuclear and CD44s membranous immunostaining of prostate cancer. *A.* High (≥50%) positive to total ratio for $p27^{kip1}$ protein in prostate cancer showing perineural invasion, in combination with normal prostatic tissue, counterstained with Haematoxylin. *B.* Low (<50%)positive to total ratio for $p27^{kip1}$ in large cribriform fields of prostatic adenocarcinoma. *C.* Strong immunostaining of CD44s in prostatic neoplastic glands, and *D.* Reduced immunostaining of the CD44s protein in prostate cancer cells



For confirmation, receiver operating characteristics (ROC) analysis was performed to determine the most appropriate cut-off level for $p27^{kip1}$ for the different clinical outcome parameters. For MIB-1 a cut-off level of 10% was chosen, again confirmed with ROC analysis. Kaplan-Meier curves were constructed for $p27^{kip1}$ to show the probability of clinical progression and cause specific death as a function of time after RRP. Association between tissue markers and known prognostic variables was calculated using the Spearman's correlation test. To identify independent prognostic factors, forward stepwise Cox regression analysis was performed by entering variables in the model that were statistically significant at the univariate level, while controlling for the other variables in the model. Backward stepwise elimination was done to verify that the same parameters remained in the final models. Statistical significance was set at $p < 0.05$.

RESULTS

Patient Cohort

For the 92 patients included in the study median age was 63 years (range, 48 - 76) and median follow-up comprised 9.4 years (range, 0 - 17). These data include the two patients who died within one month after surgery due to myocardial infarction and pulmonary embolism, respectively. No pre-operative serum PSA-levels were available. 18 Patients had clinical T_{1a-b} disease, 47 patients had clinically organ-confined disease (cT_{2a-c}), and 27 patients had clinically stage T_3. Six patients showed metastatic lymph node disease after evaluation of paraffin slides, i.e. $pT_{2-3}pN_1$, including the two patients who showed lymphogenic metastatic disease intra-operatively. No patient received pre-operative therapy of any kind. Pathological data are listed in TABLE 9.1. Of the patients with clinical recurrence eventually half (17 out of 36, at last follow-up) died of prostate cancer after a median time of 34 months after first evidence of clinical progression.

Immunohistochemistry of Prognostic Tissue Markers

For $p27^{kip1}$ mostly strong nuclear immunostaining was noted in the benign glands surrounding the tumor areas. the positive to total ratio for benign prostatic tissue was assessed to be 80 to 90% (FIGURE 9.1). In most benign hyperplastic noduli, which were occasionally present in the slides, expression of $p27^{kip1}$ protein was decreased, resulting in a decreased positive to total ratio. This is in keeping with an increased proliferative state of these hyperplastic noduli. The intensity of staining of $p27^{kip1}$ within and between tumors was highly variable. To define a marginal value of nuclear immunostaining, $p27^{kip1}$ assessment will account for interobserver variability and this may be further increased by

tumor heterogeneity and focal downregulation. Variability in staining intensity also holds true for other cycling proteins, like Ki-67. Compared to $p27^{kip1}$, tumor heterogeneity and focal clustering seemed even more outspoken for MIB-1 expression. The 50% cut-off level of $p27^{kip1}$ assessment was confirmed by ROC-curve analysis for the prediction of both disease recurrence (FIGURE 9.2) and disease specific survival (data not shown). Comparing the assessments of two observers for interobserver variability, less than 10% of cases changed category for each tissue marker (i.e. low to high expression, or vice versa).

TABLE 9.1
Tissue marker expression and tumor characteristics

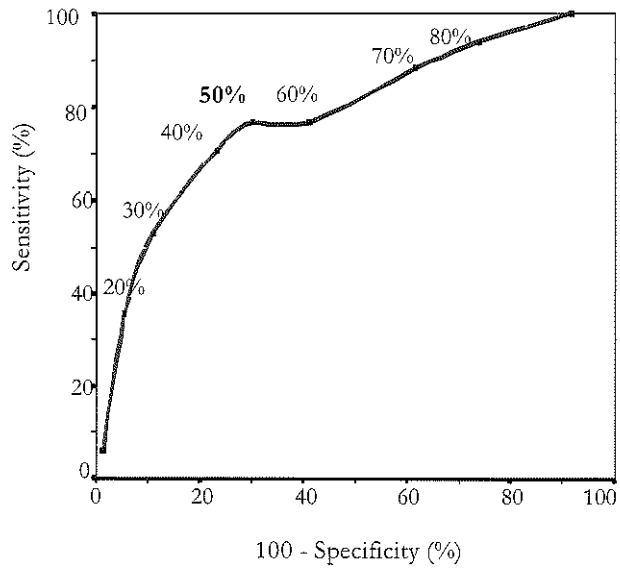
	$p27^{kip1}$ expression (positive to total ratio)		MIB-1 expression (positive to total ratio)		
Variable	0 - 49% No. (%)	≥ 50% No. (%)	0 - 9% No. (%)	≥ 10% No. (%)	Total No. (%)
Pathological stage					
T_2	4 (11)	20 (35)	18 (41)	6 (13)	24 (26)
T_{3a-b}	11 (31)	15 (26)	11 (25)	15 (31)	26 (28)
T_{3c-4}	20 (57)	22 (39)	15 (34)	27 (56)	42 (46)
RRP Gleason score					
2-6	6 (17)	19 (33)	17 (39)	8 (17)	25 (27)
7	14 (40)	27 (47)	19 (43)	22 (46)	41 (45)
8-10	15 (43)	11 (19)	8 (18)	18 (38)	26 (28)

Of the 92 tumors evaluated 35 (38%) were assessed as expressing low (< 50%) $p27^{kip1}$ protein and 16 (17%) as very low or absent (0 - 24%) expression. 13 of 35 $p27^{kip1}$ negative tumors were highly heterogeneous with focal regions of low $p27^{kip1}$ expression within large tumor fields of high $p27^{kip1}$ expression. It was noticed that cells expressing low $p27^{kip1}$ protein were often localized within large cribriform fields and within large solid tumor areas, while this was less frequently present within small groups of cells infiltrating the prostatic stroma (FIGURE 9.1). For MIB-1 10 tumors had rare, occasional, nuclear reactivity, 38 tumors showed expression in 0 - 9% of nuclei, and a total of 48 out

of 92 (52%) tumors were recorded as having high MIB-1 (≥ 10%) expression (TABLE 9.1). For expression and assessment of CD44s we refer to previously published data and to FIGURE 9.1 [23].

FIGURE 9.2

ROC-curve analysis for the prediction of disease recurrence at different cut-off levels of $p27^{kip1}$ assessment (n=92). The optimal and most appropriate cut-off level (i.e. highest sensitivity and specificity) lies between 40% and 50% positive to total ratio.



On univariate analysis low (< 50%) $p27^{kip1}$ expression, high (≥ 10%) MIB-1 expression and loss of CD44s expression were all associated with clinical recurrence and cause specific death, though MIB-1 did not reach statistical significance for the latter (TABLE 9.2). Both grade and pathological tumor stage were also highly associated with clinical outcome parameters.

TABLE 9.2
Univariate analysis of pathological prognostic markers

	Clinical follow-up data			
	Clinical progression		Disease specific survival	
Prognostic factor	χ^2	p-value*	χ^2	p-value*
Gleason Score	23.5	< 0.0001	14.5	< 0.001
pT	25.2	< 0.0001	9.3	< 0.01
pN	1.7	ns	0.8	ns
$p27^{kip1}$	23.5	< 0.0001	10.6	< 0.01
MIB-1	9.7	< 0.01	2.6	ns
CD44s	17.3	< 0.0001	5.3	0.02

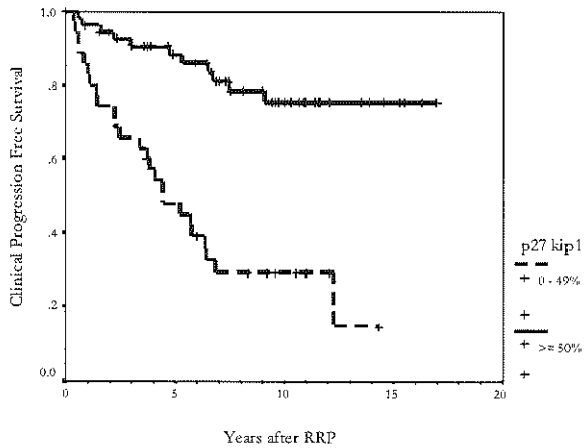
*Logrank test (for trend)
ns: not significant

FIG. 9.3 A-B show graphically the relationships between the expression of $p27^{kip1}$ and clinical outcome parameters in subsequent Kaplan-Meier curves. The probability of being free of treatment failure at 5 and 10 years of follow-up were 37% and 26% for low $p27^{kip1}$ expression, while these were 79% and 77% for high $p27^{kip1}$ expression, respectively. For MIB-1 these figures were 75% for both 5 and 10 years of follow-up for low expression and 52% and 42% for high MIB-1 expression, respectively. In Spearman's correlation analysis Gleason score of the radical prostatectomy specimen was inversely associated with $p27^{kip1}$ expression ($r = -0.26$, $p = 0.01$; TABLE 9.3). Low $p27^{kip1}$ expression was also inversely correlated with pathological tumor stage ($r = -0.24$; $p = 0.02$) and lymph node stage ($r = -0.25$; $p = 0.02$), but not with MIB-1 expression and only tended to correlate with CD44s expression ($r = 0.20$, $p = 0.06$; TABLE 9.3). Both MIB-1 and CD44s were strongly associated with Gleason score and pathological tumor stage and with each other. Pathological tumor stage was highly correlated to Gleason score (data not shown; $r = 0.55$, $p < 0.0001$).

FIGURE 9.3

Kaplan-Meier curve of the nuclear expression of the cell cycle protein $p27^{kip1}$ using a cut-off level of 50% positive to total ratio, in relation to time after retropubic radical prostatectomy (RRP), concerning **A.** Clinical progression free survival ($p<0.0001$), and **B.** Disease specific survival ($p<0.01$).

A.



B.

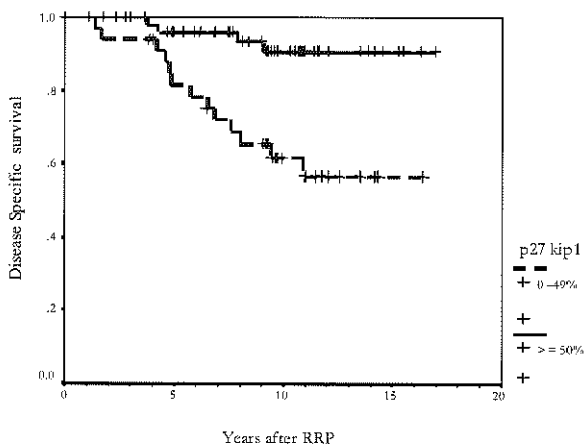


TABLE 9.3

Correlation of cell cycle proteins $p27^{kip1}$ and MIB-1, and the cell-adhesion protein CD44s with pathological characteristics. In cells are listed the correlation coefficients (r) and p-values according to Spearman's rank test

	$p27^{kip1}$		MIB-1		CD44s	
	R	p - value	r	p - value	R	p - value
Gleason score	-0.26	0.01	0.28	0.008	-0.31	0.003
pT	-0.24	0.02	0.30	0.006	-0.49	0.001
pN	-0.25	0.02	0.08	0.47	-0.16	0.12
$p27^{kip1}$	1.0	1.0	-0.17	0.11	0.20	0.06
MIB-1	-0.17	0.11	1.0	1.0	-0.23	0.03
CD44s	0.20	0.06	-0.23	0.03	1.0	1.0

TABLE 9.4

Multivariate analysis of pathological prognostic factors

	Clinical follow-up data					
	Clinical progression			Disease specific survival		
Variable	$e^{\beta,*}$	CI†	p-value	$e^{\beta,*}$	CI†	p-value
Gleason score	-	-	0.06	3.48	1.44 - 8.35	< 0.01
pT	2.85	1.50 - 5.43	< 0.01	-	-	ns
pN	-	-	ns	-	-	ns
$p27^{kip1}$	4.14	2.00 - 8.54	< 0.001	3.26	1.02 - 10.35	0.045
MIB-1	-	-	ns	-	-	ns
CD44s	0.65	0.45 - 0.95	0.02	-	-	ns

* e^{β}: Risk ratio
† CI: 95% confidence interval
ns: not significant

Multivariate analysis using the Cox regression analysis showed that low expression of the $p27^{kip1}$ protein was independent of grade, pathological tumor stage, and other tissue markers in predicting clinical recurrence ($p < 0.001$) and disease specific survival ($p = 0.045$), though with wide confidence intervals, referring to the small sample size (TABLE 9.4). Also pathological tumor stage showed to be a significant predictor of clinical failure, but not of disease specific survival. Gleason score was the most powerful predictor of disease specific survival ($p < 0.01$) on multivariate analysis, but not of disease recurrence, indicating the strong correlation between grade, stage and $p27^{kip1}$ expression. Loss of CD44s expression was an independent prognostic factor in the prediction of clinical recurrence, while MIB-1, as a marker of proliferation, failed to be a predictor of patient outcome after correction for other pathological prognosticators.

DISCUSSION

Application of prognostic tissue markers, in addition to conventional variables as serum PSA, grade, stage and surgical margins, will help the clinician in identifying biological aggressive tumors and thereby patients at risk for disease recurrence after intentive curative surgery. Correspondingly, in an effort to ensure definite cancer control, assessment of expression of prognostic tissue molecules may select candidates for adjuvant treatment, whether radiotherapy or hormonal ablation therapy. A large arsenal of molecular tissue markers have been studied in prostate cancer recently, some of which have individually proven to be of prognostic value, even independent of Gleason score and pathological tumor stage. To confirm the prognostic value of three tissue markers, i.e. $p27^{kip1}$, MIB-1 and CD44s, we related expression of each of these proteins, additive to grade and stage, to clinical outcome in a historic cohort of patients from the pre-PSA era undergoing retropubic radical prostatectomy (RRP) for prostate cancer. We show that low (< 50%) expression of the cell cycle protein $p27^{kip1}$ is an important predictor of clinical progression ($p < 0.001$) and decreased disease specific survival ($p = 0.045$) in patients with prostate cancer, additional to Gleason score, pathological tumor stage and the other evaluated tissue markers. MIB-1 (Ki-67) shows to be a significant prognostic factor on univariate analysis using a proliferation index (PI) of 10% as cut-off (TABLE 9.2), but this association was not sustained after including more powerful variables on the multivariate analysis (TABLE 9.4).

A remarkable finding in this study was that no correlation could be determined between MIB-1 expression, a profound marker of proliferative activity, and $p27^{kip1}$ expression ($r = -0.17$, $p = 0.11$; TABLE 9.3). As a protein involved in cell cycle transition cycling cells were expected to downregulate the $p27^{kip1}$ protein, as is noted in hyperplastic

BPH noduli. Apparently, in prostate cancer, being a relatively slow growing tumor, factors other than proliferation rate alone will determine tumor aggressiveness and patient outcome. Palmquist *et al.* previously reported that $p27^{kip1}$ expression not merely controls cell cycle progression, but might also be associated with other mechanisms responsible for aggressive tumor behaviour [10]. It is assumed that low levels of $p27^{kip1}$ interfere with the unability of cells to halt cell cycling, through which additional (genetic) alterations lead to an aggressive, infiltrating growth [25]. So far, few alterations and mutations in the $p27^{kip1}$ gene and the mRNA transcript have been reported [26], indicating that decreased levels of the $p27^{kip1}$ protein result from yet unknown influences on a posttranslational level. Preliminary results from our department suggest that low (< 50%) $p27^{kip1}$ expression also occurs in early-detected, preclinical (T_{1c}) cancers in radical prostatectomy specimens in a frequency comparable to that reported in the pre-PSA cohort, also after correcting for Gleason score and pathological tumor stage. Hence, low expression of $p27^{kip1}$ might be an early event in carcinogenesis. The (molecular) mechanisms by which downregulation of the $p27^{kip1}$ protein lead to aggressive tumor behavior, however, need to be further elucidated.

Recent reports concerning the prognostic role of $p27^{kip1}$, and its association to other prognostic variables, have shown conflicting results. Some groups reported an association between low $p27^{kip1}$ expression and biochemical and treatment failure [12,14], while others could not determine such an association [15]. Subsequently, an association of $p27^{kip1}$ expression and prostate cancer related survival has not been established. Differences in study design and selection of cut-off point of immunohistochemical nuclear reactivity may account for these discrepancies. For application of an independent prognostic tissue marker in routine clinical diagnostics, standardisation of marker assessment is needed. In this, the selection of a cut-off point of tumor marker assessment should meet three criteria; 1. a (sub) optimal cut-off point needs to be confirmed by ROC-curve analysis, 2. the cut-off point needs to be 'easy to assess' to reduce inter-and intraobserver variability, and 3. the cut-off point needs still to be an independent prognosticator on an independent patient series. Our study indicates that a cut-off point of 50% (low vs. high) of $p27^{kip1}$ immunostaining meets the first two criteria and is thereby justified (FIGURE 9.2). Since the study population was small in design with small number of patients in individual subgroupings, other studies need to confirm the independent value of the obtained cut-off points. Furthermore, in reducing biopsy sampling error caused by tumor heterogeneity and tumor multifocality, assessment of specific tumor areas predictive of patient outcome, rather than a time consuming cell-counting in randomly chosen high-power fields, is preferred. Identification of tumor areas with a morphologically worse appearance may be rather quick and has low risk of assessing non-representative areas within the tumor. Though, as is the case for the

Gleason grade system, tumor heterogeneity may be especially prone to result in biopsy sampling error when tumor marker expression is concerned.

In our study, Gleason score of the radical prostatectomy specimen remains the most powerful predictor of tumor related death ($p < 0.01$), while pathologic tumor stage is a strong independent prognostic factor for treatment failure and disease recurrence (TABLE 9.4). Decreased expression of the cell-adhesion protein CD44s yields additional prognostic information in the prediction of clinical progression ($p = 0.02$), independent of $p27^{kip1}$, grade and stage. Strikingly, no association could be determined between node status (pN) and patient outcome, though being a part of the pTNM-classification. An explanation for this is the selection bias, prior to surgery.

In retrospective analysis studies, the independent prognostic value of specific molecular tissue markers is well established in predicting patient outcome. Detection of molecular features indicating aggressive disease in both the radical prostatectomy specimen, as well as in the prostatic needle biopsy, may help to identify clinically relevant cancers, thereby selecting patients for (adjuvant) treatment. The other way around, absence of aggressive features in the tumor would identify those who would be suitable candidates for watchful-waiting. Though, prospective studies concerning the role of these markers on an individual basis, have not been performed, yet.

CONCLUSIONS

Our results show that reduced (< 50%) expression of the cell cycle protein $p27^{kip1}$ is a sustained independent predictor of poor patient outcome in prostate cancer, also after including powerful prognostic variables in the analysis, while MIB-1 (Ki-67) is not. Decreased expression of the cell-adhesion protein CD44s yields additional information in the prediction of clinical recurrence. These prognostic tissue markers may distinguish patients at high risk for disease recurrence after radical prostatectomy -and thus might benefit from adjuvant therapy- from those who may be curatively treated.

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CHAPTER 10

QUANTITATIVE ANALYSIS OF THE DECAY OF IMMUNOREACTIVITY IN STORED PROSTATE NEEDLE BIOPSY SECTIONS

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SUMMARY

BACKGROUND. Application of immunohistochemistry to assess presence of prognostic tissue markers is widely used. The quantitation of these markers may be hampered by a time-related loss of antigenicity in formalin-fixed paraffin-embedded tissue stored on glass slides.

METHODS. Potential loss of immunohistochemical staining intensity was examined on prostatic needle biopsy sections stored for a maximum of 4 years with antibodies against $p27^{kip1}$, CD44s, MIB-1 and AR. In benign tissue the positive to total ratio for $p27^{kip1}$ was determined, while CD44s staining intensity was assessed semiquantitatively. For MIB-1 and AR nucleair staining intensity was assessed using computed image analysis.

RESULTS. An exponential and significant decay of immunoreactivity was seen for $p27^{kip1}$ ($p < 0.01$), CD44s ($p < 0.01$), MIB-1 ($p < 0.001$) and AR ($p < 0.001$) with half-lives of 587 days, 214 days, and 290 days for $p27^{kip1}$, MIB-1 and AR, respectively.

CONCLUSIONS. Immunohistochemical assessment of prognostic tissue markers on stored slides must be considered with care in both research and clinical settings.

INTRODUCTION

Application of immunohistochemistry to investigate the expression of tissue markers on formalin-fixed, paraffin-embedded tissue is widespread and the prognostic role of some of these markers is used as a diagnostic and therapeutical decision-making tool in several cancers. The need for additional prognostic tumormarkers is a drive for considerable research efforts in many institutions. To evaluate the prognostic importance of a tissue marker by immunohistochemistry, well-fixed, adequately processed and preserved tissue material is a prerequisite for the prevention of false positive and/or negative staining outcome. In an effort to stain specific markers of interest in prostate needle biopsies, we happened to notice a potential loss of immunoreactivity over time in formalin-fixed, paraffin-embedded tissue stored on glass slides.

In most clinical and research settings it is a common practice to store precut unstained tissue sections on glass slides for reasons of direct access to positive control slides. Secondly, tissue specimens are stored on glass slides for retrospective studies in case there is too little tissue material left in the paraffin blocks after completion of routine diagnostics. This holds particularly true for prostate and mamma 18-gauge needle biopsy specimens.

Recently, several authors reported a loss, or occasionally an increase, of antigenicity in paraffin sections stored on glass slides [1-4]. Unfortunately, the results of these studies lack general applicability, because of their use of highly unorthodox tissue fixation methods. Therefore, we wanted to repeat these studies on loss of immunostaining intensity on tissue specimens, fixed and processed according to an ontime method, commonly applied in the majority of pathologic labaratories. The potential loss of immunostaining intensity of four prognostic tissue markers for prostate cancer was studied on tissue sections of prostatic needle biopsies stored for a maximum of 4 years [5-12]. The expression of antigens in stored slides was compared to that in freshly cut paraffin blocks. By this approach we demonstrated an exponential decay of immunoreactivity throughout the years of all four investigated prognostic tissue markers.

MATERIALS AND METHODS

Between 1994 and 1998 all prostate needle biopsy specimens were routinely fixed in 10% buffered formalin at pH = 7.4, embedded in paraffin, cut into 4 μm tissue sections and mounted on glass slides. Unstained tissue sections representative for the detected prostate cancer were stored for later use on glass slides coated with amino-alkylsilane

(AAS), in a dark environment at room temperature. To evaluate loss of immunostaining we immunohistochemically stained and assessed a series of 7 slides of the subsequent storage years 1994 to 1998 with antibodies against the nuclear, cell-cycle marker MIB-1 (Immunotech, France) and $p27^{kip1}$ (Novocastra, UK), the cell-cell adhesion protein CD44s (Bender MedSystems, Austria) and the Androgen Receptor (AR) (clone F39.4.1) [13]. For each marker batched series, including freshly cut specimens of prostate biopsies of 1994 were immunostained.

Immunostaining

After deparaffinization through xylene and 100% ethanol, endogenous peroxidase activity was blocked by immersing the slides for 20 minutes in a 3% H_2O_2/methanol bath. The slides were placed in a 10 mmol/l citrate buffer at pH = 6.0. Antigen retrieval was performed in a microwave oven at 700 W for 15 minutes. After cooling, the slides were placed in a Sequenza immunostaining system (Shandon, UK) and pre-incubated with 10% normal goat serum (DAKO) in PBS/BSA 5%. Then, the slides were incubated overnight at 4 °C with the primary antibody MIB-1 at an optimal dilution of 1 : 3,000, $p27^{kip1}$ at 1 : 40, anti-AR at 1 : 200 and anti-CD44s at 1 : 20 in PBS/BSA 5%. For all immunostainings the conventional avidin-biotin complex method was applied. Briefly, a 30 min incubation with the biotinylated goat-anti mouse antibody (Biogenex, San Ramon, USA) was followed by a 30 min incubation with the streptavidin-peroxidase complex (Biogenex). Subsequently, the antibody-antigen binding was visualized with diaminobenzidine hydrochloride (Fluka, Neu-Ulm, Germany) with 0.08% H_2O_2 and the CD44s and $p27^{kip1}$ stained specimens were lightly counterstained with Mayer's Haematoxylin, dehydrated and covered. No counterstaining was performed for MIB-1 and AR.

Quantitation

Staining in the benign prostatic glands was blindly assessed. For $p27^{kip1}$ and CD44s stained slides this was performed by two independent observers (ANV, THvdK). For $p27^{kip1}$ a total of 400 nuclei was counted and a positive to total ratio was calculated. For CD-44s the membranous staining was scored semiquantitatively as 0 = absent, + = weak, only in basal cells, ++ = moderate, in basal cells and sporadically also in luminal cells or +++ = intense, in basal cells and most luminal cells. For MIB-1 and AR nuclear staining intensity was assessed by a single observer (ANV) using a computer video-image analysis program (KS 400, Kontron Elektronik, GmbH., Germany). For each slide a total of 20 randomly selected color video images of 512 × 512 pixels with a resolution of 0,4348 μm

per pixel was recorded. Of all nuclei above a prefixed threshold, the inversed mean density was measured by the computer program. The detection of the immunoperoxidase product was enhanced by omission of the Haematoxylin counterstaining.

Statistical Methods

The Chi-square (χ^2) test was used to determine the significance of differences in CD44s score in the different storage years. The Mann Whitney U test was used to assess differences in mean positive to total ratio for $p27^{kip1}$, absolute immunostaining intensity and number of detected nuclei per slide for MIB-1 and AR. The level of significance was set at 0.05. For $p27^{kip1}$, MIB-1 and AR the difference between day of storage and day of immunostaining was calculated and a decay curve of relative antigen expression was generated using the formula $y_t = 100 * 2 \exp(-\alpha xt) + a_0$, in which y_t stands for the relative antigen expression as a percentage, x_t for days of storage before immunostaining and in which α and a_0 are parameters assessing the curvature and horizontal asymptote of the exponential decay curve. Using this formula, the day of immunostaining ($x_0 = 0$) stands for a relative antigen expression of 100% ($y_0 = 100$). A half-life ($x_{½}$) of antigen expression was calculated by replacing factor x_t with $1/\alpha$. TABLE 10.3 shows the exact figures for α and a_0 for the different tumormarkers.

RESULTS

For $p27^{kip1}$ there was a gradual, but consistent decrease of mean immunopositive to total ratio throughout time varying from 75.9% ± 12.49% in 1998 to 11.96% ± 30.85% in 1994 (TABLE 10.1; $p < 0.01$) with a calculated half-life of $p27^{kip1}$ antigen expression of 587 days. The freshly cut specimens had a mean ratio of 45.1% ± 30.6%. This was not statistically different from the slides stored in 1998 according to the Mann Whitney U test. The mean interobserver variance was 6.3% per slide. Similarly, for CD-44s a significant and continuous decay was recorded throughout time (TABLE 10.2; $p < 0.01$). In these slides, the discrepancy in interobserver slide assessment of more than one digit was noted in only 2 of 36 slides (6%). A significant loss of nuclear immunostaining intensity was seen throughout time for MIB-1 as was quantitated by computer-assisted image analysis (TABLE 10.1; $p < 0.001$), while the number of separately detected nuclei per slide could be kept relatively constant per storage year. For AR both the total number of detected nuclei per storage year and the mean number of detected nuclei per slide

decreased significanty with time (TABLE 10.1; $p < 0.001$), but in the remaining detected nuclei the mean measured immunostaining intensity was not different per storage year.

TABLE 10.1

Immunostaining assessment of tissue markers $p27^{kip1}$, MIB-1 and anti-Androgen Receptor (AR)

		$p27^{kip1}$†			MIB-1‡			AR§
Store year	N	Mean pos/ total ratio (%) ± SD	Nuclei measured	Nucl per slide	Inverse Mean density ± SD	Nuclei measured	Nucl per slide	Inverse Mean density ± SD
94	7	11.96 ± 30.85	440	62.9	168.3 ± 15.0	10	1.4	174.51 ± 9.18
95	7	23.11 ± 11.46	253	36.1	164.3 ± 18.9	44	6.3	191.52 ± 9.58
96	7	33.06 ± 8.56	230	32.9	165.8 ± 18.6	300	42.9	189.93 ± 6.12
97	7	51.64 ± 12.34	355	50.7	157.4 ± 19.9	1027	146.7	191.00 ± 6.31
98	7	75.90 ± 12.49	369	52.7	142.5 ± 25.0	1975	282.1	187.04 ± 8.80
94*	4	47.18 ± 30.85	66	16.4	146.9 ± 21.5	770	192.5	178.73 ± 12.79

* positive control; freshly cut specimen
† $p < 0.01$ Mann-Whitney U
‡ $p < 0.001$ Mann Whitney U
§ $p < 0.001$ Mann Whitney U

The half-life of immunohistochemical staining intensity for MIB-1 was calculated as 214 days and for AR the half-life of proportional nuclear immunopositivity comprised 290 days (FIGURE 10.1). For both MIB-1 and AR the immunostaining intensity and detection of the positive controls, the freshly cut specimens of the paraffin-blocks of 1994, were comparable with the immunostaining results of the slides stored in 1998 (TABLE 10.1), indicating maintainance of antigenicity of tissue stored in paraffin blocks.

TABLE 10.2
Immunostaining assessment of CD44s

	Immunostaining-intensity of CD44s (number of slides)			
Storage year	0	+	++	+++
94	6	0	0	0
95	3	3	2	0
96	2	2	2	1
97	0	2	0	2
98	0	0	0	6

0 = absent, + = weak, only expression in basal cells, ++ = moderate, expression in basal cells and sporadically in luminal cells, +++ = intense expression in basal cells and in most luminal cells ($\chi^2 = p < 0.01$)

TABLE 10.3
Constant variables for the tumormarkers $p27^{kip1}$, MIB-1 and the Androgen-Receptor (AR)

	α	a_0
$p27^{kip1}$	0.00170	0
MIB-1	0.00467	81
CD44s	0.003446	0

DISCUSSION

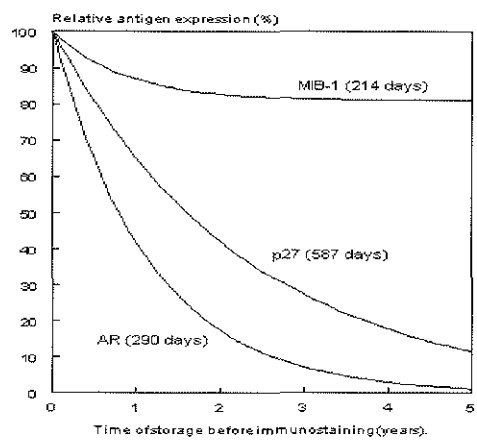
Immunohistochemical assessment of tissue markers in (pre)malignant tissue is widely used and it is presumed that the assessment of these markers will continue to play a decisive role in diagnostic and treatment decisions of patients with different kinds of tumors. For biopsy detected breast cancer, for example, the estrogen-receptor status of the tumor, as is assessed by immunohistochemical analysis, has already found an undisputed place in the treatment decision of women with this malignant neoplasm. It is anticipated that prognostic tissue markers may also have an important role in therapeutical decision making in patients with biopsy-detected prostate cancer. For immunohistochemical staining and quantification of tissue antigens standardized

methods and reproducibility are prerequisite to prevent false positive and negative test results.

We observed an exponential, significant decrease of immunoreactivity throughout time for 4 prognostic tissue markers in prostate carcinoma, i.e. $p27^{kip1}$ ($p < 0.01$), CD44s ($p < 0.01$), MIB-1 ($p < 0.001$) and AR ($p < 0.001$), in benign tissue stored on glass slides in a dark environment and at room temperature. This decay is relatively slow and shows a half-life of absolute immunostaining intensity of 214 days for MIB-1 and a half-life of proportion of immunopositive cells of 587 and 290 days for $p27^{kip1}$ and AR, respectively.

FIGURE 10.1

Exponential decay curve of immunoreactivity for $p27^{kip1}$, MIB-1 and the Androgen-receptor (AR). The y-axis showed the relative antigen expression (%) and the x-axis the time of storage before immunostaining (yrs). For MIB-1 the relative antigen expression stands for absolute immunostaining intensity, while for $p27^{kip1}$ and AR this stands for the proportion of immunopositive cells. For MIB-1 the half-life was 214 days and for AR and $p27^{kip1}$ the half-life was 290 and 587 days, respectively



Obviously, the time-course of loss of immunoreactivity depends on the antigen and probably also on the affinity of the antibody and the type of tissue. Whether a tissue marker presents itself with loss of absolute immunostaining intensity or with loss of relative immunopositivity depends on its frequency of expression in benign tissue in

combination with its ease of being detected as a separate object by the naked eye or by a computer program. MIB-1 is a cell proliferation marker and is expressed by few cells in benign prostate tissue. Therefore, MIB-1 positive nuclei were easily detected against a white background by both the naked eye and the computer program. With time still all MIB-1 immunopositive nuclei will be detected as separate objects on stored slides, though with a decreasing immunostaining intensity. Figure 10.1 shows that the MIB-1 immunostaining intensity reaches a asymptote of relative antigen expression after slide storage. The androgen-receptor (AR) and $p27^{kip1}$, on the other hand, are expressed in most nuclei in benign prostate tissue. Whereas the staining assessment of $p27^{kip1}$ was done with the naked eye, the immunoreactivity of AR was assessed using a computer program, which was set to detect all AR positive nuclei above a certain detection treshold. We observed that for AR more positive nuclei could be detected with the naked eye than could be detected by the computer program. Therefore, it is assumed that only those nuclei with an AR expression that exceeded background staining were detected as separate measurable objects by the computer program. With loss of immunoreactivity through time the proportion of AR positive nuclei, and thus the mean number of detected nuclei per slide, will decrease (FIGURE 10.1). Of course, the exact total of cells, susceptible for detection, could not be obtained in order to calculate a positive to total ratio, as was done for $p27^{kip1}$, but the expected number of cells, susceptible for detection, was kept relatively constant by recording an exact number of 20 video-images per slide.

A cause for the observed decay in immunoreactivity on stored slides cannot be given with certainty. It seems clear that the composition of tissue fixation is of utmost importance in the prevention of loss of immunoreactivity in stored slides, since Jacobs *et al.* reported a significant decrease of immunostaining of p53, factor VIII, ER and Bcl-2 within 12 weeks of slide storage in breast carcinoma specimens after fixation in 10% buffered formalin supplemented with 70% alcohol [1]. Bertheau et al. reported a loss or even increase of antigenicity in stored slides of different tissue origins after fixation in 10% formalin and postfixation with Bouin's solution [3]. Furthermore, both oxidation of the antigen and a masking of the antigen may underlie this loss of immunoreactivity. In our study antigenicity of $p27^{kip1}$, MIB-1 and AR was preserved when sections of long-term stored paraffin blocks are freshly cut. These results are in line with those of Manne et al., who observed no temporal decline in p53 and Bcl-2 expression after long-term storage of paraffin blocks [4]. Despite their unorthodox tissue fixation, Jacobs et al. showed that coating the surface of the tissue sections with a paraffin coat, to diminish contact with the ambient atmosphere, did not significantly prevent loss of immunoreactivity for p53 in breast carcinoma [1].

This result suggests that antigen degradation may only be prevented by an embedding of the tissue specimen deep in the paraffin block and by proper tissue fixation and processing. Whether storage of tissue slides in a low-oxidative environment, i.e. in N_2, in

different storage temperatures, or under a paraffin coat, will diminish the destruction of the antigen by oxidation and thereby diminish decay of immunoreactivity, is under further investigation. Alternative methods to retrieve immunoreactivity of the antigen after its decay may be an optimized microwave antigen retrieval or antigen amplification methods, like the Tyramide Signal Amplification method (TSA).

In this article we like to emphasize on the pitfall, which may occur in the assessment of immunohistochemically stained formalin-fixed, paraffin-embedded tissue stored on glass slides at room temperature, whether this is for research or clinical settings. Storage of tissue material on glass slides for future use can cause unreliable immunostaining results for an indefinite number of antigens, while this immunoreactivity is maintained when tissue is archived in paraffin blocks.

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CHAPTER 11

VALUE OF TISSUE MARKERS p27^{KIP1}, MIB-1, AND CD44s FOR THE PRE-OPERATIVE PREDICTION OF TUMOR FEATURES IN SCREEN DETECTED PROSTATE CANCER

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SUMMARY

BACKGROUND. The pre-operative prediction of prognostic tumor features in the radical prostatectomy specimen using routine clinicopathological variables remains limited. The present study evaluated the predictive value of the cell-cycle protein $p27^{kip1}$, the proliferation marker MIB-1, and the cell-adhesion protein CD44s determined on the diagnostic needle biopsy of asymptomatic men screened for prostate cancer.

MATERIALS AND METHODS. Of 81 screen-detected prostate cancers, representative biopsy cores and matched radical prostatectomy specimens were immunohistochemically stained with antibodies against the tissue markers $p27^{kip1}$, MIB-1 and CD44s. Conventional pre-operative and post-operative clinicopathological variables were assessed, and cancers were divided according to a validated tumor classification model (potentially harmless, clinically significant).

RESULTS. Low (<50%) $p27^{kip1}$ expression, high (≥ 10%) MIB-1 expression, and low (<25%) CD44s expression were considered adverse prognostic signs. Binary logistic regression analysis was performed to assess the most valuable predictors of clinically significant disease. An adverse prognostic immunostaining assessment on the biopsy was found in 10 (12.3%), 17 (21.0%), and 25 (30.9%) cases for $p27^{kip1}$, MIB-1, and CD44s, respectively. The concordance in tissue marker assessment between the biopsy specimen and matched radical prostatectomy specimens was low for all three tissue markers. The positive predictive value (PPV) of $p27^{kip1}$ was 90.0%, remarkably higher than that of MIB-1 and CD44s (41.2 and 52.0%, respectively), indicating that a low radical prostatectomy $p27^{kip1}$ score is expected if the biopsy $p27^{kip1}$ score is low. Logistic regression analysis revealed that biopsy Gleason score ($p < 0.01$) and $p27^{kip1}$ assessment ($p < 0.01$) remained the only significant predictors of clinically significant disease. All cases with low $p27^{kip1}$ expression were found to have clinically significant disease after radical prostatectomy.

CONCLUSIONS. The assessment of $p27^{kip1}$ in the biopsy specimen might add in distinguishing between potentially aggressive and potentially non-aggressive disease in prostate cancer screening.

INTRODUCTION

Despite prognostic information gained from serum prostate-specific antigen (PSA), clinical tumor stage and tumor grade on prostatic needle biopsies, the accuracy of predicting prostate cancer tumor characteristics in the radical prostatectomy specimen and with this, the final outcome of screen-detected prostate cancer, remains limited [1-4]. The vast majority of cancers is diagnosed within the PSA range 3.0 – 9.9 ng/mL, with biopsy Gleason scores 6 or 7, and with clinical tumor stage T_{1c} - T_2 [5,6]. However, the biological behavior of these tumors is highly variable. Some might have been treated unnecessarily as the post-operative prognostic tumor features proved highly favorable, while others might have too advanced disease to be cured. Therefore, refining of the prognostic information gained from pre-treatment variables, prostate cancer biopsy specimens in particular, is warranted.

Recently, several immunohistochemical studies demonstrated that the cell-cycle protein $p27^{kip1}$, the proliferation marker Ki-67 (MIB-1), and the cell-adhesion protein CD44s had independent prognostic value with respect to disease recurrence and patient survival after radical prostatectomy [7-13]. Potentially, these tissue markers might help in differentiating aggressive from non-aggressive cancers on a pretreatment basis. In the present study, we assessed whether their immunohistochemical expression on the diagnostic needle biopsy was representative for that in matched radical prostatectomy specimens. The predictive value of these tissue markers for well-established prognostic factors as pathological tumor stage, tumor grade, and tumor volume in the radical prostatectomy specimen was examined. It is anticipated that the tumor marker most suitable for application on the needle biopsy thus identified may give the clinician additional information on tumor aggressiveness and patient prognosis on a pre-treatment basis.

MATERIALS AND METHODS

Patients

Between January 1, 1998 and September 15, 1999, 99 consecutively admitted men within the screening arm of the European randomized study of screening for prostate cancer (ERSPC) underwent bilateral pelvic lymph-node dissection and radical prostatectomy at the University Hospital Rotterdam. In all screened participants prostate cancer was diagnosed on ultrasound-guided sextant transrectal biopsy of the prostate prompted by an elevated ($\geq$ 3.0 ng/mL) serum-PSA level. Neo-adjuvant (hormonal) treatment was not applied in any of the patients. No patient had pelvic lymph-node

metastatic disease neither on intra-operative examination of frozen tissue sections, nor after the examination of paraffin slides. Of 81 surgically treated patients, tissue of both the diagnostic needle biopsy and matched radical prostatectomy specimens was available for (immunohistochemical) analysis. Preoperative PSA and clinical tumor stage were obtained from the ERSPC database.

Pathological Tissue Examination

All sextant diagnostic biopsy cores were labeled and processed separately. The biopsy cores were routinely fixed in 10% buffered formalin (pH = 7.5), embedded in paraffin, freshly cut into 4 μm thick tissue sections and mounted on glass slides. H&E slides of three subsequent levels of the needle biopsy were histologically examined and a Gleason score was assigned by a specialized genito-urinary pathologist (THvdK) [14].

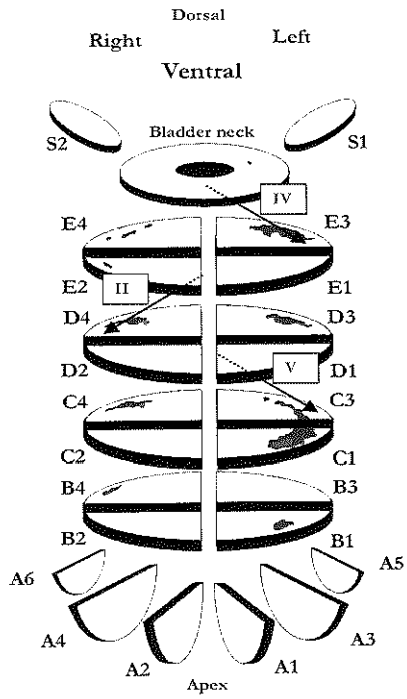
Radical prostatectomy specimens were fixed similarly, schematically cut [15], embedded in paraffin, cut into 4 μm tissue sections, and mounted on glass slides. The tumor was staged according to the TNM '97 system, and the Gleason score was determined. All tumor areas were traced and outlined on the slides. Detailed prostate maps were developed to illustrate the size, extent and location of the prostate tumor and its different histopathological grades (FIGURE 11.1). Morphometric analysis was performed to assess the tumor volume as described by Hoedemaeker and associates [16]. Finally, cancers were categorized according to a previously developed and validated prognostic tumor classification model, including pathological tumor stage, tumor volume and the proportion of high-grade cancer [17]. According to this classification model, organ-confined cancers with a tumor volume less than 0.5 mL, without Gleason growth patterns 4 and 5 were considered potentially 'harmless', while all other cancers were arbitrarily assessed as 'clinically significant' [17,18].

Selection of Most Representative Slides

The selection of the most representative biopsy core was done by an experienced pathologist (THvdK). The selection was based on the assumption that the observed tumor features within the slide would be most predictive of patient outcome. The most representative core was the core with the highest Gleason score or, when the Gleason score was not different between biopsies, the core with the most extensive tumor involvement. The biopsy slides were stored for a maximum of two-and-a-half years in a dark environment at room temperature until immunostaining was performed.

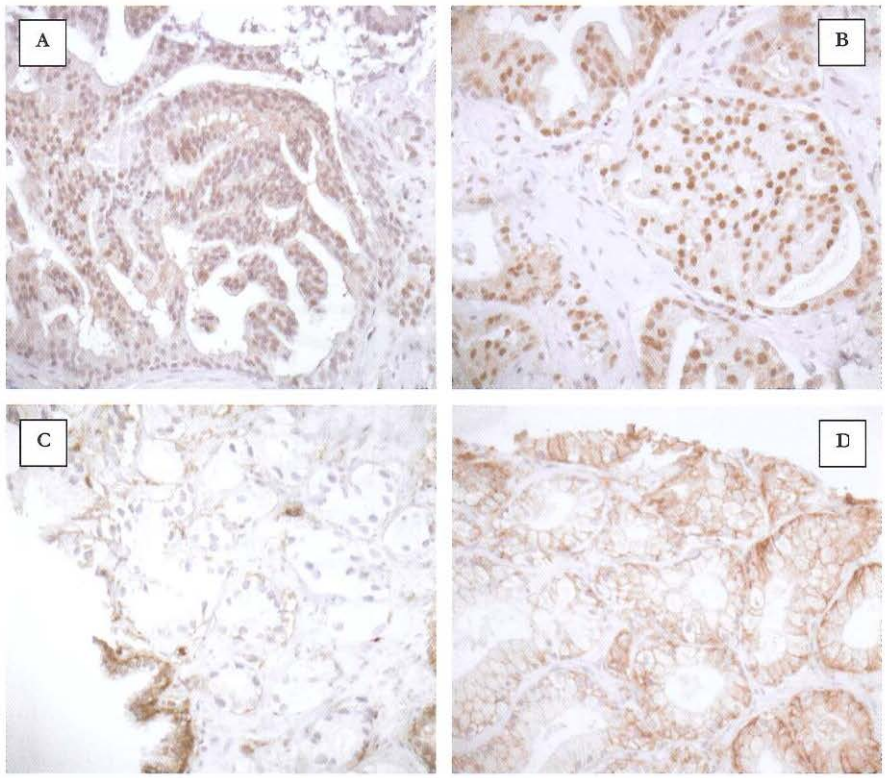
FIGURE 11.1

A prostate map showing the site, range and trajectory of the most representative biopsy core (IV) and two other biopsy cores (II and V) as well as the location of the tumor in the prostate and its corresponding grade of differentiation according to Gleason. The tumor is shown in black (Gleason growth pattern 3) and dark grey (Gleason growth pattern 4). Prostate sections C1 and D4 were assessed as the most representative of the tumor in the prostate, whereas biopsy core IV contained the highest amount of cancer (approximately 60%). Biopsy V missed the tumor completely, while biopsy II was only marginally involved with cancer (< 10%).



The selection of the most representative slides within the radical prostatectomy was performed similarly. One to three paraffin blocks with tumor tissue most representative for the whole tumor within the radical prostatectomy specimen were selected for immunohistochemical analysis. Using radical prostatectomy maps, the site, range and trajectory of the individual biopsy cores, and that of the most representative biopsy core in particular, were reconstructed (FIGURE 11.1). In doing so, one may determine whether the representative biopsy needle hit or did not hit the representative tumor parts within the radical prostatectomy specimen.

FIGURE 11.2
Immunostaining images of tissue marker expression on the diagnostic needle biopsy (magnification 400 x). *A*. Low (< 50%) tumor $p27^{kip1}$ expression. *B*. High (≥ 50%) tumor $p27^{kip1}$ expression. *C*. Low (< 25%) tumor CD44s expression, and *D*. High (≥ 25%) tumor CD44s expression.



Immunostaining

Slides with biopsy tissue and slides from the radical prostatectomy specimen were immunohistochemically stained according to similar protocols. Tissues from the radical prostatectomy specimens were freshly cut, while those of the biopsy specimens were retrieved from the storage. After deparaffinization through xylene and 100% ethanol, endogenous peroxidase activity was blocked by immersing the slides for 20 min in a 3% hydrogen peroxide/methanol bath. The slides were placed in a 10 mmol/L citrate buffer at pH = 6.0. Antigen retrieval was performed in a microwave oven at 700 W for 15

minutes. After cooling the slides were placed in a Sequenza immunostaining system and pre-incubated with 10% normal goat serum in phosphate buffered saline/bovine serum albumin 5%. The slides were incubated overnight at 4° C with the primary antibody MIB-1 (Immunotech, France) at a optimal dilution of 1 : 3,000, $p27^{kip1}$ (Novocastra, UK) at 1: 40, or CD44s (Bender MedSystems, Austria) at 1 : 200 in phosphate buffered saline/bovine serum albumin 5%. To each batch of slides, negative controls were included. For slides stained with MIB-1 and $p27^{kip1}$ the conventional avidin-biotin complex method was applied. Briefly, a 30 min incubation with biotinylated goat-anti mouse antibody (Biogenex, San Ramon, USA) was followed by a 30 min incubation with streptavidin-peroxidase complex (Biogenex). For slides immunostained with the primary antibody anti-CD44s, the catalyzed signal amplification (CSA, K1500, DAKO) system was used. After overnight incubation with the primary antibody, a 15 minute incubation with a linking antibody was followed by a 15 minute incubation with streptavidin-biotin complex, a 15 minute incubation with an amplification reagent (diluted 1 : 4 in phosphate buffered saline), and a final 15 minute incubation with streptavidin-peroxidase. Subsequently, in all slides the antibody-antigen binding was visualized with diaminobenzidine hydrochloride (Fluka, Neu-Ulm, Germany) with 0.08% hydrogen peroxide for 7 minutes. The specimens were counterstained with Mayer's hematoxylin, dehydrated and covered.

Quantitation

All slides were assessed by two independent observers (ANV, BWvR) without knowledge of matched biopsy or radical prostatectomy tumor features. In case of discrepancy between observers, the slides were reassessed in a combined session. Agreement between observers occurred in over 80% of cases for all three tissue markers. All selected sections contained benign prostatic glands, which could serve as internal positive controls. For all three tissue markers the immunostaining quantitation was similar for both the biopsy specimens and the radical prostatectomy specimens.

For $p27^{kip1}$, nuclear staining was assessed by estimating a positive-to-total ratio as previously described [7] A tumor was considered 'high' for $p27^{kip1}$ expression if 50% or more nuclei showed positive immunostaining, and 'low' if a positive-to-total ratio of less than 50% was recorded [7] (FIGURE 11.2). In case of tumor heterogeneity those parts within the tumor that showed the lowest positive-to-total ratio were assessed. For MIB-1, nuclear staining was assessed by estimating the percentage of MIB-1 positive cells [7]. Tumors with 10% or more nuclei positive for MIB-1 were considered 'high' for MIB-1 expression, whereas those with less than 10% of MIB-1 positivity were assessed 'low' for MIB-1 expression. If the tumor exhibited heterogeneous MIB-1 expression, the area with the highest density of MIB-1 positive cells was selected. Slides stained with CD44s were

assessed according to the percentage of cells showing positive membranous immunostaining (FIGURE 11.2). Since a less than 25% negative immunostaining was reported to be most predictive of clinical progression after radical prostatectomy [12], this cut-off point was taken for statistical analysis. Sllides were assessed as having 'low' (<25%) or 'high' (≥ 25%) tumor CD44s expression. A tumor CD44s score was obtained by taking the lowest assessed score within the tumor sections.

The concordance in tissue marker assessment between the biopsy and the radical prostatectomy specimen was determined for all three tissue markers. In this, an adverse prognostic assessment (i.e. low $p27^{kip1}$, high MIB-1, low CD44s) was considered a positive test outcome. The sensitivity of tissue marker assessment implies the number of adverse prognostic assessments determined on the biopsy divided by the total number of adverse prognostic outcomes in the radical prostatectomy specimen. The specificity implies the number of favorable prognostic assessments on the biopsy divided by the total number of favorable prognostic outcomes in the radical prostatectomy specimens. The positive predictive value (PPV) corresponds to the proportion of men with an adverse prognostic assessment on the biopsy who also had an adverse prognostic outcome in the radical prostatectomy. The negative predictive value (NPV) is the proportion of men with a favorable assessment on the biopsy who had a favorable outcome in the radical prostatectomy specimen as well. Similar analyses were performed with respect to biopsy tissue marker assessment and clinical significance of disease.

Statistical Analysis

Statistical analysis was performed using the statistical package for the social sciences (SPSS Inc., Chicago, IL). The association between the expression of $p27^{kip1}$, MIB-1 and CD44s on the biopsy and conventional clinicopathological parameters was evaluated by means of the Pearson chi-square (χ^2) test. The pre-operative PSA-level was categorized 3.0 - 3.9 ng/mL, 4.0 - 5.9 ng/mL, 6.0 - 9.9 ng/mL, ≥ 10.0 ng/mL, clinical tumor stage T_{1c}, T_{2a-b}, T_{2c}, Gleason score 2-6, 7, 8-10, and proportion of high-grade cancer 0%, 0–9%, 10–49%, ≥ 50%. Post-operative variables were categorized as listed in TABLE 11.1.

Binary logistic regression analysis was performed to assess the statistical significance of pre-operative variables. Clinically significant disease was taken as the dependent variable, while conventional pre-operative clinicopathological variables, and the expression of tissue markers $p27^{kip1}$, MIB-1 and CD44s on the biopsy were taken as co-variates. Variables that were not statistically significant at the univariate level were removed from the model, while controlling for the other variables (i.e. backward elimination method). Forward stepwise elimination was performed to verify that the same parameters remained

of prognostic significance in the final models. The assumption that no association existed between the variables evaluated (H0) was rejected (H1) if $p < 0.05$.

RESULTS

Patient Characteristics

All 81 patients had clinically localized disease at the time of diagnosis. The median serum-PSA level was 5.2 ng/mL (range, 3.0 - 15.1), and 71 (87.7%) had a PSA level between 3.0 –9.9 ng/mL. A total of 48 (59.3%) and 18 (22.2%) men had a Gleason score of 6 or 7 on the biopsy, respectively, and 11 (13.6%) a Gleason score 7 (4 + 3) or 8. Within the radical prostatectomy specimen, 69 (85.2%) cancers were organ-confined, 10 (12.3%) had extraprostatic extension, and 2 (2.5%) showed extensive infiltrating disease (TABLE 11.1). The Gleason score was 2 to 6 in 53 (65.4%), and a dominant Gleason growth pattern 4 or 5 was seen in 6 (7.4%). According to the tumor classification model, 23 (28.4%) cases were considered 'harmless' and 58 (71.6%) 'clinically significant'. Using prostate maps, in 14 (17.3%) cases the selected representative biopsy needle did not hit the site of the prostate that was thought to contain the representative sections within the tumor (FIGURE 11.1).

Immunostaining Assessment

Of 81 patients, 10 (12.3%) and 35 (43.2%) had a low tumor $p27^{kip1}$ expression on the diagnostic needle biopsy and radical prostatectomy specimen, respectively. These figures were 17 (21.0%) and 26 (32.1%) for high MIB-1 expression, and 25 (30.9%) and 26 (32.1%) for low CD44s expression.

TABLE 11.2 shows the sensitivity, specificity, PPV, and NPV of tissue marker assessment. The sensitivity of tissue marker assessment was low for all three tissue markers, implying that a substantial proportion of cases was incorrectly designated a favorable prognostic outcome. Nine of 10 (PPV=90.0%) cases with a low biopsy $p27^{kip1}$ expression had a low $p27^{kip1}$ expression in the prostate, whereas 26 of 71 cases designated as having a high tumor $p27^{kip1}$ expression pre-operatively, changed category after radical prostatectomy (NPV=63.4%). The PPV of MIB-1 and CD44s expression was lower than $p27^{kip1}$, while the NPVs were only slightly higher (TABLE 11.2). The expression of $p27^{kip1}$ and CD44s were nearly almost absent (i.e. low) in intraluminar growing strands of cribriform and intraductal prostate cancer (FIGURE 11.2).

TABLE 11.1

The association of the expression of $p27^{kip1}$, MIB-1, and CD44s with pathological tumor stage, Gleason score and the tumor classification model as determined on the radical prostatectomy specimen. Numbers in parenthesis are percentages.

	Tissue marker expression on the diagnostic biopsy						
Radical prostatectomy tumor features	$p27^{kip1}$ Low (< 50%)	$p27^{kip1}$ High (≥ 50%)	MIB-1 Low (< 10%)	MIB-1 High (≥ 10%)	CD44s Low (< 25%)	CD44s High (≥ 25%)	Total
Pathological stage *							
pT_{2a-c}	5 (50.0)	64 (90.1)	56 (87.5)	13 (76.5)	20 (80.0)	49 (87.5)	69
pT_{3a}	4 (40.0)	6 (8.5)	7 (10.9)	3 (17.6)	4 (16.0)	6 (10.7)	10
pT_{3b-4a}	1 (10.0)	1 (10.0)	1 (1.6)	1 (5.9)	1 (4.0)	1 (1.8)	2
Gleason score							
2-6	2 (20.0)	51 (71.8)	46 (71.9)	7 (41.1)	12 (48.0)	41 (73.2)	53
7 (3 + 4)	5 (50.0)	17 (23.9)	13 (20.3)	9 (52.9)	11 (44.0)	11 (19.6)	22
7 (4 + 3)	3 (30.0)	3 (4.2)	5 (7.8)	1 (5.9)	2 (8.0)	4 (7.1)	6
Tumor classification							
Harmless†	0 (0.0)	23 (32.4)	20 (31.3)	3 (17.6)	4 (16.0)	19 (33.9)	23
Clinically significant‡	10 (100)	48 (67.6)	44 (68.8)	14 (82.4)	21 (84.0)	37 (66.1)	58
Total	10	71	64	17	25	56	81

* pTNM 1997

† Possibly harmless disease: organ-confined cancers with a tumor volume of less than 0.5 ml that lacks Gleason growth patterns 4 and 5

‡ Clinically significant disease: All others

Association of Tissue Marker Expression with Pre-and Post-operative Variables

The association of the expression of the tissue markers on the biopsy with pre-operative and post-operative clinicopathological parameters is given in TABLE 11.3. While $p27^{kip1}$ expression was highly associated with most of the pre-operative and post-operative variables, as well as to MIB-1 and CD44s expression, the latter two tissue markers were not, or only weakly, correlated to these same parameters (TABLE 11.3). A

low expression of $p27^{kip1}$ had a high predictive value for the presence of clinically significant disease (TABLE 11.1). In fact, all cases with low pre-operative $p27^{kip1}$ turned out to have clinically significant disease after radical prostatectomy (PPV=100.0%). For biopsy Gleason scores of 7 or higher, all but one (22 of 23) cases were found to have clinically significant disease after radical prostatectomy (PPV=95.7%). Logistic regression analysis revealed that biopsy Gleason score ($p < 0.01$) and low expression of $p27^{kip1}$ ($p < 0.01$) on the biopsy were most valuable as predictors of clinically significant disease, though with wide confidence intervals (TABLE 11.4). CD44s and MIB-1 expression on the biopsy did not remain in the final models as independent predictors of clinically significant disease.

TABLE 11.2

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the expression of $p27^{kip1}$, MIB-1, and CD44s as determined on the biopsy specimen. A low (less than 50%) tumor $p27^{kip1}$, a high (10% or more) tumor MIB-1, and a low (less than 25%) tumor CD44s expression on the biopsy were considered positive test outcomes (i.e. adverse prognostic indicators).

Biopsy tissue marker	Sensitivity	Specificity	PPV	NPV
$p27^{KIP1}$ expression	25.7%	97.8%	90.0%	63.4%
MIB-1 expression	26.9%	81.8%	41.2%	70.3%
CD44s expression	50.0%	78.2%	52.0%	76.8%

DISCUSSION

In prostate cancer screening, no reliable method exists today that may identify the patients with non-aggressive disease and those with fatal disease beyond cure. Such tools are required, for it is considered that a substantial proportion of screen-detected prostate cancers may have been overdiagnosed (and subsequently overtreated), while others might not have been detected (and treated) early enough. Unfortunately, the predictive value of conventional clinicopathological parameters for powerful prognosticators as pathological

tumor stage and lymph-node metastatic disease, and with this, the identification of aggressive but curable cancers, remains limited.

TABLE 11.3
The correlation of $p27^{kip1}$, MIB-1, and CD44s with pre-operative clinicopathological parameters and tumor characteristics determined on the radical prostatectomy specimen. The figures presented are p values.

	Tissue marker expression on the diagnostic biopsy		
Variable	$p27^{kip1}$ *	MIB-1 †	CD44s ‡
PSA level	< 0.01	0.06	ns
Clinical tumor stage	0.01	ns	ns
Biopsy Gleason score	< 0.01	< 0.01	ns
Biopsy high-grade cancer	< 0.01	0.01	0.02
Biopsy $p27^{kip1}$	-	0.05	< 0.01
Biopsy MIB-1	0.05	-	0.01
Biopsy CD44s	< 0.01	0.01	-
Pathological tumor stage	< 0.01	ns	ns
Prostatic Gleason score	< 0.01	ns	ns
Tumor classification model	< 0.01	ns	ns

* Dichotomized as low $p27^{kip1}$ (less than 50%) and high $p27^{kip1}$ (50% or more) expression
† Dichotomized as high MIB-1 (10% or more) and low MIB-1 (less than 10%) expression
‡ Dichotomized as low CD44s (less than 25%) and high CD44s (25% or more) expression
ns not significant

Recent studies demonstrated that the expression of the cell-cycle protein $p27^{kip1}$, the proliferation marker MIB-1, and the cell-adhesion protein CD44s within tumors was of prognostic importance in men treated with radical prostatectomy, additional to grading and staging [7-13]. Valuable tissue markers assessed on the diagnostic needle biopsy may aid in the selection of patients to undergo (or to refrain from) radical surgery for clinically localized prostate cancer. In the current study we reported a relatively poor concordance for the expression level of $p27^{kip1}$, MIB-1, and CD44s on the diagnostic needle biopsy and representative sections of the corresponding radical prostatectomy specimen (TABLE

11.2). The sensitivity was low for all three tissue markers, indicating that prognostically adverse tumor areas within the prostate were missed in a substantial number of cases. These results are in line with those of previously published and similarly performed studies [19-21]. Furthermore, the PPV was high only for low $p27^{kip1}$ expression (PPV=90%), while these were comparably low for MIB-1 and CD44s (41.2 and 52.0%, respectively).

TABLE 11.4

Logistic regression analysis for the prediction of 'clinically significant' disease using conventional clinicopathological variables and the expression of $p27^{kip1}$, MIB-1, and CD44s on the diagnostic needle biopsy.

	Logistic regression analysis		
	e^{β}	95% CI	p value
PSA level	-	-	ns
Clinical tumor stage	-	-	ns
Biopsy Gleason score	13.01	1.78 – 96.03	< 0.01
Proportion high-grade	-	-	ns
Biopsy $p27^{kip1,*}$	8.97	1.03 - 76.92	< 0.01
Biopsy MIB-1 †	-	-	ns
Biopsy CD44s ‡	-	-	ns

95% CI	95% confidence interval
e^{β}	Odds ratio
ns	not significant
*	Low $p27^{kip1}$ (less than 50%) expression
†	High MIB-1 (more than 10%) expression
‡	Low CD44s (less than 25%) expression

Our analysis by logistic regression showed that biopsy $p27^{kip1}$ expression and biopsy Gleason score were significant predictors of clinically significant disease (TABLE 11.4). Despite wide confidence intervals due to small patient series, the observation of a low $p27^{kip1}$ expression on the diagnostic biopsy might thus be indicative for biologically aggressive disease. In our study, all men with a low (<50%) tumor $p27^{kip1}$ score on the biopsy were found to have clinically significant disease after radical prostatectomy using

the definitions of the tumor classification model. On the other hand, a high (≥ 50%) biopsy $p27^{kip1}$ score poorly predicted the presence of a prostate cancer with prognostically favorable tumor features. Therefore, the assessment of $p27^{kip1}$ expression on the biopsy is not helpful to identify patients who are most likely to benefit from conservative treatment and surveillance. Moreover, our study did not provide data whether 'aggressive' cancers identified by a low biopsy tumor $p27^{kip1}$, may be cured by the currently available treatment options, or otherwise, may already be beyond the reach of cure.

The interpretation of our results may be limited by various factors. Multifocality and tumor heterogeneity may have contributed to sampling error of the diagnostic needle biopsy, and to the poor concordance of tissue marker assessment between biopsy and radical prostatectomy specimen. As only one or two biopsy cores per patient were stained immunohistochemically, i.e. those that were assumed most representative within the biopsy sextant, tissue marker assessment may not have reflected the entire primary tumor within the prostate. On the other hand, it is not likely that an adverse prognostic immunostaining assessment would have been found in one of the 'non-representative' biopsy cores, especially when taking into account that these were mostly of lower grade and of low tumor volume. The frequency of adverse prognostic immunostaining assessments was low in our screened population (e.g. 12.3% for low $p27^{kip1}$ expression), and as a consequence, definite conclusions on the predictive value of tissue markers may only be given using larger patient series. It is likely that the proportion of adverse prognostic indicators may have been higher in other patient groups that lacked the favorable prognostic features observed in our screening group. Finally, the long-term prognostic significance of our tumor classification model remains to be established. It might well be that some men classified as having clinically significant disease in our study population would not have experienced signs or symptoms of prostate cancers ever, and conversely, that some men designated as having potentially harmless disease may still have had clinically manifest disease if not treated.

At present, the routinely performed diagnostic technique of systematic sextant prostate biopsy has a limited capability in predicting the tumor characteristics in the prostate gland, and with this, the expected biological course of disease. The present study provided some substantiation that tissue marker assessment on the biopsy, $p27^{kip1}$ in particular, might help in discriminating between potentially aggressive and potentially non-aggressive cancers in prostate cancer screening. Before the application within clinical settings is considered, our promising results on the value of $p27^{kip1}$ protein expression on prostatic needle biopsies in men with screen-detected prostate cancer will have to be confirmed, preferably in prospective, multi-institutional studies with larger number of patients.

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CHAPTER 12

FEASIBILITY OF THE ASSESSMENT OF PROMOTER METHYLATION OF THE *CD44* GENE IN SERUM OF PROSTATE CANCER PATIENTS

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SUMMARY

BACKGROUND. *CD44* is an important metastasis suppressor gene in prostate cancer patients. Downregulation of the *CD44* gene was attributed to transcription repression by methylation of CpG islands in the promoter region. The feasibility of *CD44* promoter methylation to be used as a diagnostic tool was assessed in the serum of prostate cancer patients.

MATERIALS AND METHODS. Seven serum samples of patients with prostate cancer were investigated for *CD44* promoter methylation by methylation-specific PCR. Three patients had proven metastatic disease, and 4 were free of metastases. Tissues from a variety of normal epithelia were assessed as well.

RESULTS. *CD44* promoter methylation was readily detectable in all serum samples, although no distinction could be made between patients *with* and those *without* metastatic disease on the basis of the signal intensity of methylation-specific PCR products. Remarkably, tissue specimens from different normal epithelia, especially those of the colon and rectum, repeatedly showed aberrant methylation of the promoter region of *CD44*.

CONCLUSIONS. In the serum of prostate cancer patients, assessment of the methylation status of CpG islands in the promoter region of the *CD44* gene is feasible using methylation-specific PCR. However, due to physiological promoter methylation of the *CD44* gene in normal tissues, including the colorectal mucosa, assessment of methylation of tumor-derived DNA in the serum of cancer patients lacks tissue-specificity and seems not applicable in clinical settings.

INTRODUCTION

In recent years, major efforts have been directed at the search for markers of metastatic potential in prostate cancer patients. This would be of benefit for patients with clinically unsuspected (occult) prostate cancer metastases who might have undergone inappropriate treatment with curative intent. To improve the management and treatment of patients with prostate cancer, markers of metastatic ability are needed to identify those at risk of (occult) metastatic disease.

In previous studies on prostate cancer, downregulation of CD44 expression at the mRNA and protein level was correlated with high tumor grade (i.e. Gleason score), an increased rate of progression after radical prostatectomy, and decreased disease-specific survival rates, sometimes even independent of grade and stage [1-6]. It was reported that standard CD44 (CD44s) protein expression was strongly reduced in pelvic lymph-node and distant prostate cancer metastases as well as in the corresponding primary tumors [7]. A decreased CD44 expression in tumor cells might thus identify the prostate carcinomas with an acquired metastatic potential [8]. This downregulation of the *CD44* gene was recently attributed to the methylation of CpG islands in the promoter region in prostate cancer cell lines and metastatic tissue specimens [9-11]. Repression of transcription by promoter methylation is one of the mechanisms responsible for loss of gene expression, and has been reported for other genes as well, and in a variety of other human cancers [12-15].

Recently, a sensitive method for the rapid analysis of the methylation status of CpG islands was described, i.e. methylation-specific PCR. [16]. This technique is able to detect even very low quantities of altered (e.g. methylated) DNA in different kind of solid tumors such as prostate cancer. Moreover, recent studies have indicated that tumor-derived free DNA is present in the blood of cancer patients, and that molecular genetic alterations can be detected in these blood samples [12-15,16]. In the current study, we investigated whether the assessment of promoter methylation of the metastasis suppressor gene *CD44* is feasible in human serum samples. The determination of the methylation status of the promoter region of a metastasis suppression gene in serum of cancer patients might be particularly valuable for this would help to distinguish between prostate cancers with favorable characteristics from those with an enhanced potential to metastasize.

MATERIALS AND METHODS

Patient Selection and Serum Collection

A total of 7 serum samples, collected from the blood storage facility of the Department of Urology, University Hospital Rotterdam, was analyzed. After blood withdrawal, the samples were incubated at 37°C overnight, centrifuged at low speed, and the serum was stored at −80°C before DNA extraction. Three of 7 serum samples were derived from patients with proven metastatic disease as determined by 'hot-spots' on bone scintigraphy (2 cases) or pelvic lymph-node disease (1 case), and all 3 had prostate-specific antigen (PSA) levels of more than 100 ng/mL. The other 4 samples were derived from patients with PSA levels of 4.9, 6.4, 6.6, and 7.1 ng/mL, respectively, at the time of diagnosis. They subsequently underwent radical prostatectomy for biopsy proven prostate cancer, and their PSA-levels remained undetectable (i.e. < 0.1 ng/mL) 4 years after surgery. DNA from serum samples was obtained by digestion with SDS and proteinase K at 48°C overnight, purified by phenol-chloroform extraction and precipitated with ethanol [17]. After purification, 1 mL of serum yielded an average of 50 ng of DNA, consistent with previous observations [17].

Fresh-frozen tissue specimens from a variety of organs (breast, colon, liver, lung, ovarium, prostate, rectum, and thyroid) were analyzed for promoter methylation of the *CD44* gene as well. In doing this, histologically proven benign tissue was selected. DNA of PC-346, a xenograft known to have an absent CD44 expression at the mRNA and protein level (due to *CD44* promoter methylation) was taken as a positive control and dH_2O as a negative control [9]. Genomic DNAs of PC-346 and tissue specimens were obtained according to standard procedures.

Bisulphite Modification and PCR Amplification

Isolated DNA of serum samples, tissue specimens, and PC-346 was pre-digested with *Eco*RI DNA (1 µl) in a total volume of 50 µl, denaturated by adding NaOH to a final concentration of 0.3 M, and incubated overnight at 37°C. The solution was neutralized by addition of NH_4 OAc (pH 7.0) to 3.0 M, and the DNA was precipitated with ethanol.

We designed primers to distinguish methylated from unmethylated DNA in bisulphite-modified DNA. In bisulphite modification, cytosines are converted to uracil, but those that are methylated are resistant to modification and remain as cytosine. Altered (methylated) DNA and unaltered (unmethylated) DNA can then be distinguished by methylation-specific PCR since marked sequence differences exist between these DNAs.

The method of bisulphite modification is described in detail by Herman *et al.* [16] and Verkaik *et al.* [11].

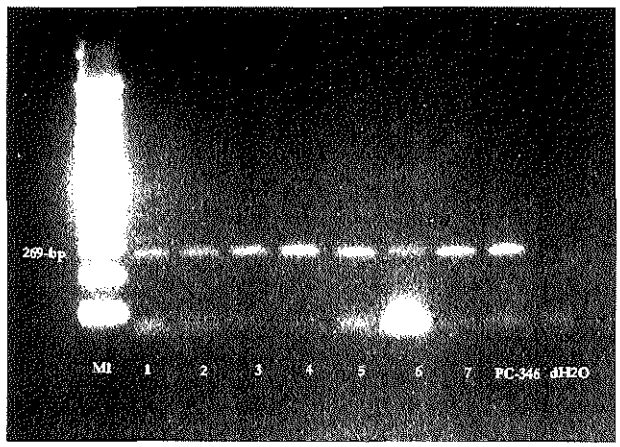
For human CD44 amplifications, primers were used based on the *CD44* DNA sequence: (F) 5' -GGTCATCCTCTGTCCTGACGCCGC- 3' and (R) 5' -GAGCGAGCGAAGGACACACC- 3' [11]. Bisulphite modified DNA (100 ng) was amplified in a PCR analysis using the primer set specific for the methylated *CD44* sequence giving a 269-bp product: (F) 5' -GGTTATTTTTTGTTTTGACGTCGC- 3' and (R) 5' -AAACGAACGAAAAACACACC- 3' and a second round of PCR using a methylated-specific nested primer set: (F) 5'- CGGAGGTATAGGTATTTCGC -3' and (R) 5' -AACGAACCCCTCTACCCCCG- 3'. In this nested PCR a 109-bp product was obtained [Ver00]. As a quality control of the bisulphite conversion process, all bisulphite-treated DNAs were also amplified with primers specific for the unmethylated *CD44* sequence: (F) 5' -GGTTATTTTTTGTTTTGATGTTGT- 3' and (R) 5' –AAACAAACAAAAAACACACC- 3'. For all PCR amplifications the conditions were as follows: 94°C for 5 min, followed by 35 cycles of 94°C, 1 min; 60°C, 1 min; 72°C, 1 min for amplifications, and a final extension for 10 min at 72° C. The PCR mixture contained 2 µl SuperTaq DNA polymerase (Sphaero Q, HT Biotechnology, Cambridge, UK), 10 µl buffer (Sphaero Q), 100 ng of each primer (1 µl), and 1 µl 100 mM dNTPs in a final volume of 50 µl. PCR products were loaded on denaturated 1% polyacylamide gels, stained with ethidium bromide and visualised under UV illumination.

RESULTS

In the 7 serum samples, human *CD44* DNA was readily detectable by standard PCR analysis. However, we found that all examined serum samples, i.e. cases *with* proven metastases and cases *without*, exhibited aberrant promoter methylation in serum DNA (FIGURE 12.1). No substantial difference in signal intensity was observed between the patients (i.e. with metastases) and controls (i.e. without metastases), indicating that the determination of the methylation status of CpG islands in the promoter region of the *CD44* gene is not likely to identify a subset of cases with unfavorable characteristics.

In all the evaluated tissue specimens, human *CD44* DNA was detectable, although the signal intensity in colon and breast tissue was lower than that of other tissues (data not shown). In methylation-specific PCR, all colon specimens repeatedly showed aberrant methylation of the promoter region of the *CD44* gene, whereas some of the evaluated breast, liver, lung, rectal and thyroid specimens showed promoter methylation as well. However, none of the tissue specimens from ovarium or prostate ever had detectable methylated *CD44* sequences (FIGURE 12.2).

FIGURE 12.1
Methylation status of CpG islands of the promoter region of the CD44 gene in the serum of patients with prostate cancer. Lane M1 is the molecular weight marker. Lanes 1, 2, and 4 correspond to patients with clinically manifest prostate cancer metastases as determined by 'hot-spots' on bone scintigraphy and/or highly elevated PSA levels. Lane 3, 5, 6, and 7 are derived from patients who underwent radical prostatectomy for biopsy proven prostate cancer, and as the PSA levels were undetectable four years after surgery were assumed free of (occult) metastatic disease at the time of diagnosis. Lane 9 is PC-346 (positive control). The size of the methylated PCR-product is 269-bp



As a control for bisulphite modification, all bisulphite treated serum samples, tissue specimens and tissue from xenograft PC-346 were amplified with primers specific for the unmethylated *CD44* sequences. All samples proved to have amplifiable sequences, demonstrating the efficacy of the bisulphite modification process.

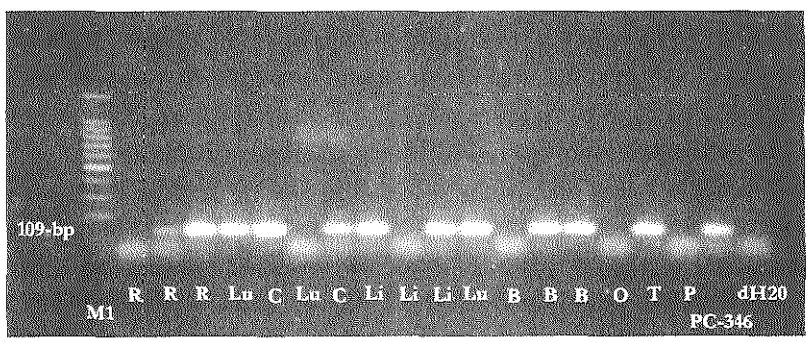
DISCUSSION

Metastasized prostate cancer carries a dismal prognosis. When treatment with curative intent is performed in patients with clinically localized prostate cancer, a proportion of these patients will experience metastatic progression later on. The serum-PSA level and the tumor grade are currently the only indicators of metastatic potential at the time of diagnosis in patients with clinically localized prostate cancer. As most patients are diagnosed with prostate cancer in the PSA range 3.0 - 10.0 ng/mL, and with biopsy

Gleason scores of 6 and 7, the distinctive capacity of serum-PSA and tumor grade for (occult) metastatic disease is limited [2]. In an attempt to adapt treatment of prostate cancer patients to the expected clinical course of disease, the search for tools that allow for an effective assessment of the metastatic potential of tumors is warranted. Recently, the attention has been directed at molecular markers with metastasis suppression ability that have the potential to distinguish metastasized cases from those that are not.

FIGURE 12.2

Methylation status of CpG islands of the promoter region of the *CD44* gene in a variety of benign tissue specimens. *Lane M1* is the molecular weight marker. *Lane 1, 2,* and *3* are normal rectal specimens (R), *Lane 4, 6,* and *11* are benign lung tissue (Lu), *Lane 5* and *7* are normal colon (C), *Lane 8, 9* and *10* are normal liver tissue (Li), *Lane 12, 13* and *14* are benign breast (B), *Lane 15* is normal ovarium (O), *Lane 16* is tissue from the normal thyroid gland (T), and *Lane 17* is benign prostate hyperplasia (BPH). *Lane 18* is PC-346 (positive control). The size of the nested methylated PCR-product is 109-bp



The standard CD44 (CD44s) protein is normally expressed in the plasma membrane of benign prostate epithelial cells. Downregulation of CD44 expression at the mRNA and protein level was associated with adverse pathological tumor features and poor outcome in several studies on prostate cancer [1-6]. For CD44, it is acknowledged that transcriptional suppression is caused by hypermethylation of CpG islands in the gene-regulatory (promoter) sequences of the gene. In benign prostatic glands, these CpG islands are unmethylated, while in metastasized prostate cancer these CpG islands are often hypermethylated [10,11]. Transcriptional inactivation by hypermethylation of the

promoter region has also been demonstrated in a variety of other malignancies and for other genes such as *E-cadherin*, *BRCA1*, *VHL*, $p16^{INK4A}$ and $p27^{kip1}$ [12,14,15]. However, promoter methylation is not a feature of malignant cells alone. Loss of gene expression by hypermethylation has been seen in physiological conditions such as female X-chromosome inactivation, genomic imprinting and during the ageing process [19-20].

Previous studies showed that genetic alterations in tumor-derived DNA are detectable in the blood of patients with various malignancies, and some study groups have suggested that the assessment of promoter methylation in the serum of cancer patients may detect free DNA sheded by cancer cells, and improve diagnostics and patient management [14,15]. Our results confirm those of other groups, and showed that the assessment of promoter methylation of the metastasis suppressor gene *CD44* indeed is possible in the serum of prostate cancer patients. Unfortunately, under the reported conditions, no distinction could be made between patients *with* and those *without* metastatic disease using the signal intensity of methylation-specific PCR products (FIGURE 12.1). This lack of distinctive capacity complicates the use in clinical settings, and quantitation of circulating aberrant tumor DNA of the *CD44* gene seems not feasible in prostate cancer patients. These unfortunate results are largely explained by our unexpected finding that methylated *CD44* was present in the blood of men *without* detectable metastatic prostate cancer, i.e. in normal physiological conditions. As we have demonstrated, a variety of evaluated tissue specimens derived from normal epithelia, including those of the colon and rectum, repeatly showed *CD44* promoter methylation, while other organs lacked this physiological DNA methylation (FIGURE 12.2). Although we cannot give definite prove, our data seem to suggest that the source of this methylated *CD44* in the blood is the normal epithelium of the rectum and colon, whereas other organs may also have contributed to this physiological *CD44* promoter methylationl. In physiological conditions, *CD44* expression is restricted both at the mRNA and the protein level in benign epithelia of the gastro-intestinal tract, and the expression of standard and/or 'splice-variants' of CD44 is upregulated during malignant transformation [21-23]. Our observations tend one to hypothesize that *CD44* silencing by methylation of CpG islands is one of the mechanisms responsible for transcription inactivation in benign cells of the colon and rectum. Indeed, it has been reported for several other genes that a physiological type of methylation occurs in the normal colon mucosa during the process of ageing [19,20]. This age-dependent methylation, and resulting gene silencing, may also hold true for the *CD44* gene in normal colorectal epithelia, although it cannot be excluded that other gene inactivating mechanisms are also involved. Furthermore, only upregulation of CD44 isoforms (most notably, the splice variant v6) has been associated with the development of distant metastases in colorectal carcinomas [21-23], whereas altered expression levels of other splice variants or standard form CD44 have only inconsistently been associated with malignant potential. Questions remain how the

different expression patterns of the standard and variant CD44 isoforms in benign and malignant cells of different tissue types can be explained by differences in DNA methylation. Understanding of the different mechanisms of promoter methylation of CpG islands in the *CD44* gene would provide further insight into the carcinogenic processes, and will probably improve the diagnostics and management of patients with cancer of different origins.

In conclusion, assessment of the methylation status of CpG islands of the promoter region of the *CD44* gene in serum is feasible using methylation-specific PCR. Since DNA methylation is not a feature of malignant disease alone, and occurs frequently in normal (colorectal) epithelia, the assessment of promoter methylation of the *CD44* gene in serum may not distinguish cases with (occult) prostate cancer metastases from those that have not. Thus, the lack of tissue and cancer specificity hampers the clinical applicability in prostate cancer patients. On the other hand, as methylation-specific PCR is a relatively easy procedure, and sensitive to detect even the smallest amounts of altered (methylated) DNA sequences, the technique may be further applied to investigate other metastatic suppressor genes in which loss of gene expression is explained by promoter methylation such as *E-cadherin*, $p16^{INK4A}$ and $p27^{kip1}$.

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Part VI

General Discussion, Epilogue and Summary

CHAPTER 13

GENERAL DISCUSSION, SUMMARY, AND EPILOGUE

By: André N. Vis

GENERAL DISCUSSION

Prostate cancer is an intriguing disease with a high socioeconomic impact, though its clinical complexity still remains difficult to understand by patients, doctors, and the general public. While the general knowledge on the epidemiology, risk factors, and dangers of lung and breast cancer is generally reasonable, prostate cancer is still surrounded by a curtain of uncertainties, taboos and misperceptions. This is worrisome as the unfortunates diagnosed with the disease may not always receive a balanced advice on proper disease management, and a potential for treatment delay is created. Two main misperceptions on prostate cancer are maintained by the lay press as well as by medically educated personnel. First, prostate cancer is often considered a disease that does not actually poses a threat to the ones diagnosed with the disease. This misperception is build on the observation that previous autopsy studies reported a 30 to 50% prevalence of disease in 80-year old males who had no evidence of clinical disease during life. With respect to these observations, malignancies that originated from the prostate gland were assumed to remain silent in their hosts during lifetime, and were unlikely to cause any bother ever. Basically, of course, this is true, but it has to be kept in mind that the microscopical prevalence of disease is not in any way similar to the prevalence of disease as reflected by the cumulative incidence of disease. This figure is calculated by adding the number of living cases with prostate cancer that have presented themselves clinically in previous years. In fact, epidemiological data prove that prostate cancer has become the 'number one' incidence cancer in males in the Netherlands in the year 2000. Moreover, a substantial proportion (30 - 60%) of these 6,500 newly diagnosed cases will be advanced at the time of diagnosis, and 20 to 25% will already be beyond the reach of cure. Also, 40% of men diagnosed with the disease will eventually die from their disease, and the mortality is substantially higher in younger (< 65 years) patients and in certain high-risk groups. Obviously, these are not the microscopical cancers detected coincidentally within autopsy studies. A second often heard misperception is that prostate cancer is considered a disease of the *very* elderly. Similar to other 'high-incidence' cancers such as colorectal cancer and breast cancer, the majority of prostate cancers is diagnosed before the age of 75 years, and one in thirteen (i.e. 500 cases a year) is even diagnosed before the age of 60 years (in the absence of screening).

Prostate cancer is better considered a malignancy with a variable and sometimes unpredictable course of disease, that because of its protracted course of disease causes itself to be preferentially found in elderly males. Indeed, the different stages of disease (i.e. locally confined, locally advanced, metastatic, death) are passed within the time frame of many years in most cases, and many men with prostate cancer will live into their seventies and eighties under adequate therapy. With sufficient time, however, the majority

of clinically diagnosed cases with prostate cancer will progress if not treated with some evolving quickly, and causing premature deaths.

These unfortunate misperceptions on prostate cancer may also influence a proper judgement making on issues like prostate cancer screening and early treatment of disease. It has been suggested previously that screening for prostate cancer could hardly be beneficial, as the application of the PSA test would lead to the detection of a large number of clinically insignificant (i.e. microscopical) cancers. Moreover, it was claimed that the performance of the screening tests was not clearly understood, and that the efficacy of early treatment has not yet been proven. For now, screening for prostate cancer is still considered a controversial issue and should not be offered to asymptomatic men within the population. Large randomized clinical trials such as the European Randomized study of Screening for Prostate Cancer (ERSPC) are presently underway to assess the impact of prostate cancer screening on disease-specific survival and quality of life. These RCTs provide a means to study the performance of the screening tests in detail in association with the biological potential of the cancers detected.

PART II. Towards Predicting the Outcome of Prostate Cancer Screening

The study of the tumor characteristics of the cancers diagnosed in screening trials may help to identify the patients who are most likely to benefit from screening efforts. Unfortunately, the final endpoint of RCTs that investigate the impact of screening on cancer-specific mortality has not yet been reached, so a distinction between those who are truly to benefit from screening efforts and those that are not, can only be estimated by examining surrogate and/or intermediate endpoints. *Chapter 2* attempted to stratify surgically treated patients in three prognostic subgroups, i.e. those that are cured from (future) aggressive disease, those that are overtreated, and those that are already beyond cure at the time of radical prostatectomy. Despite the fact that a surrogate endpoint was used in this study (PSA relapse after radical prostatectomy), we were able to stratify patients into three prognostic subgroups based on well-established pathological prognostic tumor features. **In our prognostic stratification model, approximately 20 to 25% of surgically treated cases were diagnosed with prostate cancer that may not need to be treated at the time of screen-detection, while 10 to 15% of surgically treated cases had prostate cancer that could not be cured by radical prostatectomy.** The stratification model remains arbitrarily as PSA relapse after radical prostatectomy will only predict some of the future cases that will progress clinically or the ones who are to die from the disease. So, a proper division of cancers into 'harmless', 'curable', and 'non-curable' can only be determined retrospectively, i.e. after the completion of well-performed RCTs. During the continuation of the screening study, the exact borders of the three prognostic subgroups have to be re-evaluated and adapted, and

finally, the exact 'window of opportunity' may be defined. For now, the presented stratification model only provides an estimate of the risk of PSA relapse after radical prostatectomy, thereby opting for increased surveillance or early hormonal treatment in those with an increased risk of progression, or otherwise, for a decreased visit frequency in those with a low risk of progression.

The proposed tumor classification model may be especially amenable to predictive analyses before treatment. This is needed as the number and proportion of men with presumably 'harmless' disease is remarkably high (20-25%) in our study. **Therefore, future major efforts should be directed at the prevention of treatment (and detection) of cancers with innocuous tumor features.** Using a variety of clinical, biochemical and tumor features, a reliable method should be developed that is able to predict the different prognostic subgroups before treatment. In this, artificial neural networks analysis (ANN) and correlation and regression tree analysis (CART) may be particularly valuable. Of course, the question remains whether the cancers defined as 'innocuous' in our prognostic classification model truly remain 'innocuous' if not treated. This question can only be answered by randomizing men with presumably innocuous tumor features before treatment into a group that receives treatment and a group that receives no treatment. The clinical manifestation of prostate cancer may then serve as an endpoint. The prognostic significance of the two other prognostic subgroups remains to be established as well. For instance, it might well be that some cases with advanced disease will benefit from early treatment, e.g. radical prostatectomy, or that some may profit from the early application of adjuvant hormonal therapy.

In *Chapter 3* it was demonstrated that a favorable prognostic shift was observed in the screen group of ERSPC compared to the control group. The proportion, and more importantly, the absolute number of men with metastatic disease was also lower in the screened group. Cases with distant metastases are most prone to die of their disease later on, holding strongest evidence for an indirect success of screening. As lead time has only just been passed, differences between screen and control may get more pronounced in the future. As was stated earlier, these results are only intermediate signs of success of screening trials, and do not provide by any means evidence for the assumption that prostate cancer screening reduces the mortality of disease. *Chapter 4* investigated the performance of two different biopsy techniques for the (repeated) detection of prostate cancer. For both procedures, cancers that remained undetected on repeated biopsy had most often features of clinically insignificant disease, while most of the clinically significant cancers were redetected. **Therefore, it is supposed that prostate biopsy is unlikely to detect many of the clinically insignificant cancers residing in the population.** A potential bias is raised, as it is not known how many cancers with clinically (in) significant tumor features remain after the first biopsy session.

Part III. On the Predictive Value of Prostate Cancer Precursor Lesions

Major debates have been elicited on the outcomes of the study presented in *Chapter 5*. **In population based screening for prostate cancer, the most established precursor lesion of prostate cancer, HPIN, lacked additional predictive value for the detection of prostate cancer on repeated biopsy compared to cases that had an initial benign biopsy outcome.** Moreover, we reported that the incidence rate of HPIN, and the yield for prostate cancer on repeated biopsy was lower than those reported in non-population based screening programs. Potential explanations for these discrepancies are addressed in detail in *Chapter 5* and *Chapter 6*, and are mostly explained by differences in the populations studied, differences in the initial indications for biopsy, and differences in the biopsy technique itself, both of the initial and repeated biopsy. Factors associated with the classification of precursor lesions by pathologists may have influenced the findings as well. Although we do not have any prove, it might well be that medicolegal reasons refrain (mostly American) pathologists from making a definite equifocal diagnosis in case of uncertainty. In our belief, the results of our study are particularly valuable for it concerns a population based screening study, whereas most other studies reported on opportunistic and case-finding screening studies. A population based study is known to represent a sample of the general population, and reduces the chance of all kind of biases. A further strength of our study lies in its initial design, which compared the yield for prostate cancer after a diagnosis of HPIN to that of men with an initial benign biopsy result. Only few studies on HPIN have evaluated a control group within the same study. In fact, two other study groups could not demonstrate a difference in the yield for prostate cancer on repeated biopsy in cases that underwent repeated biopsy after a diagnosis of HPIN to 'no evidence of disease'. It might well be that slight differences in the baseline characteristics of men who underwent a repeated biopsy (e.g. the PSA-level or findings on DRE or TRUS) might have influenced the outcomes of the repeated biopsy.

Part IV. Towards a Refining of Screening in Low PSA Ranges

None of the currently applied methods for prostate cancer detection, alone or in combination, works optimally as a screening tool. We do not yet have screening tests that can reliably differentiate between the presence and absence of prostate cancer, nor between aggressive and non-aggressive disease. The currently applied screening tests for prostate cancer are the PSA test, digital rectal examination (DRE) and transrectal ultrasound (TRUS). Presently, we know that the PSA blood test does rather well as an indicator for the presence of prostate cancer with respect to its sensitivity, specificity and positive predictive value. However, its validity needs to be further improved to avoid

inefficient, costly and unnecessary testing, overdiagnosis and overtreatment. The performance of DRE as a screening test for prostate cancer is known to be less accurate than PSA, and its performance is hampered by a limited reproducibility from one examiner to another. Whether this screening test for prostate cancer still has a place in the early detection of prostate cancer in population based screening is fiercely questioned. TRUS has the lowest test performance of the three with regard to its yield of prostate cancer detection. Its application is time-consuming, examiner dependent and costly. Though, improvements in its application have been described, and are to be further expected in the near future.

In *Chapter 7* we have demonstrated that the screening regimen in which DRE is applied as a screening test for prostate cancer at low PSA values (0.0 – 3.9 ng/mL) leads to a tremendous lot of unnecessary testing, both with respect to the number of DREs needed, and the number of biopsies required to detect one case of presumably clinically significant disease. We also demonstrated that when PSA only (≥ 3.0 ng/mL) was applied as a screening test for prostate cancer, the number clinically significant cancers was higher than when rectal examination only was used. Moreover, PSA based screening was far more efficient. Of course, the bothers, negative side-effects, and costs associated with the application of DRE as a screening test for prostate cancer must be balanced against the risk of missing potentially aggressive cancers within the low PSA ranges. Though, we showed that the total number of clinically significant cancers is low at low PSA values, and a substantial proportion of these undetected cancers may be detected later on in subsequent screening rounds. **With the objective of rational and efficient screening, we claim that DRE *can* and *must* be omitted as an initial screening test for prostate cancer at low PSA values (0.0 – 3.9 ng/mL).** In *Chapter 8* we demonstrated that most of the cancers detected in low PSA ranges using DRE as a screening test for prostate cancer were not detected as a result of a true-positive screening test, though rather coincidentally. **Depending on the definition, between 27 and 63% of the prostate cancers detected after the evaluation of a suspicious rectal examination (whether DRE or TRUS) at low PSA values were detected by chance only (i.e. by serendipity).**

As DRE is not routinely performed or recommended in the intermediate and higher PSA ranges (i.e. ≥ 4.0 ng/mL), this screening test for prostate cancer looses its position as a detection measure in population based screening programs. The question then remains what is the proper screening regimen in low PSA ranges, as it is known that a number of clinically significant cancers reside here. Presently, we recommend that PSA only (i.e. 3.0 ng/mL or higher) is to be used as a trigger point for biopsy at low PSA values. The collection of a blood sample is simple, readily accepted, and serum-PSA measurement is relatively inexpensive, and quite reproducible. In ERSPC, section

Rotterdam, screening efforts are presently directed at the PSA range 2.0 – 2.9 ng/mL with PSA only as the indication to biopsy. Data on the positive predictive value, the number of biopsies needed to detect one prostate cancer, and data on the tumor characteristics of the cancers detected will be given in a future report, and give further insight into the effectiveness and efficiency of screening in this debated PSA range.

PART V. On the Predictive Value of Prognostic Tissue Markers

Despite the independent prognostic value of tumor stage and the grade of tumor differentiation for the prediction of prognosis in almost every study on prostate cancer, additional markers are needed to give a more detailed and individualized insight into the expected extent of disease, the tumor aggressiveness, the risk of progression after curative treatment, and the risk of death from disease. Particularly, those tumor markers that can identify the cases with (future) metastatic disease, or those that may identify the cases that are most likely to die from the disease later on are warranted, and are and should be the subject of major research efforts. Treatment may thus be adapted to the extent of disease more often. Otherwise, tumor markers that point out the cases with potentially harmless (clinically insignificant) disease might be useful as these may reduce the risk of overtreatment.

In ***Chapter 9*** we investigated the prognostic impact for disease outcome of three prognostic tissue proteins expressed in tumors of the radical prostatectomy specimen. By immunohistochemical analysis, we found that a low cell-cycle protein $p27^{kip1}$ expression was independent of grade and stage to predict clinical disease progression, and death of disease. Also, the cell-adhesion protein CD44 was predictive for the development of local recurrence and distant metastatic disease on multivariate analysis. Potentially, these markers can be used to predict disease outcome on an individual level in surgically treated patients. Unfortunately, the expression level of these prognostic tissue markers on the diagnostic biopsy was only weakly correlated to the expression level of matched radical prostatectomy specimens ***(Chapter 11)***. Due to sampling error, the applicability of the prognostic tissue markers $p27^{kip1}$ and CD44s was also limited with respect the prediction of grade and stage of disease. Only a low (prognostically unfavorable) $p27^{kip1}$ expression could predict a worse prognostic constellation within the prostate. ***Chapter 10*** and ***Chapter 12*** indicated that the search for reliable, independent and clinically relevant tissue markers is still ongoing and impeded by all kind of problems related to proper tissue handling, reproducibility, and lack of sensitivity and specificity.

EPILOGUE

If RCTs that investigate the impact of systematic prostate cancer screening on cancer-specific mortality and quality of life eventually prove net beneficial, screening efforts should be preferentially directed at the detection of cancers within the 'window of opportunity'. The validity of the screening tests should be optimized to advocate rational, selective and (cost) efficient prostate cancer screening. When prostate cancer is diagnosed, a risk analysis should be performed before treatment to minimize the treatment of cases with 'clinically insignificant' disease. Nevertheless, cases with presumably innocuous tumor features should be followed with care as it is known that prostate cancer is an unpredictable disease, and some of the 'harmless' cancers may still progress clinically. Although not proven, it might well be that cases with advanced disease at the time of screen-detection will benefit from (early) detection as well, for instance, by radical prostatectomy, or by the early application of hormonal treatment. This has to be evaluated in future studies.

But also if RCTs do not prove net beneficial, the knowledge of the behavior of the disease, its prognostic factors, the efficacy of curative treatment options, and the management of patients with localized and advanced disease, will be tremendously increased by research efforts on prostate cancer screening. Despite the eventual (regrettable) finding that population based screening may not be warranted as a nation-wide health care policy, because the adverse effects of screening do not outweigh the benefits, it is likely that certain high-risk groups can be identified. These selected groups of patients may nonetheless profit from opportunistic prostate cancer screening efforts. In other words, even if screening trials do not prove beneficial on a population based level, the knowledge derived from screening trials may help to provide a more balanced advice to men who seek information on their risk of having this potentially lethal disease. Furthermore, when prostate cancer is diagnosed by the application of screening tools (or after the evaluation of signs and symptoms), guidelines may have been developed that help to select the appropriate treatment of patients with the different stages of disease. Also, a prediction of the long-term prognosis may be given to patients with the disease when treated or when treatment is refrained. In other words, the knowledge obtained from large-scale screening trials for prostate cancer will eventually improve the diagnostics, management and treatment of patients with the disease.

SAMENVATTING
[DUTCH]

DEEL I. Algemene Introductie

Prostaatkanker is met longkanker de meest voorkomende vorm van kanker bij mannen en is, na longkanker, de meest frequente sterfteoorzaak aan kanker (Zie: FIGUUR 1.1). Na aanleiding van het eerdere succes bij borstkanker is onlangs de vraag gerezen of ook bij prostaatkanker een bevolkingsonderzoek gezondheidsvoordeel zou kunnen opleveren. De gouden standaard met betrekking tot de vraag of een bevolkingsonderzoek ook ten goede komt aan de gezondheid van de bevolking in zijn geheel is het uitvoeren van een gerandomiseerd klinisch screenings-onderzoek. Een screeningsprogramma behelst het aanbieden van een eenvoudige en relatief goedkope screeningstest aan een grote groep mensen met als doel deze personen te classificeren als "aannemelijk" of "minder aannemelijk" voor het hebben van een ziekte of aandoening. Bij randomisatie wordt de populatie willekeurig onderverdeeld in een groep die de screeningstests ondergaat (de screeningsgroep), en een groep die op de gebruikelijke wijze wordt benaderd en behandeld (de controlegroep). Een verschil in uitkomst tussen beide groepen, bijvoorbeeld een vermindering van het aantal sterfgevallen aan prostaatkanker in de screeningsgroep, kan dan worden toegeschreven aan de toegepaste screeningstests en de vroegbehandeling van de ziekte. Voor prostaatkanker zijn diverse screeningstests beschikbaar. Ten eerste de bepaling van het serum prostaat-specifiek antigen (PSA). PSA is een eiwitstof die enkel (d.i. specifiek) door de prostaatklier wordt gemaakt en in kleine hoeveelheden naar het bloed lekt. Bij mannen met prostaatziekten, zoals bijvoorbeeld prostaatkanker, is het PSA in verhoogde concentraties aanwezig en aantoonbaar in het bloed. Wanneer in een screeningsprogramma een verhoogd serum-PSA wordt aangetoond, is verder diagnostisch onderzoek middels een prostaatbiopsie geïndiceerd. In het bij het prostaatbiopt verkregen weefsel kan prostaatkanker worden aangetoond dan wel uitgesloten. Daarnaast worden als screeningstest voor prostaatkanker toegepast het rectaal toucher (DRE) en de transrectale echografie (TRUS). De bepaling van de serum-PSA waarde is de meest gevoelige en specifieke test voor de vroegopsporing van prostaatkanker.

Het lijkt intuïtief aannemelijk dat de vroegopsporing van een ziekte gepaard gaat met een vermindering van het aantal patiënten in een vergevorderd stadium van de ziekte, bijvoorbeeld die met metastasen op afstand (d.i. uitzaaiingen), alsmede met een vermindering van het aantal personen dat uiteindelijk aan de ziekte zal overlijden. Dit is echter niet vanzelfsprekend. Screeningstests worden toegepast bij personen zonder klachten en het is niet *a priori* duidelijk welke personen kanker hebben en welke niet. In

een bevolkingsonderzoek zal de meerderheid van de gescreende personen immers geen kanker hebben en dus ook geen voordeel van de toegepaste screeningstests ondervinden. Ook wanneer in een bevolkingsonderzoek onverhoopt toch kanker wordt gediagnosticeerd, garandeert dit nog geen gunstig effect van het screeningsprogramma. Enerzijds zullen niet alle in een bevolkingsonderzoek ontdekte kankers tot klachten en sterfte leiden wanneer het bevolkingsonderzoek niet zou zijn uitgevoerd. De detectie van deze kankers heet overdiagnose en de behandeling van een overgediagnosticeerde kanker overbehandeling. Bij kankers die betrekkelijk langzaam groeien, en/of relatief laat aanleiding geven tot klachten is de kans op overdiagnose en overbehandeling groter. Ook wanneer een kanker zich relatief vaak presenteert op latere leeftijd zal vroegopsporing niet altijd gezondheidsvoordeel opleveren. Immers, personen op oudere leeftijd hebben vaak andere ziekten (comorbiditeit) en de kans dat een gescreende persoon in de tijd tussen het toepassen van de screeningstests en de klinische manifestatie van de ziekte zal overlijden aan één van deze andere ziekten (bijvoorbeeld een hartinfarct of hersenbloeding) zal vergroot zijn. Anderzijds kunnen in een bevolkingsonderzoek kankers worden gevonden, die ondanks de vroegopsporing toch aanleiding zullen geven tot klachten en sterfte. Vroegopsporing zal dan het klinisch beloop van een kanker niet veranderen, en het zal duidelijk zijn dat deze personen geen voordeel zullen hebben van het bevolkingsonderzoek; ze zullen enkel langer leven met de wetenschap van het hebben van een potentieël dodelijke ziekte. Niet alle deelnemers aan een bevolkingsonderzoek zullen dus profiteren van de screeningsinspanningen. Een uiteindelijk besluit of een bevolkingsonderzoek op prostaatkanker zal worden aangeboden aan de populatie zal worden gewogen aan de voordelen en nadelen van het bevolkingsonderzoek voor de populatie en in mindere mate aan die van het individu.

De Europese gerandomiseerde studie voor de vroegopsporing van prostaatkanker (ERSPC) is een bevolkingsonderzoek dat in zeven Europese landen wordt verricht. ERSPC heeft als doel een vermindering van prostaatkankersterfte aan te tonen van minimaal 20% in de screeningsgroep (Studie-coördinator: Prof. Dr F.H. Schröder). Om dit verschil statistisch aan te tonen is berekend dat 100.000 mannen moeten worden gescreend (met 100.000 mannen in de controlegroep). Uiteindelijk zullen we pas aan het eind van het huidige decennium weten of een bevolkingsonderzoek op prostaatkanker ook ten goede komt aan de gezondheid van de (mannelijke helft van de) bevolking. Tot die tijd zullen inspanningen moeten worden verricht om de beschikbare screeningstests meer selectief (d.w.z. enkel die groepen mannen dienen de screeningstests te ondergaan waarin de *a priori* kans op prostaatkanker het grootst is), en efficiënt te maken (d.w.z. met minimale inspanningen en met minimale kosten zoveel mogelijk prostaatkankers vinden).

Het hier gepresenteerde proefschrift rapporteert (met name) over de screeningsarm van ERSPC. Speciale aandacht is besteed aan de (tumor) karakteristieken van de in het bevolkingsonderzoek gevonden prostaatkankers en die van zijn voorlopers. Met behulp

van deze gegevens worden suggesties aangereikt die mogelijk een screeningsonderzoek meer selectief en efficiënt kunnen maken. Tevens deden we onderzoek naar enkele prognostische weefselmarkers die zijn geassocieerd met tumoragressiviteit, met de kans dat een tumor terugkeert na een in opzet curatieve therapie en de prognose van de ziekte. Weefselmarkers kunnen mogelijk bijdragen aan een meer individueel afgestemde benadering en/of behandeling van de patiënt met prostaatkanker.

DEEL II. Over het Voorspellen van de Uitkomst van Prostaatkanker Screening

Als één van de in opzet curatieve behandelingsopties voor prostaatkanker geldt de "retropubische radicale prostatectomie", het operatief verwijderen van de prostaat middels een onderbuiksincisie. Echter niet in alle operatief behandelde patiënten met prostaatkanker is de kanker ook definitief verdwenen; prostaatkanker kan lokaal of uitgezaaid terugkeren. Na een operatie is ook het PSA doorgaans niet meer aantoonbaar in het bloed, echter wanneer blijkt dat het PSA toch aantoonbaar is of wordt na een operatie, dan is dit een uiting van (biochemische) ziekteterugkeer. Het lijkt aannemelijk dat de agressiviteit en uitgebreidheid van de tumor ten tijde van de operatie zullen bepalen of de prostaatkanker kan worden genezen middels radicale prostatectomie of een hoge kans heeft terug te keren. Belangrijke determinanten die het biologisch gedrag, de agressiviteit van de tumor en de uiteindelijke prognose van de patiënt zullen bepalen zijn het tumor stadium, de graad van de tumor (de "Gleason score"), en het tumor volume, zoals die door de patholoog microscopisch kunnen worden beoordeeld in het operatie preparaat. In ***Hoofdstuk 2*** correleerden we deze pathologische prognostische tumor karakteristieken, alleen en in combinatie, met de PSA terugkeer na radicale prostatectomie. Hierbij trachtten we de door screening ontdekte kankers onder te verdelen in drie prognostische groepen: 1. De groep die het meeste baat heeft bij vroegopsporing en vroegbehandeling, aangezien er een aantal prognostisch ongunstige (agressieve) factoren aanwezig is, maar waarbij de tumor niet (biochemisch) terugkeert na radicale prostatectomie. Deze groep kankers heeft, als ze niet zou zijn ontdekt door screening, een aanzienlijke kans om in de toekomst te leiden tot ziekte en sterfte. 2. Een groep die een prognostisch zeer gunstig profiel heeft en die mogelijk niet direct een gevaar voor de patiënt zou hebben opgeleverd. Deze kankers hebben een lage groeisnelheid, een lage kans op uitzaaiingen, en zouden hoogstwaarschijnlijk tot aan volgende screeningsronde(s) niet hebben geleid tot klachten. 3. De groep die zeer agressieve tumor karakteristieken heeft ten tijde van de operatie en waarbij het PSA snel terugkeert na radicale prostatectomie. Mogelijk waren deze kankers al uitgezaaid ten tijde van de operatie, al kan dit niet met zekerheid worden aangetoond.

Hoofdstuk 3 geeft de voorlopige resultaten van de vergelijking tussen de screeningsarm en de controle arm van ERSPC. In de screeningsarm is de Gleason score, zoals bepaald op het naaldbiopt van de prostaatkankers, gunstiger dan in de controlearm, terwijl ook het aantal patiënten met uitzaaiingen in zowel absolute zin als relatieve zin lager was in de screeningsgroep. Deze resultaten geven dus een eerste aanwijzing voor de veronderstelling dat een bevolkingsonderzeok op prostaatkanker voordelen kan opleveren met betrekking tot de sterftereductie aan de ziekte. Het aantal patiënten in de controlegroep met uitgezaaide ziekte slechts was klein en definitive conclusies kunnen nog niet worden getrokken. Tien patiënten presenteerden zich initieël met uitgezaaide ziekte ongeveer 5 jaar na randomisatie. Wanneer de gegevens over enige tijd worden herbeoordeeld is het aannemelijk dat het verschil tussen screeningsgroep en controlegroep meer geprononceerd is.

DEEL III. Over de Voorspellende Waarde van Voorloper Lesies

Naast de diagnose "definitief geen prostaatkanker" of "aangetoond prostaatkanker" wordt in een screeningsonderzoek door de patholoog soms ook de diagnose "voorloper lesie van prostaatkanker" of "afwijking, verdacht voor kanker" gesteld. Voorloper lesies zijn microscopisch waarneembare afwijkingen die worden beschouwd als de laatste fase in de ontwikkeling van een kanker voordat eigenschappen van maligne ontaarding (kanker) optreden. De meest erkende voorloper lesie van prostaatkanker is hooggradige prostatische intra-epitheliale neoplasie (HPIN). Een "afwijking, verdacht voor kanker" is een microscopisch waarneembare afwijking die cellulaire en histologische overeenkomsten vertoont met prostaatkanker, maar niet in die mate dat de patholoog ook definitief de diagnose "prostaatkanker" durft te stellen. Omdat de hoeveelheid verkregen weefsel in een screeningsprogramma relatief gering is, komt deze diagnose meer voor dan buiten screeningsprogramma's. Het is vanzelfsprekend dat een "fout-positieve" diagnose verregaande consequenties heeft voor een deelnemer aan een screeningsonderzoek en deze moet dus worden vermeden. Bij de diagnose "HPIN" of "prostaatbiopt verdacht voor maligniteit" wordt geadviseerd het biopt te herhalen binnen 6 weken om prostaatkanker aan te tonen dan wel uit te sluiten. In ***Hoofdstuk 6*** toonden we aan dat in een bevolkingsonderzoek voor de vroegopsporing van prostaatkanker de diagnose "HPIN" relatief weinig frequent wordt gesteld (0.8% van de 4.057 gebiopteerde mannen) en dat vervolgdiagnostiek niet vaker leidt tot de diagnose "prostaatkanker" dan wanneer het eerste biopt zou zijn afgedaan als "definitief geen prostaatkanker". In 3 van de 30 (10.0%) hergebiopteerde mannen met HPIN troffen we prostaatkanker aan in het herhalingsbiopt, tegen 51 van de 462 hergebiopteerde mannen met een eerdere "benigne" biopsie uitslag. De aanvullende voorspellende waarde voor prostaatkanker van HPIN is

dus relatief gering of zelfs afwezig. Bovendien toonden we aan dat de tumor karakteristieken van de 3 gevonden kankers prognostisch zeer gunstig waren. Anderzijds kwam de diagnose "afwijking, verdacht voor kanker" vaker voor dan "HPIN" (2,6% van de 4.057 gebiopteerde mannen) en in een aanzienlijk groter deel (36 uit 93; 38.7%) van de hergebiopteerde mannen troffen we prostaatkanker aan in het herhalingsbiopt. De tumor karakteristieken van de gevonden kankers kwamen nagenoeg overeen met die van de kankers die op het eerste naaldbiopt werden gevonden. We kunnen concluderen dat een herhalingsbiopt bij mannen met HPIN niet direct geïndiceerd is, terwijl mannen met een verdachte lesie een herhalingsbiopt dienen te ondergaan.

***DEEL IV.* Over de Waarde van Screening bij lage PSA Waarden (0.0 – 3.9 ng/mL)**

De "American Cancer Society" en de "American Urological Association" adviseren vanaf het begin van de jaren '90 om bij elke man vanaf de leeftijd van 50 jaar jaarlijks een PSA bepaling en een DRE als screeningstest voor prostaatkanker uit te voeren. Naar analogie van deze Amerikaanse screeningsaanbevelingen werden aanvankelijk in ERSPC alle mannen in de screeningsgroep uitgenodigd voor een PSA bepaling en, in hetzelfde bezoek, het ondergaan van een DRE. In de lage PSA waarden (0.0 – 3.9 ng/mL) vormt enkel een afwijkend DRE een indicatie voor naaldbiopsie. Recent is gebleken dat de positief voorspellende waarde van een DRE (d.i. het percentage gevonden kankers per afwijkende screeningstest) voor de detectie van prostaatkanker beperkt is in de lage PSA regionen. Echter, wanneer een groot deel van de op deze manier gevonden kankers agressief zou zijn en mogelijk in de toekomst zou leiden tot klachten of sterfte, dan zou een relatief inefficiënt screeningsbeleid gerechtvaardigd kunnen zijn.

Op geleide van eerder gepresenteerde onderzoeken (Hoofdstuk 2) konden de door screening ontdekte prostaatkankers worden onderverdeeld in "klinisch irrelevant" (kankers die vanwege hun relatief onschuldige tumor karakteristieken mogelijk niet direct detectie en behandeling behoeven) en "klinisch relevant" (kankers die vanwege hun agressieve tumor karakteristieken mogelijk aanleiding hebben gegeven of zullen geven tot klinisch manifeste ziekte en/of sterfte). In ***Hoofdstuk 7*** toonden we aan dat het aantal door DRE ontdekte klinisch relevante kankers in de lage PSA regio's relatief gering is en bovendien evenredig steeg met de serum-PSA waarde. We konden berekenen dat een zeer groot aantal mannen een DRE (289 mannen bij PSA < 3.0 ng/mL) moest ondergaan om één klinisch relevante kanker te vinden en tevens dat slechts een klein deel van de mannen met een afwijkende screeningsstest ook daadwerkelijk werd gediagnosticeerd met prostaatkanker op het naaldbiopt (1 op de 46 bij PSA < 3.0 ng/mL). Bovendien bewezen we in ***Hoofdstuk 8*** dat een niet gering deel (tussen 27 en 63%) van de ontdekte kankers niet op basis van een juist-positieve screeningstest werd

ontdekt, maar op basis van toeval. In andere woorden, de voorspellende waarde van de screeningstest voor prostaatkanker (DRE) bij lage PSA waarden wordt overschat.

DEEL V. Over de Voorspellende Waarde van Prognostische Weefselmarkers

Naast de differentiatiegraad van de tumor en de stadiering is er behoefte aan aanvullende merkstoffen die een betrouwbare uitspraak kunnen doen over de agressiviteit van de tumor voor het individu, de kans dat de tumor curatief kan worden behandeld middels radicale prostatectomie en/of de uiteindelijke prognose van de patiënt. Met name tumor markers die voorspellend zijn voor het hebben van of het in de toekomst krijgen van metastasen op afstand zijn gewenst aangezien deze de behandeling van de ziekte meer kunnen afstemmen op de uitgebreidheid van de ziekte. De studie gepresenteerd in ***Hoofdstuk 9*** onderzocht de prognostische waarde van drie weefseleiwitten, te weten $p27^{kip1}$, MIB-1 (cel cyclus eiwitten) en CD44s (celmembraan eiwit) met betrekking tot het optreden van klinische progressie en sterfte aan prostaatkanker in een groep patiënten die een radicale prostatectomie onderging tussen 1980 en 1988. Uit eerdere studies was reeds gebleken dat een verlaagde immunohistochemische expressie van $p27^{kip1}$ en CD44s en een verhoogde expressie van MIB-1 samengaan met een slechtere prognose na radicale prostatectomie. In onze studie bleken de drie eiwitten sterk geassocieerd met tumor graad en tumor stadium, en een verlaagde expressie van $p27^{kip1}$ was een onafhankelijk statistisch significante variabele voor het optreden van klinische progressie en prostaatkankersterfte. Ook een verlaagde expressie van CD44s bleek een onafhankelijke voorspellende factor, echter alleen voor klinische progressie. Deze beide factoren kunnen dus naast graad en stadium een betrouwbaarder uitspraak doen over de prognose van de individuele patiënt.

We onderzochten in ***Hoofdstuk 11*** of de drie weefselmarkers konden worden gebruikt om reeds voor een behandeling iets te zeggen over de expressie in de prostaat en over de te verwachte graad en het stadium van de tumor. Hiervoor vergeleken we in een groep mannen, die een radicale prostatectomie had ondergaan, de expressie van de drie weefselmarkers op het diagnostisch biopt met die in de tumor in de prostaat. Tevens vergeleken we de expressie van de tumormarkers met de graad van de tumor en het tumor stadium. Doordat het diagnostisch biopt soms prognostisch belangrijke delen in de tumor mist was de correlatie tussen de expressie van de weefselmarkers in het biopt en die in de prostaat alsook met de graad en het stadium van de tumor gering.

In ***Hoofstuk 10*** en ***Hoofstuk 12*** toonden we aan dat de zoektocht naar betrouwbare, statistisch onafhankelijke en klinisch relevante weefselmarkers nog steeds doorgaat, en dat het toepassen van weefselmarkers in de kliniek nog steeds wordt belemmerd door allerlei problemen gerelateerd aan adequate weefsel fixatie, reproduceerbaarheid en een gebrek aan sensitiviteit en specificiteit.

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LIST OF PUBLICATIONS

From this Thesis:

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CURRICULUM VITAE

[ENGLISH]

The author of this thesis was born on October 9th 1970 in the city of Den Helder, the Netherlands. After graduating from college at the Alfrink College in Zoetermeer, he started the academic study of Biomedical Sciences at the University of Leiden in 1989. At this same University, he started medical school in 1990. During his medical education, the author worked as a teaching assistant at the laboratory of macroscopic Anatomy of the Medical Faculty of Leiden. A pre-residency was done at the department of Plastic, hand and reconstructive surgery of the Hospital Leyenburg, Den Haag (supervisor dr. A.R. Koch, Plastic surgeon). Here, a multicenter study was performed on the efficacy and safety of the 'four-corner arthrodesis' in the treatment of patients with 'scapholunate advanced collapse' (SLAC) wrists.

On October 25th 1996 he graduated from medical school to become a medical doctor (M.D.). From January 1997 until September 1998 the author worked as a resident at the department of Cardiopulmonary surgery at the Leids Universiteit Medisch Centrum (LUMC), Leiden. In October 1998 he started working on the present dissertation at the department of Clinical Pathology and the department of Urology as a member of the European randomized study of screening for prostate cancer (ERSPC). The thesis project was accepted as AGIKO project (i.e. a resident in clinical research) by the Dutch Urological Association (DUA) and the Dutch Society of Scientific Research (NWO). The concept of this thesis was accredited by the DUA as the best patient-oriented clinic scientific research project of 2000 – 2001 with the 'Prof. Dr M.A. Moonen'-prize. During this preferentially scientific period, the author of this thesis worked as a clinical trial doctor in the treatment of patients with metastatic prostate and renal cell carcinoma in co-operation with the department of Clinical Oncology, Dijkzigt Ziekenhuis, Rotterdam.

From October 1st 2001, the author of this thesis is working at the department of General Surgery at the Rijnland Hospital, Leiderdorp (clinical supervisor: Dr J.F.W.B Rijksen, General Surgeon) within the framework of the two-year residency training on General Surgery. On January 1st 2004 he will return at the Dijkzigt Hospital, Rotterdam, for the continuance of his Urological training (clinical supervisor: Prof. Dr F.H. Schröder, Urologist).

CURRICULUM VITAE
[DUTCH]

De auteur van dit proefschrift werd geboren op 9 oktober 1970 te Den Helder. Na het behalen van het VWO eindexamen aan het Alfrink College te Zoetermeer werd in 1989 begonnen met de studie Biomedische Wetenschappen aan de Rijksuniversiteit van Leiden. In 1990 werd aan deze zelfde universiteit gestart met de studie Geneeskunde. Ten tijde van de studie was de schrijver van deze thesis gedurende drie jaar werkzaam als student-assistent macroscopische anatomie van borst-buik-bekken aan de Medische Faculteit van Leiden. Een keuze co-schap werd doorlopen op de afdeling Plastische, hand-en reconstructieve chirurgie aan het ziekenhuis Leyenburg te Den Haag (supervisor dr. A.R. Koch, Plastisch chirurg). Daarbij werd een multicenter onderzoek verricht naar de effectiviteit en veiligheid van de "four-corner" arthrodese bij de behandeling van patiënten met 'scapholunate advanced collapse' (SLAC) polsen.

Op 25 oktober 1996 werd het artsexamen behaald. Vanaf januari 1997 tot september 1998 was hij werkzaam als AGNIO op de afdeling Thoraxchirurgie van het Leids Universiteit Medisch Centrum (LUMC). Op oktober 1998 werd begonnen met een promotie-onderzoek op de afdeling klinische Pathologie en Urologie binnen de Europese gerandomiseerde studie voor screening op prostaatkanker (ERSPC). Het promotie-onderzoek werd door de Nederlandse vereniging voor wetenschappelijk onderzoek (NWO) en de Nederlandse vereniging voor Urologie (NVU) gehonoreerd als AGIKO project. Door de NVU werd het concept voor dit boekje geaccrediteerd als beste klinisch patientgericht onderzoek van 2000 - 2001 met de "Prof. Dr M.A. Moonen" prijs. Gedurende de wetenschappelijke periode was de promovendus tevens werkzaam als klinische trial arts bij de behandeling van patiënten met gemetastaseerd prostaat-en niercelcarcinoom in samenwerking met de afdeling klinische Oncologie van het Dijkzigt Ziekenhuis.

Vanaf 1 oktober 2001 is de schrijver van dit proefschrift als AGIO werkzaam in het Rijnland Ziekenhuis te Leiderdorp (opleider Dr J.F.W.B Rijksen, Chirurg) in het kader van de vooropleiding Algemene Heelkunde, en na afloop, op 1 januari 2004, zal hij terugkeren in het Dijkzigt Ziekenhuis, Rotterdam, voor het vervolg van de Urologische opleiding (opleider Prof. Dr F.H. Schröder, Uroloog).

DANKWOORD
[DUTCH]

Wanneer je mij vijf jaar geleden had gevraagd of ik ooit zou willen promoveren, dan zal ik waarschijnlijk met een volmondig '*nee*' hebben geantwoord. Echter, tijden en meningen kunnen veranderen en het bewijs ligt voor je. Als ik zo het boekje inkijk dan merk ik dat enkel de hoofdstukken 9 en 11 in het oorspronkelijke promotieconcept zaten. De ideeën voor de andere hoofdstukken zijn pas later, tijdens de onderzoeksperiode, ontstaan en verder uitgewerkt. Het schept dan ook niet de verbazing dat het onderzoek een flinke wending heeft genomen. Terugkijkend denk ik dat drie jaar wetenschappelijk onderzoek mijn klinisch denken en vorming in sterke mate in gunstige zin heeft beïnvloed. Naarmate het onderzoek vorderde ontdekte ik dat er een breed inzicht in de epidemiologie, kliniek en behandeling van prostaatkanker was onstaan, alsmede in de vele aspecten van de vroegopsporing van de ziekte.

Een proefschrift schrijf je uiteraard niet alleen. Velen hebben in meer op mindere mate een bijdrage geleverd aan de totstandkoming van dit boekje, en de hierin opgenomen artikelen en was niet op zuiver wetenschappelijk niveau, dan was het wel op praktisch, inspirerend en/of vriendschappelijk vlak. Ten eerste wil ik natuurlijk *Bas van Rhijn* ('Bas') bedanken. Al op de eerste dag dat we begonnen aan ons promotie-onderzoek waren we verknocht aan de wetenschap, alhoewel we uiteraard de kliniek nog steeds hoog in het vaandel hadden staan. Drie jaar hebben we vertoefd in een kamer waar tientallen coupe-dozen, honderden artikelen, poster-rollen, de microscoop en de computer(s) soms letterlijk onontloopbare attributen waren. Hier hebben we het traject van de opleiding uitgedacht, en met succes. Ik hoop dat je als mijn paranimf wilt fungeren. Ten tweede is er *Robert Hoedemaeker* ('Hoed'), mijn voorganger. Veel van heb ik te danken aan het feit dat de infrastructuur voor dit onderzoek reeds was gelegd en dat ik, zoals gezegd, op een rijdende trein kon springen. Als mijn voorganger had je de nodige kennis en vaardigheden in huis om mij in hoge mate te inspireren. Het feit dat je in veel van mijn artikelen als 'tweede' staat spreekt voor zich. Naarmate de screeningsstudie voortschrijdt in de tijd worden de bevindingen steeds interessanter en makkelijker publiceerbaar en dat zal waarschijnlijk ook voor mijn opvolger(s) gelden.

De beide promotoren: *Prof. Dr Th.H. van de Kwast* ('Theo') was mijn meest directe begeleider, gaf me een uitgebreid en gedetailleerd inzicht in de pathologie van prostaatkanker, leerde met het 'Gleason scoren' en gaf me een enorme impuls tot het doen van degelijk en gefundeerd wetenschappelijk onderzoek. Je enorme laagdrempeligheid en vele discussies deden me veel deugt. *Prof. Dr F.H. Schröder* ('Zeer geachte professor Schröder', 'FHS'). Ik heb U leren kennen als een uitermate aimabel

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