Population Based Screening for Prostate Cancer: prognostic findings of two subsequent screening rounds

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Bevolkingsonderzoek naar prostaatkanker: pathologische bevindingen in twee opeenvolgende screenings rondes

Proefschrift

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Contents

General introduction

Prostate cancer is nowadays the most common non-cutaneous cancer in men in the Western world. Since the introduction of Prostate Specific Antigen (PSA) testing in the last decade, prostate cancer incidence increased dramatically. In addition, the population is aging, and prostate cancer incidence increases with higher age. The dilemma of prostate cancer is that more men die with prostate cancer than from prostate cancer, as reflected by the observation that in 70% of men who are 80 years or older prostate cancer is diagnosed histologically on autopsy. As a consequence of this high incidence on autopsy, it may be anticipated that a large proportion of old men are diagnosed with prostate cancer when undergoing prostate biopsy and a great proportion of prostate cancers detected in screening programs may be over-diagnosed. It is as yet unclear whether PSA based screening reduces prostate cancer mortality. Due to screening with PSA most cancers are diagnosed in an early stage and therefore possibly in a curable stage. As a result, cancer is removed in an early stage, before the tumor is able to metastasize. It is conceivable that population-based early prostate cancer screening will reduce prostate cancer mortality. In order to investigate this further, randomized clinical trials have been introduced. In the USA the Prostate Lung Colorectal and Ovary cancer (PLCO) study investigates if prostate cancer screening is justified. In Europe the European Randomized Study of Screening for Prostate Cancer (ERSPC) is conducted in 8 European countries to study whether prostate cancer screening can reduce prostate cancer specific mortality at affordable costs and quality of life. The European screening centers differ with regard to the screening procedure but they share PSA testing and most other features (Table 1). At the Rotterdam section of the ERSPC the screening protocol comprises serum PSA testing followed – in case of an elevated serum PSA level- by six lateralized needle biopsies, three from each side of the prostate (systematic sextant biopsy) in men aged between 55 and 75 years. Every four years the same cohort of men is screenend. Men are excluded from screening in the 2nd round if a previous diagnosis of prostate cancer is made and those with interval carcinoma (i.e. cancers detected after the 1st round, but during the 4-year screening interval period. Unfortunately, the final outcome of the ERSPC will not be here until the end of 2008 or later. This thesis is restricted to an analysis of the data from first and second screening rounds at the Rotterdam section of the ERSPC.

Awaiting the final outcome of the ERSPC, the investigations collected in this thesis are aimed to provide insight in 1) intermediate endpoints, concerning stage and grade of prostate cancer in subsequent screening rounds and the forthcoming therapy choices, 2) the efficiency of the screening protocol employed at the Rotterdam section of the ERSPC and 3) the natural biology of prostate cancer and its possible premalignant lesions.

Table 1

country	number of men randomized	age (years)	screening interval (years)	PSA cut-off (ng/ml) for sextant biopsy
Netherlands	42376	55-75	4	≥3.0[1]
Finland	80458	55-65	4	≥4.0 or abnormal DRE at PSA ≥3.0
Sweden	32298	50-66	2	≥3.0
Belgium	9284	55-74	7	≥4.0 or abnormal DRE or TRUS
France	Randomization ongoing	55-69	2	≥3.0
Spain	4278	45-70	4	≥2.9[2]
Italy	14577	55-70	4	≥4.0 or abnormal DRE and/or TRUS ≥2.5
Switzerland	Randomization ongoing	55-70	4	≥3.0

PSA prostatic specific antigen, **DRE** digital rectal examination, **TRUS** transrectal ultrosonography

[1]Since May 1997, before that PSA ≥4.0 ng/ml and abnormal DRE and/or TRUS used to be indicators for sextant biopsy.

[2]Since May 1998, it used to be ≥4.0 ng/ml

Part 1 Introduction to prostate cancer, pathology and clinical features

Chapter 1 provides a general background for the understanding of prostate cancer as a disease, including a description of the anatomy of the prostate, prostate cancer staging and grading, and current methods for detection of prostate cancer. In addition, an overview of the worldwide prostate cancer incidence, the effect of PSA testing on prostate cancer incidence and prostate cancer screening is given. This chapter also reviews the results of studies that have been published so far on prostate cancer screening.

Part 2 Importance of lesions with potentially increased risk for subsequent detection of prostate cancer

Premalignant lesions are considered as precursor lesions for cancer, as it is thought that these lesions would in time progress to prostate cancer. In literature high-grade prostatic intra-epithelial neoplasia (PIN) is generally accepted as the precursor lesion of prostate cancer, both on the basis of morphological resemblance of the cells composing PIN and the similarity of genetic alterations in PIN and prostate cancers. Recently attention was drawn to atrophy as another potential premalignant prostate lesion. It was thought that atrophy could be a precursor lesion because of the adjacent location near prostate cancer and the resemblance of morphology of some forms of atrophy

to that of prostate cancer. In addition, some studies report a similarity of molecular changes in atrophy and prostate cancer.

The biopsy diagnosis "lesion suspicious for prostate cancer (LSPC)" is made if the pathologist is not able to deliver with certainty a definite diagnosis of prostate cancer, because the lesion lacks some of the criteria necessary to make the diagnosis. In case of a diagnosis of PIN or LSPC made on the biopsy, the participant in the screening program will be asked to undergo a repeat biopsy. Repeat sextant biopsies are performed in these cases in order to increase the chance of detection, as these lesions are considered to be associated with a concurrent prostate cancer. It is important to have quantitative data on the frequency of these lesions in a screened population. They reflect the general population and directly impact on the costs related to screening and the accompanying increased stress, associated with repeat biopsy, which possibly lowers quality of life. In addition, we wished to investigate if the frequency and diagnostic relevance of PIN and LSCP would change during the subsequent screening round, four years after the first round. This part of the thesis reports the incidence of these lesions diagnosed in a screening population and the related incidence of prostate cancer. In addition, the possible relationship between atrophy and subsequent prostate cancer detection was investigated.

Chapter 2 outlines the incidence of PIN and LSPC in men screened for prostate cancer during the first (prevalence) and second screening round. The incidence of prostate cancer after repeat biopsies for these precursor lesions was compared with the incidence of prostate cancer in men biopsied one year after a benign biopsy outcome.

Chapter 3 deals with the recently proposed premalignant lesion: prostatic atrophy. We distinguished three variants of atrophy and we investigated the incidence and extent of each variant of atrophy and the frequency of subsequent prostate cancer during a follow-up period of 8 years.

Part 3 Intermediate endpoints to determine the efficacy of the screening protocol

In this part of the thesis the efficacy of the screening protocol used in the Rotterdam section of the ERSPC is monitored. This was done by investigation of the incidence of potentially advanced and focal cancers in the 2nd screening round. If screening during the 1st round were effective, we would expect that after a 4-year screening interval the cancer detection rate would decrease. We also expected a decrease in potentially advanced cancers and an increase of focal cancers. If we did not see a change towards the incidence of favorable cancers after a 4-year screening interval, tumors would have enough time to re-grow to the same level as they were diagnosed in the prevalence screening round and the screening interval might be considered as too long. This might also be reflected in the tumor volume in subsequent screening rounds. Another aspect of testing the screening protocol would be a comparison between the incidence, stage and grade of cancers of the screening and control arm. In the control arm, men might for instance, undergo opportunistic

screening (undergoing a PSA test upon request) or might be diagnosed incidentally with a prostate cancer on trans-urethral resection of the prostate (TURP) or cystoprostatectomy. Both the opportunistic screening and incidental finding might lead to a proportional increase of favorable tumors in the control arm and thus lower the prostate cancer specific mortality. In addition, a comparison of incidence, stage and grade between screening and control arm was made.

Chapter 4 compares the incidence of potentially advanced tumors on sextant biopsies in the 1st and 2nd screening round. Potentially advanced cancers were defined as high-grade (Gleason score 8-10) prostate cancers, or cancers largely composed of poorly differentiated tumor (Gleason pattern 4). These cancers carry a poor prognosis. Stage, grade and follow-up of these potentially advanced cancers are reported.

Chapter 5 deals with the incidence of focal cancer on sextant biopsy in the 1st and 2nd screening round. A focal cancer is a minimal focus of well-differentiated (Gleason score 3+3) cancer in one biopsy core. These cancers carry a favorable prognosis and might be an indicator of potential over-diagnosis. The follow-up of men treated with radical prostatectomy and watchful waiting was reported. For men managed with watchful waiting PSA doubling times were calculated.

Chapter 6 describes the differences between 1st and 2nd screening round findings, concerning therapy choice and radical prostatectomy. The follow-up of men treated with radical prostatectomy is reported together with prognostic factors for biochemical recurrence in men treated with radical prostatectomy.

In **Chapter 7** the clinical outcome of all men diagnosed with prostate cancer in the screening arm are compared to men diagnosed in the control arm. Follow-up of men who underwent radical prostatectomy is given.

Part 4 Natural history of screen detected prostate cancers

Prostate cancer is a heterogeneous and often multi-focal cancer, which is characterized by different Gleason patterns in one tumor. Two opposing hypotheses may explain the presence of both high-grade and low-grade components in prostatic cancer growth. 1) During its development a prostatic cancer starts to grow and to dedifferentiate from a well differentiated (low Gleason score) to a poorly differentiated (high Gleason score) tumor, or 2) the combination of low and high grade Gleason scores evolves from pluri-potent stem cells that shed throughout the prostate and grow in different sites of the prostate to form a heterogeneous cancer, which eventually collides to a big tumor with low-and high-grade components. Both mechanisms might also occur at the same time.

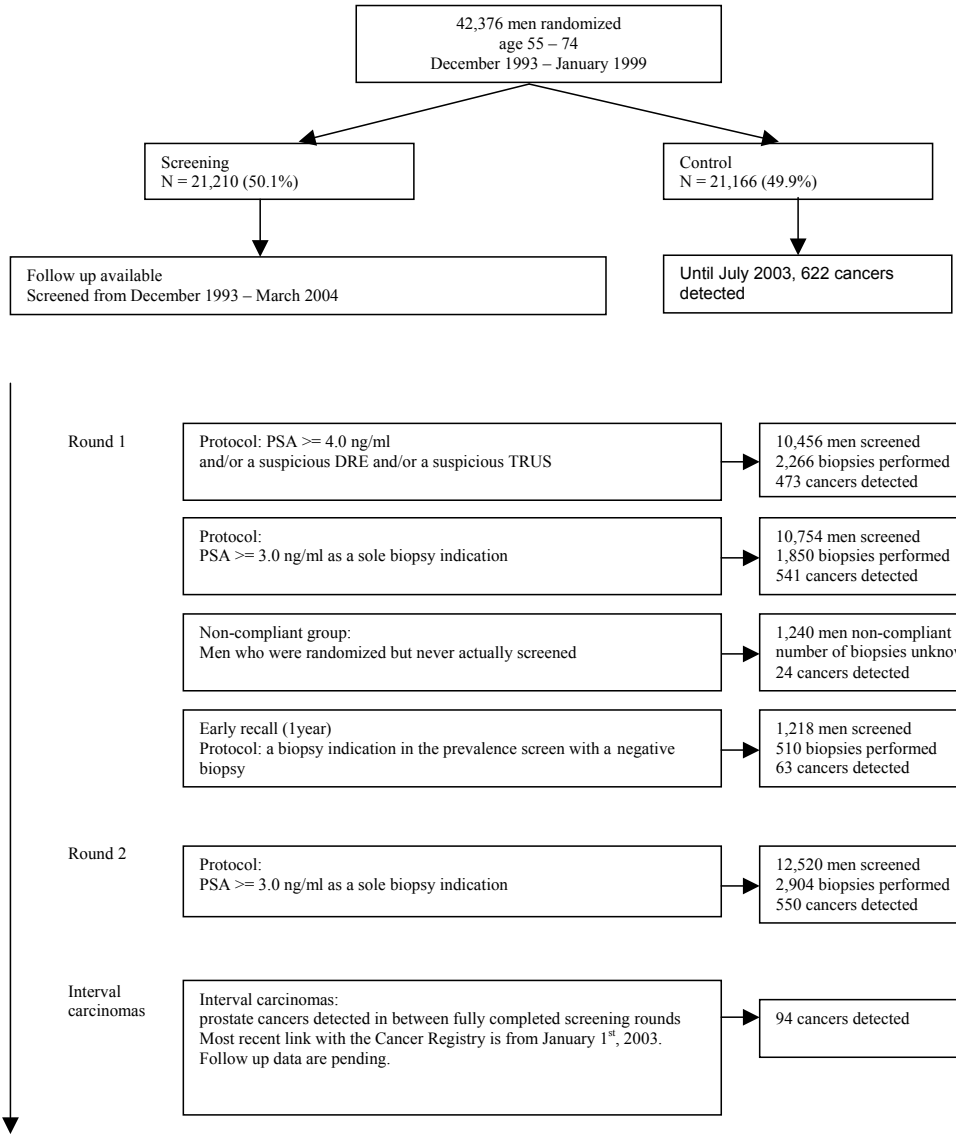
Chapter 8 This chapter examines the relation between age and Gleason score of cancers detected in the different screening rounds and the control arm. To support or to reject the hypothesis of dedifferentiation, the MISCAN model was used, which is a computer model that simulates screening. Two MISCAN

models were fitted (testing 1 and 2) to investigate which model would fit best to the screening situation.

Chapter 9 investigates whether there were significant genetic differences in well differentiated (Gleason score 6) and moderately differentiated (Gleason score 7) screen detected prostate cancers. As Gleason scores 6 and 7 are composed of Gleason pattern 3+3 and 3+4, it was investigated whether the Gleason patterns 3 from either Gleason score 6 or 7 prostate cancer, differed from each other and whether there were significant differences between a Gleason 3 and 4 pattern of a Gleason score 7 tumor.

Chapter 10 investigates the level of apoptosis measured by immunohistochemistry. Several studies have shown that prostate cancer could be prevented by drugs that induce apoptosis. Several studies reported a decreased apoptosis rate in advanced prostate cancer. However, no reports have been documented on early prostate cancer as far as we know. In this chapter the apoptosis activity is measured in screen detected prostate cancer. For this purpose a tissue micro-array (TMA) was used. A TMA consists of multiple small cores of tissue located on one slide. The TMA presented here contained screen detected prostate cancer and benign tissue. The screen-detected cases were composed of well differentiated and poorly differentiated (different Gleason scores) cancer and small and larger tumors (tumor volume). Tumor volume and differentiation were investigated, because it was thought that bigger and poorly differentiated tumors would have a different apoptosis level, compared to smaller and well differentiated tumors.

Figure 1 ERSPC Consort Diagram



Part 1

Chapter 1

Introduction to prostate cancer, pathology and clinical features

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Introduction

Over the last decade, considerable debate has occurred about whether screening for prostate cancer should be performed in asymptomatic men. PSA testing is mainly responsible for the changing statistics in prostate cancer. Presently, two large randomized clinical trials for prostate cancer are ongoing, one in Europe (European Randomized Study of Screening for Prostate Cancer, ERSPC) and one in the USA (Prostate Lung Colorectal and Ovary cancer, PLCO). The trials differ considerably in design. The answer with adequate evidence whether screening reduces mortality from these randomized clinical trials will not be available before 2008 or later. Until this time the debate about PSA based screening will go on. The goal of the ERSPC is to evaluate whether population based screening reduces mortality from prostate cancer at an acceptable price in terms of quality of life and costs. Within the ERSPC, approximately 193,000 men in eight European countries were recruited and randomized [1].

For justifying population based screening for disease, Wilson and Jungner developed ten criteria (Table 1). The Wilson and Jungner [2] table is the backbone of this review.

Table 1 Ten criteria to justify population based screening for a disease (Wilson and Jungner)

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

Anatomy, Morphology and function of the prostate

The normal prostate is a glandular organ with the size of a chestnut and surrounds the urethra as it exits the bladder, below the bladder neck (Figure 1). The prostate can be subdivided in several biologically distinct regions, a transition, central and peripheral zone (Figure 2). In the transition and central zone the most prominent lesion that occurs is hyperplasia, also referred to as benign prostatic hyperplasia. The incidence of these lesions increases with age, reaching 90% by the eighth decade. It results in enlargement of the prostate and in some cases can cause urinary obstruction. The peripheral zone is the site of origin of 70-80% of the carcinomas [3].

Figure 1

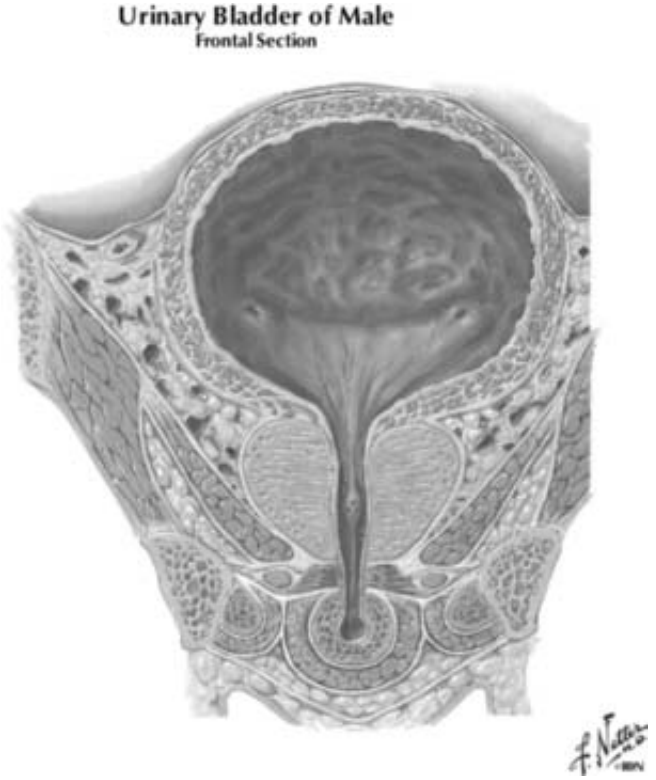
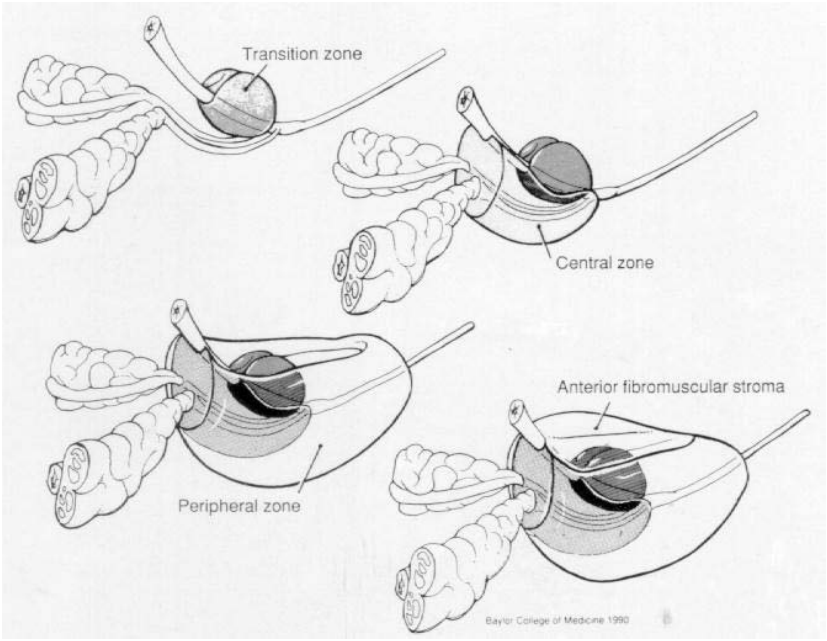
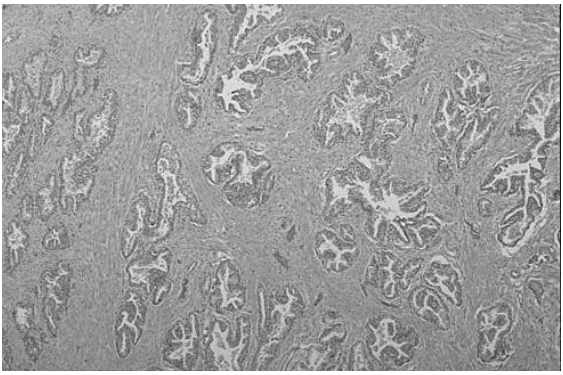


Figure 2 Zones of the prostate (Baylor college of medicine)



The prostate consists of groups of exocrine glands, which produce substances that are added to the spermatozoa. One major component is prostate specific antigen (PSA), a member of the kalikrein family, which are proteins with a proteolytic function. PSA influences the viscosity of the ejaculate. The histology of the normal prostate is depicted in figure 3. A double epithelial layer lines the exocrine glands of the prostate. The inner or luminal secretory epithelial cells have a cuboidal shape. They produce an array of proteins (including PSA) that are released in the ejaculate. The outer layer is a continuous layer consisting of mostly flattened basal epithelial cells. The glands are surrounded by a dense stroma, rich in smooth muscle fibers, whose function is to squeeze out the prostate secretion [3].

Figure 3



Prostate cancer

Epidemiology of prostate cancer- an important health care problem

Prostate cancer is the now the most common non-cutaneus cancer in the western world. It occurs mainly in men who are older than 50 years; prostate cancer incidence is highest in men of 75 years and older [4]. It is estimated that prostate cancer will be diagnosed in 232,090 (life time risk 1 in 6 men) men in the USA in 2005 and that 1 out of 8 men will die from the disease [5]. The importance of prostate cancer as a health care problem worldwide is further illustrated in Table 2. Prostate cancer incidence varies widely between countries and ethnic populations. These variations can be attributed for a large part to the frequencies of PSA testing. When standardized for age in the world population, the incidences of different world parts can be compared.

Table 2 Incidence and mortality from prostate cancer worldwide

part of the World/Country	incidence	ASR (World)	SIR (%)	**mortality incidence**	**SMR (%)**
United States of America	140.8	104.3	472	**26.2**	**227**
Canada	121.1	83.9	388	**26.1**	**216**
China	1.5	1.7	8	**0.9**	**13**
Northern Europe	80.1	45.4	218	**36.3**	**254**
Western Europe	94.5	54.9	260	**34.3**	**245**
South Africa	18.4	42.8	194	**11.1**	**333**
World	17.8	21.2	100	**6.7**	**100**

(Mortality) Incidence is noted as rate of prostate cancers or prostate cancer deaths per 100,000 person years of observation

ASR Age standardized rate, the prostate cancer incidence per 100,000 person years that a population would have if it had a standard age structure. The world population was taken as a standard.
SIR/SMR standardized incidence/mortality ratio, observed number of prostate cancer cases/prostate cancer deaths by the expected number, using the age specific incidence/mortality of the world as standard. i.e. a 100% SIR means the observed incidence is equal the expected incidence, standardized by age.

The prostate cancer incidence is highest among countries where regular PSA testing is recommended (i.e. USA and Canada). According to the standardized incidence ratio the incidence in the USA and Canada is almost 5 and 4 times higher compared to the world standardized incidence ratio, respectively. They are followed by western en Northern Europe. In Europe, the prostate cancer incidence decreases when heading towards the Mediterranean Sea. The lowest incidence is in China and India (data not shown). Black men are at increased risk for prostate cancer as compared to white men. The risk of dying from prostate cancer is higher in Europe compared to Northern America. Worldwide the chance of dying from prostate cancer however, is small [6].

Tools for clinical detection of prostate cancer

Digital rectal examination (DRE)

Before the PSA era, in 1990, a digital rectal examination (DRE) was the traditional method for detection of prostate cancer, when the posterior surface of the prostate is palpated digitally. The result of a DRE is considered suspicious for prostate cancer when an induration, or a discrete hard nodule of the prostate is found [7]. The DRE remains the simplest and least invasive method of assessing patients for prostate cancer. The inter-observer and reproducibility varies among urologists, urologists in training and general practitioners and therefore the prostate cancer incidence would differ when DRE was used as the only screening tool [8,9]. The positive predictive value of DRE is low, especially in the low PSA range. In the ERSPC the positive predictive value of DRE in the PSA range 0-2.9 ng/ml was 4-11% [10]. The limited use of DRE is also illustrated by the concordance between clinical and pathological staged prostate cancers. Of 172 patients in the ERPSC who underwent radical prostatectomy, 50 (29%) had impalpable disease on DRE [11].

Transrectal ultrasonography (TRUS)

Adenocarcinoma of the prostate can be visualized as a hypoechogenic area at TRUS [12]. The value of TRUS is limited in detecting prostate cancer. In a study where TRUS was evaluated for its' potential benefit, a lesion was detected in 37% of the prostate cancer cases [13]. Even if TRUS is combined with DRE, the value is limited . TRUS is, however, useful in calculating the size of the prostate. This would allow the calculation of PSA density (PSA/prostate volume). In prostates with a limited prostate volume brachytherapy might be considered and therefore TRUS is useful [14].

Prostatic specific antigen (PSA)

The test, that among other things changed the prostate cancer incidence worldwide and in addition is used in the follow-up after therapy for prostate cancer, is the determination of serum concentration of prostate specific antigen (PSA). PSA is synthesized in the luminal cells of the prostate. The exact mechanism of PSA leakage is as yet unclear, generally it is assumed that PSA leaks through the walls of imperfectly built cancerous glands, and eventually enters the bloodstream by way of diffusing through the capillary membranes [7]. An elevation of serum PSA may be caused by prostate cancer, however, also prostatitis, benign prostatic hyperplasia (BPH) and other conditions (i.e. ejaculation, prostate biopsy and surgery) have been shown to increase serum PSA . Therefore, PSA, although predominantly prostate specific, is not cancer specific. PSA is not only produced by the prostate. It is reported that female breast tumors, ovarian tumors and even benign breast tissue are able to produce PSA .

Tumor classification Tumor stage and grade

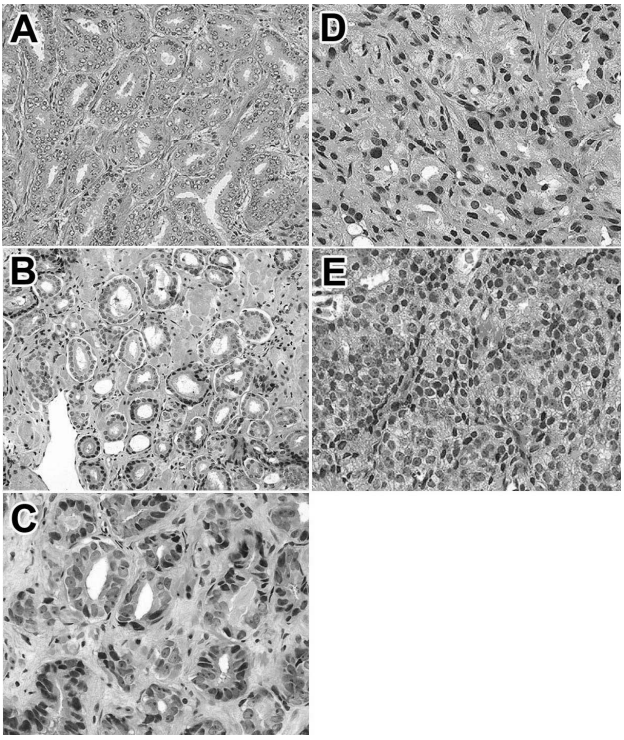
The extent of prostate cancer is reported according to TNM classification [15] (Table 3). Initially, prostate cancers are staged clinically, by means of DRE and TRUS. After radical prostatectomy, with concomitant lymph node dissesection a pathological T and N stage can be given. In the TNM stage, T1 is sub classified in T1a, T1b and T1c. Clinical stage T1a and T1b is not possible, because T1a and T1b are diagnosed incidentally at TURP. On the contrary, pathological stage T1c is not possible, because it is diagnosed when no abnormalities on DRE and TRUS are present, but prostate cancer was diagnosed on biopsy.

Clinical staging of prostate cancer is difficult as described above, due to the low predictive value of DRE and TRUS. Once the prostate is eligible for pathologic staging, 25-50% of the cancers were initially under staged, meaning the final stage is higher compared to the initial stage [16]. The grading of prostate cancer used in the ERSPC is the Gleason score system [17], which is based on architectural features. Generally more than one growth pattern is present in the tumor. The Gleason score system combines the two most prominent patterns. However, when a growth pattern occupies less than 5% of the tumor, it will not be noted in the Gleason score. Growth patterns are divided in five categories (figure 4 and 5). Therefore Gleason score ranges from 2-10. With Gleason score 2 as the most well differentiated tumor and Gleason score 10 as the least differentiated tumor. Generally Gleason score 2-6 is considered as a well-differentiated tumor. Gleason score 7 is intermediate and Gleason score 8-10 is considered as poorly differentiated . Internationally it is agreed that a needle biopsy is not graded under Gleason score 6 [18]. Gleason score is an important predictor for prostate cancer specific survival [19].

Table 3 TNM classification (UICC 2002) [15]

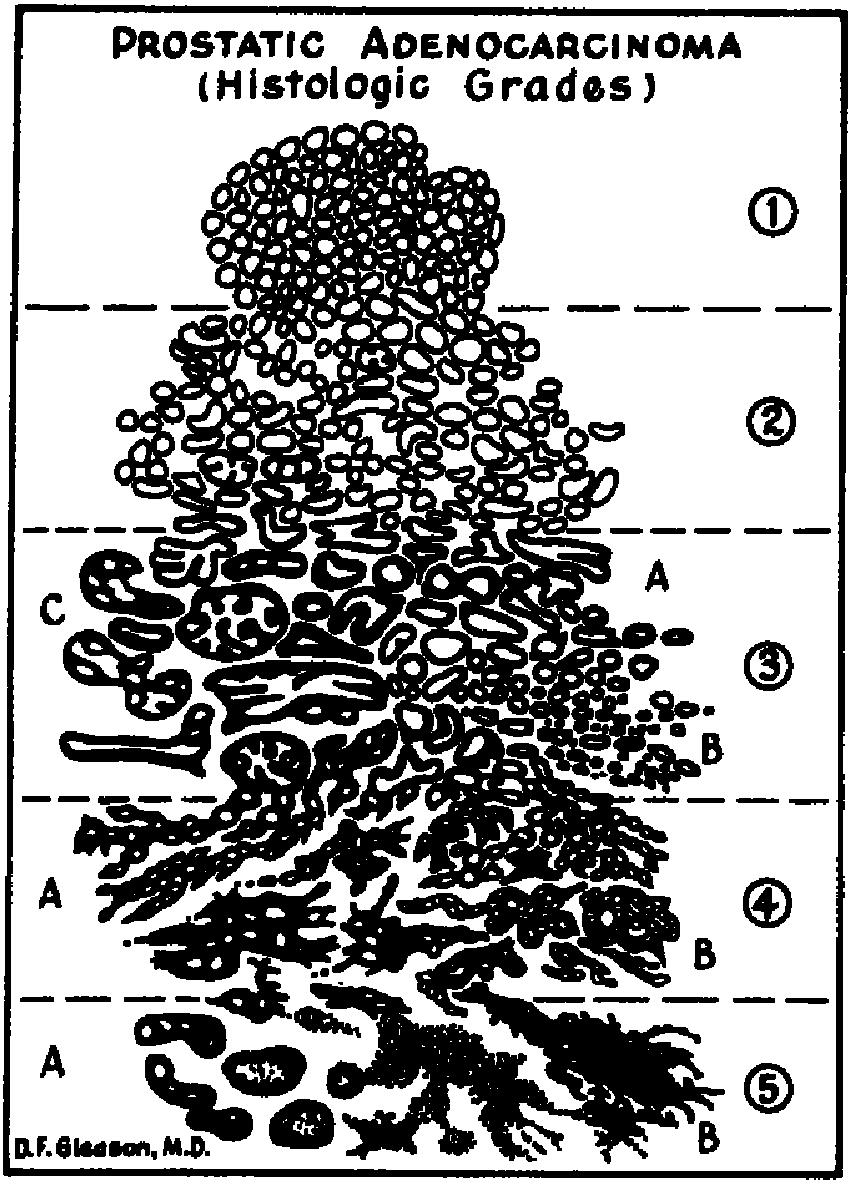
Tumor category **T**	
Tx	No information on primary tumor possible
T0	No indication for a primary tumor
T1	No clinical evidence of tumor (non palpable, not visible on TRUS)
T1a	Tumor diagnosed incidentally at trans-urethral resection in ≤5% of TURP tissue
T1b	Tumor diagnosed incidentally at trans-urethral resection in >5% of TURP tissue
T1c	Tumor diagnosed on needle biopsy
T2	Tumor is confined to the prostate
T2a	Tumor in one side of the prostate with the maximum side of half a lobe
T2b	Tumor in one side of the prostate, larger than half a lobe, not in both lobes
T2c	Tumor in both sides of the prostate
T3	Tumor with extra-prostatic extension
T3a	Tumor extends into the periprostatic tissue one- or both sides
T3b	Seminal vesicle invasion in one- ore both sides
T4	Tumor is fixated into nearby organs, other than seminal vesicle: bladder neck, levator musculature, external sphincter or pelvic wall invasion
Node category **N**	Invasion of regional lymph nodes
Nx	No information on lymph nodes possible
N0	No regional lymph node metastases
N1	Metastases in to regional lymph nodes
Metastasis category **M**	
Mx	No information on distant metastasis possible
M0	No distant metastases
M1	Distant metastasis
M1a	Metastases to non-regional lymph nodes
M1b	Bone metastases

Figure 4 *a* Gleason pattern, *b* Gleason pattern 2, *c* Gleason pattern 3, *d* Gleason pattern 4, *e* Gleason pattern 5



The modified Gleason score does take into account the Gleason pattern occupying less than 5% of the tumor, which is normally not included despite their probable prognostic significance. A modified Gleason score comprising the conventional Gleason sore and tertiary patterns of higher grade has been proposed, because it appears superior to the conventional Gleason score in identifying patients at increased risk of disease progression [20]. Gleason score can artificially be higher in patients who received hormonal therapy, because it changes the morphology, resembling high Gleason scores [21]. Overall the consensus view is that one should not report histologic grade after hormonal therapy .

Figure 5 Schematic overview of Gleason pattern



Prostate biopsy

The definite diagnosis after PSA elevation can only be made by needle biopsy of the prostate. Systemic sextant biopsy used to be the most commonly used biopsy strategy. This systematic sextant biopsy procedure has now become controversial, because it has been reported that 20-30% of cancers are missed by this procedure [22]. Vashi et al. recommended to increase the number of biopsy cores as prostate volume increases and prostate cancer volume decreases, i.e. for a prostate of 40 ml or greater at least 12 cores are needed to detect a tumor of 1 ml. The final decision on the appropriate extent of biopsy procedures will depend on a better understanding of the balance between overdiagnosis and the prevention of prostate cancer deaths.

The prostate biopsy can bring about complications. Within the ERSPC after performing over 5000 sextant biopsies, hematuria and hematospermia still present after 3 days occurred in 23% and 50% of men, respectively. More severe side effect such as fever (3,5%) and urinary retention (0.4%), wherefore hospitalization was needed (0.5%) were far less frequent [23].

Histology of prostate cancer and lesions which potentially increase the risk for subsequent detection of prostate cancer

Prostate cancer histology

The diagnosis of prostate cancer is preferably made on needle biopsy, this provides more specific information about grade and extent of tumor in comparison to fine-needle aspiration. Morphologically prostate cancer is difficult to diagnose, because only a few histological findings are specific for prostate cancer [24]; 1) architectural changes, 2) the absence of basal membrane cells and 3) nuclear abnormalities. Other less important criteria include mitosis, hyperchromasia, and the presence of amorphous substance in the lumina . Microscopically, prostatic adenocarcinomas can exhibit a wide spectrum of appearances ranging from anaplastic tumors to highly differentiated neoplasms. The well-differentiated lesions are composed of small glands that infiltrate the adjacent stroma. Glands are not separated by stroma, but lay back to back. The basal cell layer, seen in benign glands is absent. The neoplastic glands are lined by a single layer of cuboidal cells with prominent nucleoli. Prostate cancer is multi-focal in 75-85% of the radical prostatectomy specimens . When doubting the diagnosis of prostate cancer on histology, immunohistochemistry may help to make a definite diagnosis. An antibody against high molecular weight cytokeratin (i.e. 34βE12) is a frequently used basal cell marker. Absence of this basal cell marker in a lesion histologically suspicious for cancer supports a diagnosis of prostate cancer. However, absence of basal cells demonstrable by basal cell immunohistochemistry is not always conclusive for prostate cancer. Some benign prostatic lesions may have inconspicuous or even lack basal cell lining focally . The recently described marker P504S, or α-methylacyl CoA racemase (AMACR), has great promise in this regard. Positive staining AMACR can be used to support a diagnosis of cancer on prostate needle core biopsies when the focus in question is <1 mm in maximum dimension .

Prostate cancer spreads initially in the prostate itself (i.e. ducts and acini, fibromuscular stroma and blood vessels) before invading the seminal vesicles and apex of the prostate and bladder. Metastatic spread occurs mainly to the skeleton and lymph nodes. Bone metastases are usually multiple and are osteoblastic [3].

Lesions which potentially increase the risk for subsequent detection of prostate cancer

High-grade prostatic intra-epithelial neoplasia (PIN) can be found adjacent to invasive carcinoma in 80% of the cases . Based on this finding and because PIN has similar cytogenetic aberrations PIN was found as a precursor lesion for prostate cancer.

Recently, atrophy has been considered as lesions predictive for associated prostate cancer. This is because some forms of atrophy mimic prostate cancer morphology and genetic abnormality. In addition, atrophy is a proliferative lesion associated with inflammation. Inflammation has been linked to cancer progression in other organs. Therefore it is thought that atrophy is a pre-cancerous condition .

Lesions that are suspicious for prostate cancer (LSPC), but not diagnostic for cancer, are no precursor lesions for prostate cancer. They are sometimes referred to as atypical small acinar proliferations (ASAP), however, they lack

histologic criteria to make a definitive diagnosis of prostate cancer. Despite not being a precursor lesion, the chance of concurrent prostate cancer is high and therefore repeat biopsy is required.

Treatment of prostate cancer

Due to PSA testing, an increasing proportion of men are detected with early-stage prostate cancer, allowing curative treatment in these men. Roughly, there are four treatment options for early stage prostate cancer available; surgery, radiotherapy, hormone therapy, and observation, also known as watchful waiting, or active surveillance. The advantages of radiotherapy and radical prostatectomy are obvious; the intention of treatment is usually curative. However side effects of both curative treatments are significant. Aside from sexual dysfunction, which are obvious in both treatments, pre- vs. post-therapy effect sizes in radical prostatectomy and radiotherapy were large and medium for urinary function inconveniences, small and large effect on bowel function, in localized prostate cancer patients, respectively. Changes were significantly different pre-vs. post-treatment for urinary function in radical prostatectomy and bowel function in radiotherapy [25]. Despite differences of side effects, no association between primary therapy and health related quality of life could be found . Within the ERSPC patients treated with radiotherapy reported decreased quality of life compared to patients treated with radical prostatectomy, however patients treated with radiotherapy were older . Patients in the ERSPC with screen-detected and clinically diagnosed prostate cancer reported similar health-related quality-of-life after treatment with radical prostatectomy or radiotherapy. Contrary to this, Steineck et al. published on the different effects in patients treated with radical prostatectomy or watchful waiting, these authors found that erectile dysfunction (80 vs. 45%) and urinary leakage (49 vs. 21%) were more common after radical prostatectomy as compared to watchful waiting. Apparently, the knowledge of being diagnosed with prostate cancer also influences sexual function even when no treatment is given (watchful waiting). The optimal curative therapy, considering disease recurrence, prostate cancer specific survival and side effects is as yet unclear. There have been very few randomized clinical trials for treating prostate cancer. These trials come along with difficulties and long follow-up is needed. Retrospective analysis of surgery, external beam radiotherapy and brachytherapy showed similar results for early stage prostate cancer . A randomized clinical trial comparing radical prostatectomy and watchful waiting showed a significantly decreased prostate cancer specific survival in men managed by watchful waiting (discussed below)

Radical prostatectomy

Radical prostatectomy allows a full Radical prostatectomy allows a full pathologic assessment of the tumor. Extra capsular extension, seminal vesicle invasion, extension to surrounding structures and presence of positive surgical margins can be assessed. Apart from Gleason score, which is mostly

upgraded in the pathologic assessment of prostate cancer, upstaging is not uncommon [33-35]. Both can be explained due through sampling bias. After prostatectomy PSA is undetectable, any rise in PSA is suspicious for disease recurrence (biochemical recurrence). This contrasts the follow-up after radiotherapy where a PSA rise not always indicates disease recurrence. Despite these advantages, radical prostatectomy is a major surgical procedure and requires the patient to be able to tolerate the procedure without substantial risk. In addition, quite some side-effects can occur; urinary leakage, and impotence. Because radical prostatectomy requires an anastomosis between the bladder and the urethra distal to the prostate, urinary incontinence can develop. Diminished or absent potency can occur after surgery because neurovascular bundles are manipulated. The proportion of patients that regained urinary continance was 92%. Depending on whether unilateral or bilateral nerve sparing surgery was offered. Impotence ranged from 32 to 53%, respectively{Catalona, 1999 #385}.
Robotic-assissted laparoscopic prostatectomy has been introduced as a less invasive surgical approach. In the hands of experienced surgeons, hospital stay, operation time, postoperative pain and intra-operative bleeding are more advancious compared to convential radical prostatectomy. The oncologic outcome in centres that have more familiarity in laparoscopic approaches, surgical margin status is comparable to centres were a high volume of conventional prostatectomy is performed. Wheter urinary incontinance and erectile dysfunction is improved as compared to conventional radical prostatectomy is as yet inconclusive {Smith, 2005 #386}.

Radiotherapy
The advantage of radiotherapy is that it involves no risk of anesthesia therefore is suitable in men with extensive co-morbidities. After radiotherapy, the prostate is in place and continues to produce PSA, with the possibilities for benign PSA increases as described above [29]. A PSA rise after radiotherapy therefore not indicates prostate cancer recurrence. Brachytherapy involves placements of radioactive sources in the prostate. It is more localized compared with radiotherapy resulting in a reduction of the radiation dose to surrounding structures [30].

Watchful waiting
Watchful waiting, which indicates active surveillance of a man with prostate cancer by means of PSA testing, might be appropriate for men with low life expectancy and low grade disease [19,31]. In recent years, as more early stage cancers are diagnosed in younger men, discussion about watchful waiting in young men as initial treatment came up with the possibility to deferred treatment. A randomized clinical trial considering radical prostatectomy versus watchful waiting in clinically localized prostate cancer favored radical prostatectomy with an adjusted hazard rate of 0.45 for death from prostate cancer. However, no significant difference was found in overall mortality. In this study, a limited proportion of patients with poorly differentiated Gleason

scores (i.e. Gleason pattern 4 (25%) and 5 (5%)) were included in both arms and therefore these men were at increased risk of dying from prostate cancer without curative treatment . Two studies mentioned in the natural history section reported good follow-up results in well-differentiated tumors of patients managed with watchful waiting (discussed below) [19,31].

Adjuvant therapies/ other therapies

One of the advantages of radical prostatectomy is that adjuvant radiotherapy can be given. Salvage radiotherapy after biochemical recurrence for radical prostatectomy has a 4-year progression-free survival of 45% in high risk patients [32]. A recent clinical trial randomizing patients with a pT3 stage prostate cancer in adjuvant radiotherapy and surveillance, showed a significantly lower PSA progression free survival rate in the surveillance group. However after a 10-year follow-up, no differences were seen in presence of metastasis and overall survival . Whether adjuvant radiotherapy for positive surgical margins is beneficial, is now being investigated in a randomized clinical trial. Salvage radical prostatectomy might also be performed after local recurrence after radiotherapy. Long-term results show a 5-year progression-free survival of 55% (95% confidence interval, 46-64%) [33].

Cryosurgery is a relatively new treatment for prostate cancer, it is a technique that involves using freezing by inserting probes into the prostate in order to destroy the prostate cancer cells. Cryosurgery is suitable for patients who are unwilling to undergo radical prostatectomy and radiotherapy, even in high risk patients [34]. In a longer follow-up of 590 patients with localized or locally advanced prostate cancer 7-year biochemical disease-free survival was between 61-68% for low-, medium- and high-risk patients, respectively [35].

High-intensity focused ultrasound (HIFU) can kill tissue through coagulative necrosis. HIFU can be used for localized prostate cancer or for recurrent prostate cancer or as adjuvant treatment. The 5-year disease free survival rate was 65% in one study [36] the overall population. The main side effects are incontinence and bladder neck stenosis. The treatment repeatability remains an unique advantage of this option [37].

Treatment of metastasized prostate cancer

Metastasized prostate cancer can be treated with hormones (endocrine therapy), because most prostate cancers depend on androgens for their growth. Metastatic prostate cancer shows a rapid response to surgical or medical castration, with improvement in bone pain, regression of soft-tissue metastases, and a decline in serum prostate-specific antigen (PSA) levels. Nevertheless, in virtually all patients the tumor ultimately becomes androgen-independent , because most tumors will eventually acquire mechanisms for independent growth [38,39]. This happens after castration at a median of 18 to 24 months. Chemotherapy studies have been carried out in these hormone refractory prostate cancers. Two trials reported the benefit of docetaxel given every 21 days. It significantly elongated survival with two months. Currently,

docetaxel should be advised in androgen-independent prostate cancer for the time being [40,41].

Natural history

The natural history of prostate cancer is for a great deal uncertain, because men are much more likely to die with, rather than of, prostate cancer [42]. From autopsy studies it is known that prostate cancer can be found in 55% of men in their 5th and 64% in their 7th decade, respectively [43]. Furthermore incidental prostate cancer diagnosed in trans urethral resection of the prostate (TURP) or at cystoprostatectomy is found in 10 and 46%, respectively [44,45]. This illustrates the substantial proportion of prostate cancers that are not likely to kill.

Clinical symptoms of prostate cancer are rare. When a prostate cancer is extensive it will give complaints mimicking BPH. These include weak urine stream, dribbling at the end of urination, hesitation before urine flow starts, a sense that the bladder has not emptied completely .

Albertsen et al. [31] describe the natural history of prostate cancer. They followed patients with localized prostate cancer (n=767) for 20 years who were not treated curatively. They found a mean prostate cancer specific mortality of 7%, 14%, 27%, 45% and 66% in the age category 55-74 years with Gleason score 2-4, 5, 6, 7 and 8-10, respectively. In this study prostate cancer was diagnosed between 1971 and 1984, by clinical means. In this era, PSA was not yet in use and accurate staging was lacking in many patients, therefore patients with occult metastasis might have been included in the analysis. Also endocrine therapy was given in different protocols (immediate or delayed) and not in every patient . Anyway, the data are likely to overestimate risks with respect to screen-detected cancers. Reliable information on the natural history on screen detected prostate cancer is not yet available. Johansson et al.[19] recently studied 223 patients with organ-confined prostate cancer who were not initially treated. Orchidectomy or exogenous estrogens were offered if progression to symptomatic disease occurred. The authors found that after a median follow-up time of 21 years, in total 91% of the patients died, but prostate cancer was the cause of death only in 16% of the entire cohort, 40% had progression of disease of whom almost half developed distant metastasis. The most significant predictor for mortality was a poorly differentiated tumor (prostate cancer specific survival of less than 30% after a 5 year follow-up). When however diagnosed with an organ confined well-differentiated tumor the cause specific survival after a 20-year follow-up was 72%, without initial treatment. Even with this relatively good cause specific survival in men with favorable and known tumor characteristics managed by watchful waiting, it remains difficult to predict tumor progression in the individual patient. Gleason scores in biopsies are frequently under graded as is clinical staging when compared to the Gleason score and pT-stage in radical prostatectomy specimens . Still, Gleason grading in biopsies as well as in radical prostatectomy specimens have been shown to be predictive for disease specific survival after radical prostatectomy [46].

Controversy of screening

Although the benefit of prostate cancer screening has not yet been established in randomized clinical trials, the American Cancer Society Recommends yearly PSA testing and digital rectal examination, beginning at age 50 in every healthy man [47]. The NCCN (National Comprehensive Cancer Network) even recommends PSA testing in men beginning at age 40 with a PSA cut-off level for further screening of 0.6 ng/ml, and when the biopsy result is negative in the PSA range between 2.6-4.0 ng/ml re-screening should be done within 6-12 months . Even without screening men more often die with prostate cancer than from prostate cancer. Screening will increase the risk of over-diagnosing prostate cancer, which was calculated as high as 48% within a screening population with a 4-year screening interval [48]. McGregor et al. [49] calculated only one in 8 screen-detected cancers is likely to kill its carrier if left untreated. Over diagnosis results in over-treatment. In addition, the serious adverse effects of radical prostatectomy and radiotherapy must be taken into account if reduced mortality is seen in a screened population [48].

Primary prevention

Apart from regional differences, which partly depend on hereditary factors (in the USA African-Americans are at increased risk for prostate cancer), there are also external risk factors that influence the transformation to prostate cancer. For example, if a Chinese man living in China moves to the USA, the risk of developing prostate cancer increases with 50% compared to if he continued living in China [50,51].

The prostate cancer risk is two to three-folds higher in men who reported a history of prostate cancer in first-degree relatives. In monozygotic twins there is a higher concordance in prostate cancer incidence compared to dizygotic twins, suggesting a genetic influence of 42% [52]. It is estimated that 5-10% of all prostate cancer cases may have a hereditary basis [53].

Diet is one of the external risk factors, associated with prostate cancer. A diet rich in lycopene (tomatoes), vitamin D and E, high consumption of fish and soybean products was associated with a decreased prostate cancer risk. A high consumption of diary products, meat and fat, however were associated with an increased prostate cancer risk [54]. Despite the promising epidemiological association between prostate cancer and vegetables and fruit, a recently reported prospective study could not find a significant association between the intake and a decreased prostate cancer incidence [55]. Further prospective studies are needed to establish significant associations between diet and prostate cancer incidence. Alcohol intake has neither a positive nor negative effect on prostate cancer development[56]. Epidemiological evidence exists that there is a link between infections (i.e. sexual transmitted disease) and inflammation (prostatitis) and prostate cancer . Some authors might suggest that post-inflammatory atrophy would be a pre-cancerous condition . In addition to this hypothesis non-steroidal anti-inflammatory drugs (NSAIDs) are protective for prostate cancer . A large trial was set up (ViP) with rofecoxib (a COX-2 inhibitor (NSAID family)) to prevent prostate cancer

[57]. However, rofecoxib was withdrawn of the market because of cardiovascular side effects [58].

Hormonal factors (androgens) appear to play a role in the development of prostate cancer, the disease does not occur in eunuchs castrated before puberty, and the incidence is low in patients with hyperestrogenism (i.e. liver cirrhosis) [59]. Because hormones were associated with prostate cancer development, the prostate cancer prevention trial was conducted . In the prostate cancer prevention trial, participants were randomized into a study and placebo group to study the effect of finasteride (inhibitor of the conversion of testosterone to dihydrotestosterone, the primary androgen in the prostate) on the prostate cancer incidence. There was a 24.8 % prostate cancer reduction over a seven-year period in the study group compared to the placebo group. It remains however unknown whether this finding will translate into a decrease of prostate cancer mortality or if finasteride only causes a delay in prostate cancer diagnosis. Higher Gleason scores compared to men within the placebo group accompanied the substantial reduction in prostate cancer incidence in men with positive biopsies in the study group. Finasteride also adversely influences sexual function and this needs to be weighed if finasteride is considered to be given to men as a protective agent.

Secondary prevention: rationale for screening and results of studies

Worldwide epidemiological surveys demonstrate decreased prostate cancer mortality since 1993 in several countries (Table 4). This phenomenon can be seen in areas of the world where screening is prevalent and not prevalent . However, this decrease in mortality rates is more impressive in countries where screening is more common, as in the USA and Canada. The fall in prostate cancer mortality in the USA was mostly attributable to the reduction in the number of men who were initially diagnosed with distant metastases and eventually died rapidly. Nonetheless, temporal and geographical differences provide inconclusive evidence suggesting the potential benefits of PSA screening. Coldman et al. studied the incidence and mortality in Canada and confined that the incidence of prostate cancer increases with more PSA use. Strikingly, regions with the smallest increases in incidence had the largest declines in mortality. This suggests that mortality reduction may be (in part) due to factors other than PSA screening. Notably decreases in mortality were only in part related to prostate cancer.

Table 4 Relative survivals in percentages during three time periods by cancer site

Site	1974-1976 (%)	1983-1985 (%)	1992-1999 (%)
prostate	67	75	98
colon and rectum	50	57	62
lung and bronchus	12	14	15
pancreas	3	3	4

To date, final outcome of one randomized screening trial has become available. The authors claim a 62% reduction in disease specific mortality . However, an earlier report of this study was heavily criticized, because of methodological flaws. This large reduction in mortality resulted from a "screening received" analysis, disregarding randomization. The intention to screen" analysis resulted in a 16% increased risk of death in the screen arm (RR=1.16). The participation in the screen arm was only 23%. In addition, the time from randomization to screening was 3 years in the screening arm and therefore the time to observe mortality was 3 years shorter compared to the control group, because men diagnosed with prostate cancer before the screening date were excluded .

The Tyrol mass-screening study wherein PSA testing was freely available in the federal state of Tyrol showed promising results when prostate cancer mortality was compared to other parts of Austria where PSA testing was not freely available and to expected death rates in Tyrol. However, this was not a randomized controlled trial [60]. Several case-control studies were conducted in the USA with DRE and PSA screening that also support the benefit of screening, showing an inverse association between the screening test and the prostate cancer mortality (odds ratio 0.7). Nevertheless, the 95% confidence interval was 0.5-1.1 and the DRE screening effect could not be separated from the PSA screening effect .

Within the ERSPC results will not be available until 2008. However, there is confirmatory evidence that screening introduces more favorable prostate cancer characteristics. Within the Finnish section of the ERSPC, the proportion of clinically organ confined prostate cancer in the screening arm was 82% compared to 65% in the control arm [61]. In the Rotterdam section of the ERSPC, radical prostatectomy specimens of patients not in a screened population were compared with patients from a screened cohort. Metastasis was not seen in the screened cohort, whereas 18% of the control population had metastases. Gleason score and pathological stage was also significantly lower in the screened cohort as compared to the not screened cohort. This report presents clear evidence for favorable disease stages in prostate cancer in the screening arm [62].

Screening may miss its original goal and cause more harm than benefits. Screening introduces lead-time: this means that through screening, prostate cancer is diagnosed earlier than based on clinical incidence. The calculated lead-time for prostate cancer ranges between 4.5 and 12 years. Lead-time depends on age and aggressiveness of the cancer. Younger men and indolent prostate cancers have longer lead times, compared to older men with high grade disease .As yet the optimal screening interval in trials is unknown, notwithstanding, yearly PSA testing is recommended in the USA as mentioned above [47]. Thornblom et al. who screened with PSA >10.0 ng/ml and/or abnormal DRE and TRUS, found no over diagnosis at all after 12 years of follow-up, when screening was only performed once. All centers in the ERSPC, except Sweden and Belgium have a screening interval of 4 years. The lead time at the ERSPC section in Rotterdam was estimated at a median

of 10.7 years, which would imply that a screening interval of 4 years is sufficient .

Despite discouragement of PSA testing in Europe due to the lack of beneficial convincing evidence, PSA testing has become very common. The French urological association recommended recently PSA screening [63]. In a population where the National Health Service does not approve screening, PSA testing was calculated as high as 36% in men in the UK between 1994-1999. Miller et al. recently reviewed over 50,000 patients from the American College of Surgeons National Cancer Data Base (NCDB) and found that 69% of cases were diagnosed without symptoms and the majority of these men (78%) presenting with prostate cancer in the absence of symptoms was 59 years or younger. In men 80 years and older, 46% were diagnosed asymptomatically, which probably indicates detection trough screening. This "contamination" may also occur in the control arms of randomized clinical trials and might blur their outcome.

Recently for the Dutch part of the ERSPC, Otto et al. showed that the contamination rate in the control arm, which was measured in a 3-year follow-up amounted to 20.2% of men in the control-arm who had their PSA tested. Only 7-8% of men with PSA ≥3.0 ng/ml underwent prostate biopsy. This translates into a contamination rate in the control arm of the ERSPC of 3% per year which may be considered as low and is taken into account by the sample size calculation [64].

Screening with PSA

Despite the lack of specificity, PSA has been found suitable as a screening tool. The most favored cut-off to use PSA as a screening tool, is the PSA range <4.0 ng/ml. Catalona et al. [65] initially used this cut-off as an indicator for biopsy and biopsies were also performed if patients had a digital rectal examination (DRE) suspicious for cancer. In the ERSPC a cut-off of PSA 4.0 ng/ml was initially used as an indication for sextant biopsy as well as abnormal DRE and TRUS in the PSA ranges 1.0-3.9 ng/ml. However, it turned out that the relative sensitivity and positive predictive value (PPV) of DRE and TRUS was only 37% and 9.7% in the PSA range <4.0 ng/ml, based on the "a priori prevalence assessment" [66] (Table 5 shows the sensitivity and specificity of the DRE test alone of men who underwent sextant biopsy in two PSA ranges).

Table 5 Sensitivity, specificity, positive predictive value (PPV) and negative predicting value (NPV) of the digital rectal examination (DRE) in two PSA ranges in the 1st screening round of the ERSPC. Every percentage is relative since in this table only men who underwent sextant biopsy (n=4117) were included.

PSA range ng/ml	relative sensitivity %	relative specificity %	PPV %	NPV %
PSA<3.0	62	42	0.9	55
PSA<4.0	50	54	16	86

In addition to these unfavorable test characteristics it was shown that the proportion of cases with favorable prognostic factors increases with lower PSA values [10,66]. At the same time evidence accumulated that in the PSA range 2.0-4.0 ng/ml up to 65% of the cancers were missed by DRE and TRUS. With the (unproven) assumption that most of cancers present, and detectable in the PSA range 1-3 ng/ml, would still be detectable in a curable stage at re-screening and after having shown that the overall detection rate remained unchanged in this PSA range [67], it was decided to omit DRE and TRUS as screening tests and to biopsy all men with PSA values in the 3.0-4.0 ng/ml range. In table 6 the incidence of prostate cancer of the 1st screening round is shown in different PSA ranges.

Table 6 positive predictive value (PPV) and detection rate per PSA range in the 1st screening round of the ERSPC

PSA range ng/ml	No. men screened	No. prostate cancer	No. biopsies	% PPV biopsy	% PPV detection rate, PSA range
0.0-0.9	7139	4	185	2.2	0.06
1.0-1.9	6205	45	510	8.8	0.75
2.0-2.9	2508	30	221	13.6	1.20
3.0-3.9	1426	179	792	22.6	12.55
4.0-10.0	2235	526	2006	26.2	23.53
>10	457	230	403	57.1	50.33
total/mean	19970	1014	4117	21.7	5.08

After the initial cut-off of <4.0 ng/ml in 1994, Catalona et al. now recommend a PSA cut-off level of 2.6 ng/ml as an indictor for biopsy. They found a prostate cancer incidence of 22% in the 2.6-4.0 ng/ml PSA range (in biopsies). Tumors in this PSA range had favorable characteristics, they were significantly smaller and more often organ confined (88 vs. 63%) as compared to tumors detected in the PSA range 4.0 ng/ml and greater . Punglia et al. recently reported on the effect of verification bias, which occurs when the "relative" sensitivity and specificity is studied in a population which was only for a part exposed to the test (i.e. not everyone with an increased PSA value will undergo prostate biopsy to confirm prostate cancer). Verification bias masks the true sensitivity and specificity of PSA. Adjusting for verification bias (i.e. lowering the PSA threshold for biopsy recommendation from 4.1 to 2.6 ng/ml in men younger than 60) simply significantly improved the

estimated sensitivity and specificity of the PSA test for a screening population. However, even after adjusting for verification bias, the bias still exists, if not all men were tested. In the prostate cancer prevention trial of Thompson et al. (discussed above) every man in the placebo group was offered a prostate biopsy after the 7-year study period, therefore this study was not subject to verification bias. In the PSA range ≤ 4.0 ng/ml, the prostate cancer incidence was 15% and of these tumors 15% contained Gleason pattern 4, which indicates that high grade cancer in the low PSA range is not a rare finding. Similarly, a side study performed in the screening arm of the ERSPC in the PSA range 2.0-3.9 ng/ml the prostate cancer revealed an incidence of 17% in sextant biopsies, and the detection rate was 14% four years after the initial screen .

Detection and radical treatment of the large proportion of organ confined prostate cancers in this low PSA range, does not automatically lead to lower prostate cancer mortality rates: the high incidence of prostate cancer in sextant biopsies in the age range (61-91 years, with a median of 69 years) of the men in the study of Thompson et al. is not surprising, it includes the possibility that these biopsies identified autopsy cancers [68]. Configuring this possibility, Hoedemaeker et al. reported organ confined tumors (pT2) in 93% of which 86% were small tumors (<0.5ml) in the PSA range <4.0 ng/ml in the ERSPC. Apart from being diagnosed clinically, half of the radical prostatectomy specimens contained Gleason pattern 4, in line with Thompson et al.

To improve the test characteristics of PSA, various parameters related to PSA change were introduced (i.e. PSA velocity, PSA doubling time, PSA slope). PSA velocity was put forward as a promising screening tool by Carter et al. . They found a PSA velocity of 0.75 ng/ml per year significantly associated with clinical prostate cancer. However, in the screening arm of the ERSPC, PSA velocity was 0.62, 0.46 respectively 0.03 ng/ml per year in prostate cancer patients, men with negative biopsy outcome and men without a biopsy indication (PSA ≤3.0), respectively. When PSA velocity as well as PSA doubling time were tested in receiver operating characteristic analyses (ROC curves), the areas under the curve were only moderately greater than 0.5 (equal to chance) and therefore of very limited use in predicting biopsy outcome in the screened population of the ERSPC . Particularly, in the low PSA range in the ERSPC, PSA velocity was not a predictive variable in a multivariate analysis to predict prostate cancer in sextant biopsies .

When only the PSA <1.0 ng/ml range was considered, as in the Spanish section of the ERSPC, it was found that if these men were repeatedly PSA tested after a 4-year screening interval, only 4 prostate cancers were diagnosed. In concordance with this, within the Dutch section of the ERSPC 0.9% of men with a PSA <1.0 ng/ml showed progression to PSA levels ≥3.0 ng/ml, however if that occurred a PPV of 19% was found [69], (dr. F.H. Schröder, Erasmus medical center, Rotterdam, submitted)

In the ERSPC, TRUS determined prostate volume was a negative predictor for biopsy outcome after multivariate testing; the smaller the

prostate, the higher the prostate cancer incidence . Therefore PSA density might be predictive for prostate cancer.

In addition to PSA dynamics, other molecular forms of PSA have been under research. The ratio of free to total PSA improved the relative specificity in detecting prostate cancer in the PSA range 4-10 ng/ml by Catalona et al. [70]. This was configured in several studies, including the Swedish part of the ERSPC . Pro-enzyme of PSA (Pro-PSA) and Benign PSA (BPSA) are formed in the peripheral and transitional zone of the prostate. The amount of pro-PSA is increased in prostate cancer whereas BPSA is increased in BPH patients . However when samples of the ERSPC were tested for Pro-PSA as an individual marker to distinguish between BPH and cancer it did not improve the specificity further than free PSA .

Genetic abnormalities in prostate cancer

Numerous genetic abnormalities have been described in prostate cancer, but only a few genes involved in progression of prostate cancer are shown to carry genetic alterations. The most frequently occurring tumor suppressor genes in prostate cancer are PTEN and p53. Both represent markers of advanced prostate cancer. Loss of PTEN is associated with increased Gleason scores and increased risk of recurrence, while p53 gene mutations are most common in metastasized and androgen –independent prostate cancer. These markers provide no additional prognostic information to conventional prognosticators of prostate cancer [20]. Genes involved in prostate cancers are genome-wide mapped by characteristic large chromosomal alterations (losses, gains, and translocations) in the tumor. Chromosomal losses are an indication for a localization of a tumor suppressor gene, whereas gains and translocations are an indication for an oncogene. The most frequent genetic changes in prostate cancer progression and/or prostate cancer recurrence are gain of the long arm off chromosome 8 and 11 (8q and 11q) and loss of the short arm of chromosome 8 (8p) .

Conclusions

Annual PSA testing was recommended since 1993 in the USA but, 11 years later in 2004, there is still no conclusive evidence that PSA screening is beneficial. When going through the points of Wilson and Jungner (Table 1) requirements 1-4 and 6 can be met. Point 5, a suitable screening test can be met partly, because the PSA test is a test with limited sensitivity. Better tests urgently need to be found. The ideal situation would be a test that distinguishes between significant or insignificant prostate cancer. The Natural history of prostate cancer is partly understood (point 6), however, we do not know what the natural history is of screen detected prostate cancer. Point 8, the policy of whom to treat as patients is discussed thoroughly in this introduction, because since increasingly more localized prostate cancers are diagnosed, over-diagnosis and therefore over-treatment accompanied with adverse side effect seriously influences quality of life. The answer if screening for prostate cancer reduces disease specific mortality and therefore can be

used continuously or as "a once and for all project" reflected in point 10 is still unknown. At the moment there is no scientific basis for population based prostate cancer screening outside randomized clinical trials designed to assess its effectiveness and identify men who might benefit from screening.

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Part 2
Chapter 2

Lesions predictive for prostate cancer in a screened population: first and second screening round findings

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Abstract
Objective: We evaluated the incidence of prostate cancer, high-grade prostatic intraepithelial neoplasia (PIN) and lesions suspicious for prostate cancer (LSPC) in sextant biopsies in two subsequent screening rounds at a 4-year interval and their predictive value for subsequent prostate cancer detection.
Methods: In the Rotterdam section of the European Randomized Study of Screening for Prostate Cancer (ERSPC), 4117 men underwent sextant biopsy in the 1st screening round. The 2nd round was performed at a 4-year interval and biopsies were taken in 1840 men.
Results: The incidence of prostate cancer, LSPC and PIN in the 1st, respectively 2nd round were 24.6, resp. 19.9% (p=0.001), 2.7, resp. 2.8% and 0.8, resp. 2.5% (p<0.0001). Prostate cancer incidence after repeat biopsy for LSPC in the 1st, resp. 2nd round was 36.7 and 17.0%, and after repeat biopsy for PIN 13.3% in both rounds, respectively. Men with a benign biopsy in the 1st round had a significantly lower prostate cancer incidence in the 2nd round compared to men who did not undergo biopsy in the 1st round (10.7 vs. 22.7%, p<0.0001).
Conclusions: The decrease of prostate cancer detection in the 2nd round was associated with an increase in the incidence of PIN. Strikingly, LSPC diagnosed during the 1st round, but not during the 2nd round were predictive for prostate cancer, while isolated PIN was never predictive for prostate cancer. PIN shoud not be an indication for repeat biopsy in a screening population. Importantly a 1st round benign biopsy outcome proved to be a negative predictor of subsequent prostate cancer detection.

Introduction

Elevated serum prostate specific antigen (PSA) tests and to a lesser extent abnormal digital rectal examination (DRE) and transrectal ultrasonography (TRUS) are indicators for prostate biopsy. So far histopathologic examination of prostate needle biopsies is the only possibility to establish the diagnosis of prostate cancer. Some prostate biopsy lesions, like high-grade prostatic intraepithelial neoplasia (PIN), or lesions suspicious for prostate cancer (LSPC) have been postulated as lesions predictive for the subsequent detection of prostate cancer [72,73].

A LSPC is characterized by a proliferation of prostatic glands with abnormal architectural patterns, but lacking sufficient cytonuclear atypia to make a definite diagnosis of prostate cancer [74]. LSPC have been described by a wide variety of terms, including atypical small acinar proliferation (ASAP) [75]. The incidence of LSPC is 1.5-4.8% of prostate biopsies [76,77] and the chance of finding prostate cancer on repeat biopsies ranges from 39% up to 60% [78,79].

PIN is characterised by atypical glandular cells with prominence of nucleoli lining larger acinar structures surrounded by an interrupted basal cell layer. The incidence of isolated PIN in prostate needle biopsy is 5% [24]. The chance of finding prostate cancer on repeat biopsy after an initial diagnosis of isolated PIN is 23-50%. [80-83]. The current opinion is that PIN represents a precancerous state for prostate cancer, and close follow-up of these cases is recommended.

In the current study we analyzed the frequency of prostate cancer in sextant biopsies, PIN, and LSPC in sextant needle biopsies in two subsequent prostate cancer screening rounds after a 4-year interval. In addition, the predictive value of prostate biopsies with benign outcome, PIN or LSPC for subsequent detection of prostate cancer was evaluated.

Material and methods

In the Rotterdam section of the European Randomized Study for Prostate Cancer (ERSPC) 42,376 participants, aged 55-75, were randomized in a screening (n=21,210) and a control arm (n=21,166). The first round of screening (prevalence screen) took place from November 1993 until December 1999. A total of 19,970 men actually underwent screening. Screening for prostate cancer in this study was done by PSA determination, DRE and TRUS. Systematic sextant prostate needle biopsy was recommended for participants who had either an elevated PSA level (≥ 4.0 ng/ml), abnormal DRE or abnormal findings on TRUS. The protocol was simplified on May 1997, when sextant biopsy was recommended if PSA was ≥3.0 ng/ml. Abnormalities on DRE and/or TRUS were no longer indicators for biopsy. Until March 1996, an interim screening was performed. One year after the 1st screening round men with benign biopsy outcomes at the prevalence-screening round were re-invited at an interim screening round for PSA measurement. In this interim screening round there were 495 men biopsied.

The second screening round, using PSA ≥3.0 ng/ml as a cut-off value for sextant biopsy, started in November 1997 and is still ongoing. Until September 2003, 11,654 men were screened
PSA determinations were done with a Hybritech Tandem E assay (Hybritech Beckman-Coulter Corp., San Diego, Cal), and after January 2000, the automated version was used (Beckman-Access, Beckman-Coulter Inc. Fullerton CA, USA). All men had signed informed consent prior to their participation to the screening study.

Systematic sextant biopsies were obtained during longitudinal and cross-sectional ultrasonographic scanning of the prostate. A 7th biopsy was taken if a hypo-echogenic lesion was visible at TRUS. Repeat biopsy strategy for PIN was repeat sextant biopsy and a 7th if a hypo-echogenic lesion was visible at TRUS and for LSPC, 4 biopsies were taken from the region of the prostate were the lesion was diagnosed. One pathologist (T v/d K) reviewed all biopsies with cancer, PIN and LSPC in order to avoid inter-observer variation. During this review, the number and size of biopsies were recorded as well as proportion of tumor involvement, and proportion of each Gleason grade.

Statsistical analysis
Statistical analysis was done with the SPSS software package (SPSS Inc., Chicago, IL). $P<0.05$ was considered significant. Chi-square tests were used for ordinal variables, student T-test for linear variables, i.e. PSA and age.

Results
Prostate cancer incidence 1st, interim, and 2nd screening round
In the 1st screening round 19,970 men had a PSA test, of whom 4117 underwent sextant biopsy. In total, after repeat biopsies for PIN and LSPC were performed, 1014 men with prostate cancer were diagnosed (biopsy incidence 24.6%). One year thereafter, 514 men were re-invited for sextant biopsy in the interim screening round (including 15 men who did not show up for biopsy in the 1st screen). In this round 63 prostate cancers were diagnosed after performing repeat biopsies. After exclusion of 3 men with prostate cancer detected in the interim screening round who did not undergo sextant biopsy in the 1st round, it could be calculated that prostate cancer incidence dropped in the interim screen, as compared to the prevalence screening round from 24.6 to12.1% ($p<0.0001$). So far, in the 2nd screening round 1840 men underwent sextant biopsy and 366 prostate cancers were diagnosed (incidence 19.9%), which was significantly lower compared to the biopsy detection rate in the 1st round ($p<0.0001$).
In Table 1 the diagnoses of men biopsied in the 1st and 2nd screening rounds are shown.

Table 1 Biopsy Findings in 1st and 2nd Screening Round

	initial biopsy n (%)		diagnosis after repeat biopsy n (%)		cancer detection rate n (%)	
biopsy diagnosis	1st round	2nd round	1st round	2nd round	1st round	2nd round
Benign	3004 (72.8)	1391 (75.6)	3078 (75.1)	1457 (79.5)	60 (12.1)[1]	NA
PIN	34 (0.8)	46 (2.5)	3 (0.0)	6 (0.0)	9 (13.3)	6 (13.3)
LSPC	108 (2.6)	50 (2.7)	5 (0.0)	2 (0.0)	35 (36.5)	8 (17.0)
PC	970 (23.6)	353 (19.2)	1014 (24.7)	367 (20.0)	NA	NA
other malignancy[2]	1 (0.0)	0 (0.0)	1 (0.0)	0 (0.0)	1 (0.0)	0 (0.0)
total (%)	4117 (100)	1840 (100)	4101[3] (100)	1832[3] (100)	1014(24.6)	367 (19.9)

NA Not Available, **PIN** Prostatic Intraepithelial Neoplasia, **LSPC** Lesion Suspicious for Prostate Cancer, **PC** Prostate Cancer

[1]Prostate cancer detection rate after repeat biopsy in interim screening round, selected from 3078 benign biopsies in the 1st screening round

[2]Leiomyosarcoma of rectal wall

[3]16 men in the 1st and 8 men in the 2nd screening round declined repeat biopsy after a diagnosis of PIN or LSPC (see figure 1 and 2)

PIN in two subsequent screening rounds

In the 1st screening round 34 of 4117 biopsied men had a diagnosis of isolated PIN (0.8%). Thirty men underwent repeat biopsy, of whom 4 were diagnosed with prostate cancer (13.3%). In the remaining 26 men, who underwent repeat biopsy, only 2 men were again diagnosed with PIN and 2 other men with LSPC.

In the 2nd round, 46 men were diagnosed with PIN (2.5%), 41 of whom underwent repeat biopsy. Six men were diagnosed with prostate cancer (13.3%). Of the remaining 35 men, four respectively one man were diagnosed with PIN respectively LSPC, after repeat biopsy. Except for one man who was biopsied 3 times and showing PIN each time, men with PIN in their repeat biopsy were not rebiopsied a 3rd time in the 1st and 2nd round. Compared to men who underwent repeat biopsy for an initially benign diagnosis, men with an initial diagnosis of PIN did not have a higher probability for detection of prostate cancer (12.1 vs. 13.3%). Although men with PIN in the 1st round were significantly older compared to men with benign diagnoses, this was not the case in the 2nd round. The incidence of PIN increased significantly from the 1st to the 2nd round ($p<0.0001$). Potential predictive factors for detection of prostate cancer after repeat biopsy, i.e. the length, bilaterality and multi-focality of PIN in biopsy cores, age, PSA, DRE and TRUS findings, did not reach significance, neither in the 1st nor in the 2nd round.

Prostate cancer yield after repeat biopsy for a LSPC

Hundred-eight men were diagnosed with a LSPC in the 1st round (2.6%). A total of 96 men underwent repeat biopsy and 35 of them were diagnosed with prostate cancer (36.5%). Compared with the biopsy detection rate of cancer in the interim screening round, prostate cancer incidence after repeat biopsy in men with a 1st round LSPC was significantly higher ($p<0.0001$). Two men diagnosed with PIN in their 1st biopsy were diagnosed with LSPC after repeat biopsy.

In the 2nd round 50 men were diagnosed with LSPC (2.7%), 47 men underwent repeat biopsy and 8 of them were diagnosed with prostate cancer (17.0%), which was significantly lower, compared to the 1st screening round ($p<0,0001$). Similarly, when the prostate cancer incidence on repeat biopsy in the 2nd round was compared with the interim screening round prostate cancer incidence no significant differences could be found.

Slightly more men had LSPC in the 2nd screening round compared to the 1st screening round. Men with LSPC had significantly higher PSA values (6.3 vs. 4.5 ng/ml) compared to men with benign diagnoses at biopsy in the 1st round ($p<0.0001$), but not in the 2nd round. Despite higher PSA values in the 1st round in men with LSPC, their PSA level, age, DRE, and TRUS findings were not predictive for prostate cancer after repeat biopsy in both rounds.

Prostate cancer yield during subsequent screening rounds after initial diagnosis of PIN or LSPC

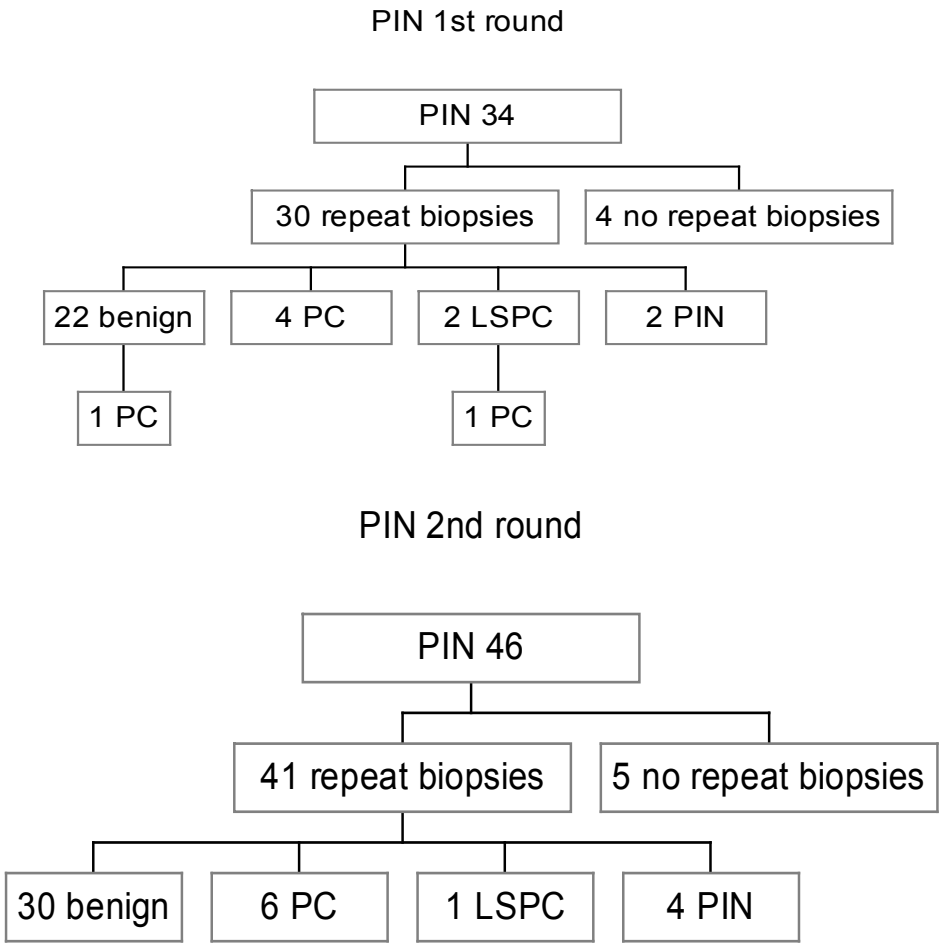
In Figure 1 and 2, the flowcharts of PIN and LSPC diagnoses in the 1st and 2nd screening round can be seen. In total 2 out of 22 men diagnosed with PIN in the 1st round were diagnosed with prostate cancer in the follow-up. One man with an initial PIN diagnosis, followed by a lesion suspicious for malignancy after repeat biopsy in the 1st round was diagnosed with prostate cancer in the interim screen. Another man with PIN in his 1st sextant biopsy and benign diagnosis in his repeat biopsy was incidentally diagnosed with prostate cancer at transurethral resection of the prostate (TURP) 3 years later. Three men with a PIN-diagnosis in the 1st round were invited in the interim screening round. They had benign diagnoses after repeat biopsy for PIN in the 1st as well as in the interim screening round. Three men with a PIN diagnosis in the 1st round (of whom 2 also underwent screening in the interim screening round) had a benign biopsy outcome in the 2nd round.

In total 7 out of 108 men with an initial LSPC diagnoisis in the 1st round were detected with prostate cancer after a 4-year follow-up. Four men with benign biopsy outcome after repeat biopsy for LSPC in the 1st round, were diagnosed with prostate cancer in the 2nd round. Three of the 4 had a benign diagnosis in their repeat biopsies. The 4th man was diagnosed with prostate cancer after repeat biopsy, prompted by a PIN-diagnosis in the 2nd round. One man did not undergo repeat biopsy in the 1st round, but was diagnosed with prostate cancer in the 2nd screening round.

Two men with LSPC in the 1st round had an interval carcinoma just before the 2nd screening round. One of them declined repeat biopsy in the 1st

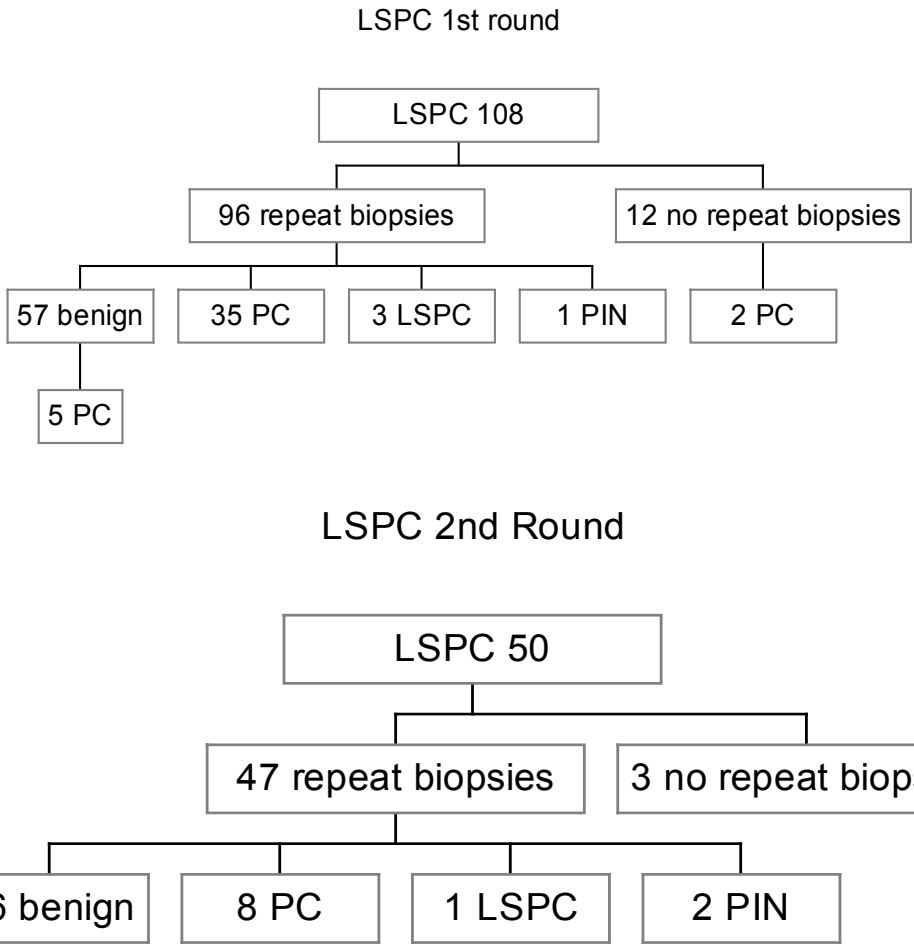
screening round. The other interval carcinoma was detected after a rising PSA.

Figure 1 PIN in 1st and 2nd scfeening round



Benign: benign diagnosis after repeat biopsy
PC: prostate cancer
LSPC*:* lesion suspicious for prostate cancer
PIN: prostatic intraepithelial neoplasia

Figure 2 LSPC in 1st and 2nd round



Benign: benign diagnosis after repeat biopsy
PC: prostate cancer
LSPC*:* lesion suspicious for prostate cancer
PIN: prostatic intraepithelial neoplasia

Prostate cancer yield after benign biopsy outcome in the first and/or interim screening round

After (repeat) sextant biopsies in the first round, 3078 men had benign diagnoses, and 745 of them underwent a biopsy in the 2nd screening round. Prostate cancer was found in 86 men. However, one man was diagnosed with PIN and 5 men with LSPC in their first biopsy in the 2nd round. When excluding these 6 men, there was a chance of 10.7% of being diagnosed with prostate cancer in the 2nd round after a benign biopsy in the first round. The 2nd round prostate cancer incidence in men who were biopsied in the 1st as well as in the interim screen, (n=192) was 10.9% after exclusion of 2 men who had initially LSPC in the 2nd round and prostate cancer after repeat biopsy. The prostate cancer incidence in men who were not biopsied in the 1st round was 22.7%, which was significantly higher ($p<0.0001$). Eight men were found with an interval carcinoma between the 1st and 2nd screening round. Of those men with a benign biopsy in the 1st round, significantly more men with abnormal DRE and TRUS in the_2nd round were at risk for detection of prostate cancer as compared to men with normal DRE and TRUS findings ($p=0.004$ and $p=0.007$). PSA, PSA-velocity and age however, were not predictive for prostate cancer after biopsy in the 2nd round.

Discussion

Here we report on the incidence of prostate cancer in sextant biopsies and the chance of finding prostate cancer after repeat biopsy for different lesions predictive of cancer studied in two subsequent screening rounds with an interval of 4 years. The prostate cancer incidence in sextant biopsies declined significantly from 24.6% to 19.9% in the 1st and 2nd screening round, respectively. This is associated with the previously reported drop in the amount and grade of tumor in sextant biopsies in the 2nd screening round [84]. Also the proportion of potentially advanced prostate cancers (Gleason score ≥4+3 in sextant biopsy) significantly decreased in the 2nd screening round [85].

The incidence of PIN in sextant biopsies increased significantly from the 1st to the 2nd round (0.8% vs. 2.5%). Especially in the 1st screening round the proportion of isolated PIN is low as compared to data in literature. DeMarzo et al. recently published a PIN incidence of 5% [24]. This was, however, after reviewing 439 cases where-in 6 cases of PIN were missed and another 6 cases were previously diagnosed as low-grade PIN. The PIN incidence before review was 2.7%, which is comparable to our 2nd round data. The incidence reported in our series was without reviewing and the actual PIN incidence might therefore be higher than reported here. As a result of reviewing sextant biopsies for PIN, its incidence is expected to increase, because the reviewer will be more focused on the detection of PIN instead of prostate cancer. Inter-observer variation for diagnosing PIN is reported to be "moderate" (kappa value 0.61-0.81), [86] which could result in a higher PIN incidence by chance. Other explanations for the discrepant low PIN incidence in our study include:

1) in a population based screening lower PIN incidence rates may occur compared to a referred urological population. 2) "only" sextant biopsies were performed, which represent a limited sample size. 3) Tissue preparation/staining variables may influence the pathologic features used to diagnose PIN [87].

Since all PIN diagnoses were reviewed by one uro-pathologist (T.v/d .K) and biopsy and preparation methods were equal in the subsequent screening rounds, the observed rise of the PIN-incidence in the 2nd round may be considered realistic. An explanation for the increased frequency in PIN may be the aging of the screened population. Indeed, in an autopsy study, it has been shown that PIN occurs more commonly in older men [43], a phenomenon also observed during the prevalence screening round in our study (data not shown). The incidence of LSPC remained stable during the two subsequent screening rounds (2.6% to 2.7%). This incidence is comparable to previous literature data ranging from 1.5-4.8% [76,77]. Generally, much less variation in the incidence of suspicious lesions is reported compared to PIN.

During the 1st screening round LSPC was highly predictive for detection of prostate cancer after repeat biopsy. Strikingly, this was not the case for men diagnosed with a LSPC in the 2nd screening round, although the proportion of detected prostate cancers tended to be higher as compared to that in men with initially benign diagnosis in the first screening round undergoing biopsy one year later (17.0 vs. 12.1%). This difference was not significant. Men with PIN in the 1st and 2nd round were not at increased risk for detection of prostate cancer after repeat biopsy as compared to men with a previous benign biopsy diagnosis (13.3 vs. 12.1%). One other large study also has reported that PIN is not a risk factor for prostate cancer detection after repeat biopsy [88]. The limited predictive value of isolated PIN is further illustrated by prostate cancer in only two men with a prior PIN diagnosis (and benign diagnosis after repeat biopsy) after a 4-year interval. If these two men were added to the men who were diagnosed with prostate cancer after repeat biopsy for PIN, there still would be no significantly increased prostate cancer risk for men with PIN in the 1st round. This implies that in this screening study the only lesion significantly predictive for subsequent prostate cancer is a diagnosis of LSPC, particularly in the 1st screening round.

The finding that PIN and possibly LSPC in the 2nd round do not represent a significant risk factor for subsequent detection of prostate cancer is not comparable to previous literature, where PIN, respectively LSPC is reported to be associated with 23-51% [80-83], respectively 39- 60% [78,79] chance of prostate cancer after repeat biopsy. This may be due to differences in the populations under investigation. For example, the population of Park et al. [83] in which the prostate cancer detection rate is 50% after repeat biopsy for PIN, does not represent a clear screening or referred urological population. However, the number of repeat biopsies and cores in their population (19% of the population had a 3rd sextant biopsy with at least 6-10 core biopsies) explains a higher prostate cancer incidence. The higher detection rate of

prostate cancer after repeat biopsy for LSPC reported by some authors [88] may similarly be the consequence of a different repeat biopsy protocol. In our center, only 4 cores were taken, directed at the site where the LSPC was found. The intention of our repeat biopsies was just to confirm or to disprove that the previously detected LSPC represented an adenocarcinoma. Allen et al. [89] reported that prostate cancer was found on the same and adjacent sites of the atypical small canard proliferation in 85% of the time and the remaining were found at other sites. Extrapolating this data would imply that 15% of cancers are missed by our biopsy strategy. This percentage corresponds well with the ``background`` prostate cancer incidence of 12.1% in men with prior benign diagnosis.

Another explanation for our lower prostate cancer incidence after repeat biopsy for PIN and LSPC could be down-staging and reduction of tumor volume in our cohort of men. Tumor stage in prostate cancer declined considerably after introduction of prostate cancer screening [62]. The chance of diagnosing prostate cancer obviously decreases with lower tumor volumes [90]. From literature we know that in up to 99% of cystoprostatectomies because of bladder cancer contained PIN, whereas in only half of these prostates also concurrent prostate cancer was diagnosed [91]. This indicates that the "volume" of PIN involved glands in a prostate may be larger as compared to the volume of cancer in case screen-detected prostate cancers. Thus, during subsequent screening rounds the chance of finding prostate cancer on sextant biopsy decreases and the chance of finding PIN increases. Nevertheless, detection of PIN is subject to a considerable sampling bias as becomes apparent from the observation that in the minority (2 of 24, i.e. 8.3%) of men with an initial diagnosis of PIN, this lesion was found again during repeat biopsies.

A large proportion of the men (i.e. 40.5%) who underwent sextant biopsy in the 2nd round of our screening study also underwent sextant biopsy in the 1st round. Strikingly, men with a benign biopsy outcome in the 1st round, including those who underwent a repeat biopsy, were at significantly decreased risk of prostate cancer compared to men who never had a sextant biopsy before (10.7 vs. 22.6% $p<0.0001$). This is comparable to the observation in men who underwent "repeat" biopsy in the interim screen: Their prostate cancer incidence dropped to 12.1% (1 year after a benign diagnosis in the 1st round). The prostate cancer incidence in the 2nd round of men who were previously biopsied once (1st round) or twice (1st and interim screening round) was 10.7 vs. 10.9%, respectively. Apparently, a screening interval of 1- or 4 years after a benign biopsy outcome did not influence the prostate cancer incidence in sextant biopsies. In literature a range from 10-30% prostate cancer incidence has been reported for repeat biopsies after a prior benign diagnosis [92-95]. Djavan et al. found a 10% prostate cancer incidence after doing repeat biopsy (sextant biopsy and 2 additional transition zone biopsies) within 6 weeks, a comparable figure with ours, but Fleshner et al. reported a prostate cancer incidence of 30% after repeat biopsy. This high incidence may be attributed to the low number of biopsies (< 6 biopsy cores) in almost 20%

of the men during the initial biopsy procedure. Comparison of our data with literature is confounded by differences in biopsy procedures (i.e. different number of repeat biopsies, number of biopsy cores and regions in the prostate biopsy) and patient populations. Also the quality of the pathology performance may influence the biopsy detection rate of prostate cancer after an initial biopsy with benign outcome [96].

Conclusions

The significant decrease of prostate cancer incidence in sextant biopsies in the 2nd screening round was associated with an increase in the proportion of biopsies with isolated PIN. Strikingly, during the 1st round, but not during the 2nd round LSPC was highly predictive for subsequently detected prostate cancer, while PIN was not predictive for prostate cancer in repeat biopsy neither in the 1st nor 2nd round. This might be explained by downsizing of the tumor volumes in the 2nd round. No clinical variable could help predict the outcome of repeat biopsy diagnostics. Furthermore, an initially negative biopsy in the 1st screening round proved to be "protective" for prostate cancer in the 2nd screening round. DRE and/or TRUS abnormalities were now able to predict subsequent prostate cancer after an initially benign diagnosis. The finding of isolated PIN in a screened population had no predictive value for prostate cancer within 4 years and therefore this lesion by itself should not be considered an indication for repeat biopsy. For a diagnosis of LSPC in the 2nd screening round we continue to recommend a repeat biopsy, since a repeat biopsy is intended to clarify the nature of the previously diagnosed LSPC. Additionally, abolishment of repeat biopsies after a diagnosis of LSPC could increase the pressure on the pathologists to give a less ambiguous diagnosis.

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Chapter 3

Atrophy in prostate needle biopsies and its relationship to prostate cancer incidence in screened men

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Theo H. van der Kwast

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Abstract
Objective: To evaluate whether the incidence of atrophy reported on sextant biopsies is associated with subsequent prostate cancer detection. To obtain a more thorough analysis of the different categories and extent of atrophy a review of benign biopsies was performed.
Methods: In the Rotterdam section of the European Randomized Study of Screening for Prostate Cancer (ERSPC) 4117 and 1840 men underwent sextant biopsy in the 1st and 2nd screening round (4-year interval), respectively. Sextant biopsy was prompted by elevated PSA. For review, randomly taken benign sextant biopsies (n=202) with a follow-up of at least 8 years were chosen.
Results: Before review, atrophy was reported in biopsies in 11.4% and 8.7% in the 1st and 2nd round, respectively. Prostate cancer incidence during 8-year follow-up after an initial diagnosis of atrophy was 10.4%, which was not significantly higher than the 12.3% of cancers detected after a benign diagnosis without reference to atrophy. After review the incidence of simple atrophy, post atrophic hyperplasia and sclerotic atrophy in sextant biopsies was 91, 47 and 9%, respectively. Extensive atrophy was observed in 5% of biopsies. Only 2 (4.7%) men in the reviewed group had a subsequent diagnosis of prostate cancer in the 8-year follow-up. Additionally, prostatic intraepithelial neoplasia (PIN) was diagnosed in 3 men (7.0%) in the 2nd screening round.
Conclusions: Atrophy, especially its simple form, is a very common lesion in prostate biopsies (94%). Atrophy in an asymptomatic population subject to screening was not associated with a higher prostate cancer or PIN incidence during subsequent screening rounds.

Introduction

Prostate cancer is the most common non-cutaneous malignancy in the Western world[4]. So far histopathologic examination of prostate needle biopsies is the only possibility to establish the diagnosis of prostate cancer. Histological examination of prostate biopsies can be difficult and a conclusive diagnosis cannot always be rendered, since some lesions may resemble prostate cancer [24]. A lesion suspicious for prostate cancer, and high-grade prostatic intraepithelial neoplasia (PIN) and, more recently, atrophy have been considered as lesions predictive for associated prostate cancer [72,73]. It is hypothesized that atrophy is the consequence of chronic inflammation. Chronic inflammation has been linked to cancers other than prostate cancer (i.e. the liver (hepatitis C), stomach (Helicobacter pylori) and colon (colitis ulcerosa)). Also epidemiological evidence exists for a link between infections (i.e. sexual transmitted disease) and inflammation (prostatitis) and prostate cancer [97]. The observation of reduced frequency of prostate cancer in men using non-steroidal anti-inflammatory drugs (NSAIDs) is consistent with the hypothesis that there is an association between inflammation and prostate cancer [98].

Several forms of prostatic atrophy have been described. Ruska et al. distinguished two categories of atrophy, i.e. simple atrophy (SA) and post atrophic hyperplasia (PAH). A third form, sclerotic atrophy, was also suggested to represent a pre-neoplastic lesion, however this is a seldom found diagnosis [99]. Because atrophy was believed to originate from inflammation, McNeal et al.[100] used the term post-inflammatory atrophy for the above mentioned (SA and PAH) lesions [97]. DeMarzo et al. introduced the term "proliferative inflammatory atrophy" (PIA) for PAH and SA, since both tend to be highly proliferative lesions, associated with inflammation. They also suggested that PIN lesions might arise from PIA [101,102].

Atrophy has been linked to prostate carcinogenesis by some authors, because of its spatial relationship with cancer in radical prostatectomy specimens [103]. Some molecular changes in prostate cancer and PIN are shared with atrophy; the frequency of p53 mutations in PAH was equal to the frequency in PIN, in a small numbers of cases [104]. The incidence of atrophy in prostate biopsies is unknown. Nevertheless, a recent study reported the occurrence of atrophy in autopsy prostates: of the examined prostates 66% contained atrophy with inflammation and 22% contained atrophy without inflammation, respectively [105].

This study focuses on the frequency of atrophy reported in sextant biopsies during two subsequent prostate cancer screening rounds with a 4-year interval. To make a distinction between the different forms of atrophy, their extent in needle biopsies and their role as a predictor for prostate cancer, 202 biopsies reported with a benign outcome (including atrophy), were reviewed.

Material and methods

Study population

Our study population consisted of 41,919 participants, aged 55 to 74 years, who were randomized to screening and non-screening. The prevalence screening was done between June 1994 until December 1999 and a total of 19,970 men were actually screened. Screening for prostate cancer was done by PSA determination, digital rectal examination (DRE) and transrectal ultrasonography (TRUS). Systematic sextant needle biopsy was recommended for participants who had either an elevated PSA level (≥ 4.0 ng/ml) or abnormal DRE and/or abnormal findings on TRUS. After November 1997, sextant biopsies were suggested in all men who had a PSA value of 3.0 ng/ml or more, and DRE and TRUS were omitted as a screening tool. The 2nd screening round, using the PSA ≥3.0 ng/ml screening protocol after a 4-year interval, started in June 1998 and finished in December 2003.

Diagnosis of atrophy without pathology review

In the routine prostate cancer screening setting (before review), community pathologists could, in the absence of cancer, report atrophy as a separate diagnostic entity on sextant biopsies. However, no guidelines regarding the criteria for such a diagnosis are employed in the laboratory.

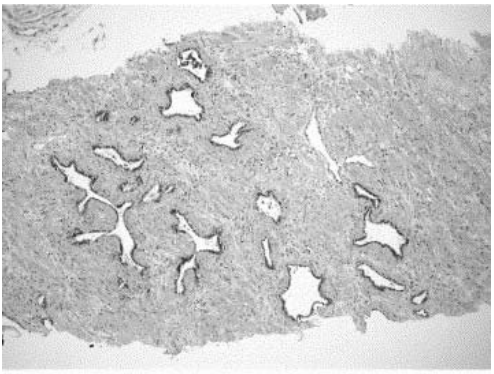
Categories of atrophy after pathology review

For review, one uro-pathologist (T.v.d.K), who was blinded for follow-up details, reviewed a random sample of sextant biopsies (n=202) with a benign outcome (including atrophy) for the incidence and category of atrophy. The sextant biopsies dated from 1995, in order to achieve a follow-up of at least 8 years. The diagnostic entity "atrophy" was split up in three different categories, which are shown in figure 1: 1) Simple atrophy, 2) post atrophic hyperplasia, and 3) sclerotic atrophy. Each category was further graded in arbitrary extent categories: 1) no atrophy, 2) 1-3 biopsy cores containing one or more separate small (<5 mm) foci of atrophy, 3) >3 biopsy cores containing one or more separate small (<5 mm) foci of atrophy, 4) >5 mm of continuous atrophy (adjacent area) in 1 or 2 biopsy cores, 5) >5mm of continuous atrophy (adjacent area) in >2 biopsy cores.

Statistical analysis

Statistical analysis was done with the SPSS software package (SPSS 11.0 Inc., Chicago, IL). $P<0.05$ was considered significant. Chi-square tests were used for ordinal variables, student T-test for linear variables, i.e. PSA, age and prostate volume. Multinomial regression was used to evaluate the association between the extent of atrophy and age, PSA and prostate volume.

Figure 1 *a* Simple atrophy, with whole biopsy core involved, *b* Post-atrophic hyperplasia (black arrow), *c* Sclerotic atrophy, with whole biopsy core involved



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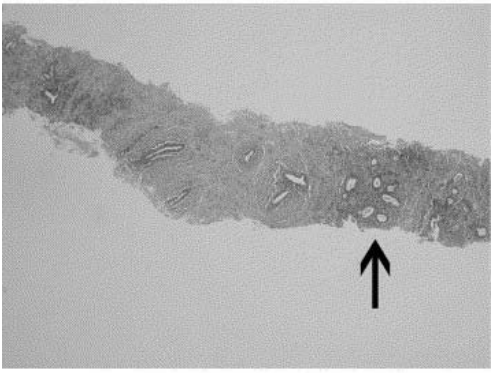

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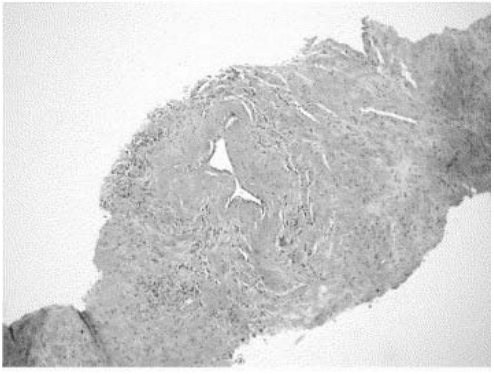

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Results

Atrophy incidence before review

In the 1st and 2nd screening round 4117 and 1840 men underwent sextant biopsy. Atrophy was reported in 11.4% (468) and 8.7% (160) of sextant biopsies in the 1st and 2nd round, respectively ($p<0.0001$). Of those 468 men reported with atrophy in the 1st round, 135 were again biopsied in the 2nd screening round. Prostate cancer was found in 14 (10.4%) men. Of those men whose 1st biopsy showed benign pathology without atrophy and biopsied again in the 2nd screening round (n=661), 12.3% were found to have prostate cancer, which was not statistically different from the prostate cancer incidence after an initially reported diagnosis of atrophy (10.4%). Thirty-nine patients were diagnosed with prostate cancer before the scheduled 4-year screening interval (interval carcinomas). Of these 39 patients, 5 and 34 had atrophy and benign diagnoses reported in the 1st screening round. If these numbers of patients were added to the 10.4% and 12.3% prostate cancer incidence in the 2nd screening round, there still was no significant difference in prostate cancer incidence in men with atrophy as compared to those with benign diagnosis during the 1st round. The PIN incidence in the 2nd round in men with a 1st round atrophy and benign diagnosis was 5.4 and 3.3%, respectively, which was a non-significant difference.

Men with a reported atrophy diagnosis were significantly older and had larger prostate volumes (59 vs. 51 ml) as compared to men with a benign, no atrophy biopsy diagnosis ($p<0.001$). PSA levels did not differ significantly between these two groups

Atrophy incidence after review

Two hundred-two consecutive sets of sextant biopsies from the 1st screening round reported as benign, including atrophy, diagnoses were reviewed for the presence of 3 different types of atrophy and their extent. The results are listed in table 1.

Table 1 Different forms of atrophy in prostate needle biopsies

	simple atrophy (SA) n (%)	post atrophic hyperplasia (PAH) n (%)	sclerosing atrophy n (%)
no atrophy	18 (9)	106 (53)	184 (91)
1-3 cores with focal atrophy	114 (56)	77 (38)	17 (8)
>3 cores with focal atrophy	42 (21)	4 (2)	1 (1)
>5 mm atrophy in 1 or 2 cores	21 (10)	12 (6)	0 (0)

In total 94% of the men were diagnosed with some form of atrophy of which simple atrophy was the most common form. Extensive atrophy (a continuous

area of >5 mm in more than 2 cores) was seen in 5% of men, predominantly in the simple atrophy form. In total, 43 men with an atrophy diagnosis after review underwent biopsy in the 2nd screening round of whom two (4.7%) and 3 (7.0%) men were diagnosed with prostate cancer and PIN. The 2 men diagnosed with prostate cancer had both simple-and post atrophic hyperplasia. The extent of atrophy in these two men was intermediate to large for simple atrophy (atrophy category 3 and 4 for man 1 and 2) and small for post atrophic hyperplasia (atrophy category 1 and 2 for man 1 and 2). No interval carcinomas or cancers diagnosed after the age of 75 were found in these men.

Men with atrophy after review were significantly older, compared to men without atrophy (67 vs. 62 years of age, P=0.008). Although not significant, men with atrophy had higher prostate volumes and PSA levels compared to men without atrophy. Men with extensive (atrophy category 4 and 5) post atrophic hyperplasia had higher PSA levels compared to men with extensive simple atrophy, however this was also not significant. The extent of atrophy was not significantly associated with a higher prostate cancer incidence.

Discussion

In this study we reported the incidence of atrophy before and after review and the prostate cancer incidence in sextant biopsies in the subsequent screening rounds after a 4-year screening-interval.

Before review, the incidence of reported atrophy was 11.4% and 8.7%, in the 1st and 2nd screening round, respectively. The prostate cancer incidence in the 2nd screening round in men with a diagnosis of atrophy (10.4%) and in men with a benign (not explicitly atrophy) diagnosis (12.3%) in the 1st screening round, did not differ significantly. During the next year of follow-up a limited number of additional carcinomas was detected which did not influence the outcome.

Because the true incidence of atrophy and its different categories in prostate biopsies was unknown, 202 sextant biopsies reported as benign, including atrophy were reviewed. After review, 94.0% of these biopsies contained some form of atrophy. Due to the under diagnosis of atrophy on the routine pathology reports, we will focus on the results after review. After a diagnosis of atrophy during review, the prostate cancer incidence within the 8-year follow-up was 4.7%, which was comparable to the prostate cancer incidence in men with benign biopsies, who also underwent a biopsy after a 4-year screening interval. Apparently, neither before nor after review atrophy was associated with an increase of prostate cancer incidence.

The incidence of atrophy after review reported here is comparable to the autopsy study of Billis et al. [105] who reported a total atrophy incidence of 88% compared to 94% in our study. The prostate cancer incidence in their study was equal in prostates with and without atrophy and they concluded that atrophy was not associated with prostate cancer. This is in contrast to views expressed in recent literature, where it is hypothesized that atrophy, i.e.

PIA (PAH and SA) [101] may be a precursor or risk factor for prostate cancer. It was also suggested that PIA might progress to PIN. However, in our study we were also not able to find a significant association between a diagnosis of atrophy and the presence of PIN in subsequent screening rounds. It could be argued, however, that one or more categories of atrophy represent a lesion, which predisposes to prostate cancer on the very long run. According to this view only young men (e.g. < 40 years) with atrophy would be at risk for development of prostate cancer at older age. Direct evidence for this view will be hard to obtain. On the other hand, our findings do not contradict such a hypothesis although we would expect that the extent of atrophy at age > 55 would be a manifestation of atrophy at young age. The lack of an association of (extensive) atrophy and subsequent prostate cancer might then be considered as an argument against the role of any kind of atrophy in prostate carcinogenesis.

The dramatic discrepancy between the atrophy incidence before and after review can probably be explained by the possibility that the pathologist is less focused on the importance of the diagnosis, because in our institution atrophy was not regarded as a clinically relevant lesion for prostate cancer. In routine practice a diagnosis of atrophy might only be rendered, if this lesion was present in a prominent way due to the lack of guidelines for diagnosing atrophy. Also a high inter-observer variation will be present among pathologists for the diagnosis of atrophy due to the lack of guidelines for diagnosis atrophy.

Men with atrophy were significantly older, compared to men with a benign, no atrophy diagnosis. As a result of this, we expect that the incidence of atrophy during the subsequent screening rounds in the ERSPC will increase. No significant differences were found in prostate volume and PSA between men with and without atrophy. As expected, because atrophy as a whole was not associated with the detection of subsequent prostate cancer, also the category and extent of atrophy was not associated with an increased prostate cancer incidence.

In the absence of guidelines, consensus about diagnosing atrophy needs to be established worldwide, before linking atrophy with prostate cancer.

Conclusions

Atrophy is a common lesion and is present in 94% of sextant biopsies. An atrophy diagnosis in our asymptomatic population was not predictive for prostate cancer nor PIN in subsequent screening rounds (8 year follow-up). Neither the separate atrophy categories (i.e. simple atrophy, post atrophic hyperplasia, and sclerotic atrophy) nor their extent in sextant biopsies were associated with an increase in prostate cancer incidence in subsequent screening rounds as compared to men with benign diagnoses.

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Part 3

Chapter 4

Potentially advanced cancers detected by screening after an interval of 4 years

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Abstract

Objective: At the Rotterdam section of the European Randomized Screening program of Prostate Cancer (ERSPC) a cohort of 19,970 men aged between 55-75 years is screened at an interval of 4 years. This includes a systematic sextant needle biopsy procedure in men with elevated PSA and/or positive DRE or transrectal ultrasound. Detection during the second screening round of a large number of cancers mainly composed of high grade (Gleason grade 4/5) and/or a high amount of cancer might be considered as a failure to identify these tumors at an early stage during the prevalence screening.

Patients and methods: Men diagnosed during the second screening round with a potentially advanced cancer (PAC), i.e. prostate cancer Gleason score 7 (4+3, or 3+4 with a >30% cancer involvement) or 8-10 in their biopsy, were identified. Clinical data, including PSA values in de prevalence screen, biopsy history, clinical staging and follow-up were retrieved. In radical prostatectomy specimens the features of the tumor were further analyzed.

Results: During the second screening round 503 cancers were detected in 11,210 participants, including 30 (6.0%) with features of PAC in their diagnostic biopsy. Curative treatment was offered to 26 patients. Prostatectomy showed organ-confined cancer in 11 of 12 specimens, and tumor volumes ranged from 0.11-7.93 ml (median 1.05ml). PSA failure was noted in 6 of 22 patients offered a curative therapy.

Conclusions: PAC is a rare finding in the 2nd screening round after a 4-year screening interval. A substantial proportion of 2nd round detected PAC represents organ-confined cancer. The data suggest that the currently employed screening protocol is sufficiently effective to detect > 95% of cancers at a stage before they have developed features that render them incurable.

Introduction

In the western world the incidence of prostate cancer is increasing due to general availability of serum tests for prostate specific antigen (PSA) and aging of the population[106]. Early detection of prostate cancer by PSA testing may result in a decrease in mortality from prostate cancer, but definite evidence for this effect is not yet given. The European Randomized study of screening for prostate cancer (ERSPC) is an international multi-center population based trial that investigates the impact op PSA testing for cancer mortality [107]. Participants are randomized in a screening and control arm and final outcome of this study with regard to endpoint parameters like mortality from prostate cancer is not expected before year 2008.

Analysis of intermediate parameters in order to assess the efficacy of the current screening protocol of the Rotterdam section of the ERSPC seems to be warranted for a few reasons. Previously, it was claimed that annual PSA testing in combination with digital rectal examination (DRE) would lead to the detection of all cancers at a curable stage [108]. Indeed, the American Cancer Association recommends yearly PSA testing [109]. The screening interval of 4 years employed in the Rotterdam section of the ERSPC, may therefore be considered a too long time period. In addition, according to the ERSPC protocol the diagnosis of prostate cancer is established on a systematic sextant biopsy. This systematic sextant biopsy procedure has now become controversial, because it has been reported that about 30% of cancers are missed by this procedure [110]. The earlier finding by the Rotterdam section of the ERSPC that the incidence of prostate cancer did not drop significantly in the 2nd round of screening (3.9%) as compared to the first screening round (4.3%) may support this view [84]. Especially for large prostates and small tumors a more extended biopsy procedure is recommended [111]. For above reasons it may be anticipated that in a screening program with a 4-year interval using systematic sextant biopsies prostate cancers may be missed, which could lead to the manifestation of clinically advanced prostate cancers during the 4-year interval or to their detection at the second screening round.

In order to generate a simple parameter for the efficacy of screening for prostate cancer several attempts were made to categorize the outcome of prostate needle biopsies in subsets with a different prognostic impact [84,111-113]. It was reported that among biopsies with Gleason score 7 those with dominant Gleason pattern 4 (4 + 3) indicate the presence of more adverse pathological findings compared to those with Gleason score 7 with a smaller pattern 4 component (3 + 4)[112]. An increased prostate cancer mortality in patients with Gleason score 7 and even higher in those with score 8-10 was reported compared to patients with Gleason score 6 in their biopsy [113]. Some others demonstrated a relationship between extent of cancer in the biopsy and pathologic stage in the prostatectomy specimen [114,115]. Thus, an arbitrary categorization model may be constructed defining "potentially advanced" prostate cancer (PAC) on the basis of Gleason score and amount of tumor present in sextant biopsy [84]. Following such an arbitrary model, we considered the detection during the second screening round of a significant

number of prostate needle biopsies with PAC, defined by a predominance of Gleason pattern 4 and/or 5 or needle biopsies with >30% cancer involvement by Gleason score 7 (3 + 4) cancers as an indication for a potential inadequacy of our current screening protocol.

In this study we analyzed the frequency of PAC in sextant biopsies during the second round of screening for prostate cancer and we compared these findings in the corresponding prostatectomy specimens and with clinical stage and follow-up. Furthermore, the screening history of these men was analyzed in order to find an explanation for failure of early identification of these potentially advanced cancers.

Patients and Methods

In the Rotterdam section of the ERSPC 42,376 participants, 55-75 years old, were randomized in a screening (n=21,210) and a control arm (n=21,166). In the first round, which took place from October 1991 until December 1998, a total of 19,970 were actually screened of whom 4,243 men underwent prostate biopsy.

These numbers also include participants from the pilot study, in which in case of PAC the same screening protocol was used. Screening for prostate cancer in this study was done by PSA determination, digital rectal examination (DRE) and transrectal ultrasonography (TRUS). Systematic sextant prostate needle biopsy was recommended for participants who had either an elevated PSA level (≥ 4.0 ng/ml), abnormal DRE or abnormal findings on TRUS. The protocol was simplified on May 1997, when sextant biopsy was recommended if PSA was ≥3.0 ng/ml. Abnormalities on DRE and/or TRUS were no longer indicators for biopsy. The second screening round, using the latter screening protocol, started in October 1995 and is still ongoing. Until March 2003, a total of 11,210 of the expected 13,390 participants were screened of whom 2,607 men had undergone prostate biopsy. Of the men with a negative biopsy in the 1st screening round (n=3,151), 60% were screened in the 2nd round. Those men who were not screened anymore in the 2nd screening round either refused biopsy (12.6%), were too old (16.0%) or died (4.0%). In 7.4% of the men the reason was unknown. When there was an invitation for sextant biopsy in the 2nd screening round, 12.6% of the participants did not undergo biopsy; including 10.9% refusals and 1.7% of the participants, who were not allowed to undergo a biopsy due to anticoagulation therapy. The expected number of participants in the second round is based on the total number of participants of the first round, after exclusion of 1) participants with prostate cancer already found in the first screening round, 2) those with an age over 75 and 3) those died during the 4-year screening interval.

PSA determinations were done with a Hybritech Tandem E assay (Hybritech Beckman-Coulter Corp., San Diego, Cal), and blood samples were stored to allow repeat determinations. All men had signed an informed consent prior to their participation to the screening study.

During the second round of the screening program two side studies were performed: 1) the impact of PSA doubling on the detection of (clinically

relevant) prostate cancers on 5,658 men. If PSA increased twice or more since the first screen, men were invited for sextant biopsy.
2) Investigation of the incidence of prostate cancer in the PSA 2-4 ng/ml range performed on 885 men. Men in this side study were invited for sextant biopsy. The participants were also included in the analysis.

Systematic sextant biopsies were obtained during longitudinal and cross-sectional ultrasonographic scanning of the prostate. A 7th biopsy was taken if a hypoechogenic lesion was visible at TRUS. One pathologist (T v/d K) reviewed all biopsies with cancer in order to avoid inter-observer variation in Gleason grading. During this review, the number and size of biopsies were recorded as well as proportion of tumor involvement, and proportion of each Gleason grade.
Slides from radical prostatectomies of participants with prostate cancer were retrieved from the archives of the pathology laboratories of the Erasmus Medical Center and surrounding hospitals of the Rotterdam region. There was one single protocol for total embedding of the prostates in use in all pathology laboratories allowing accurate measurements of tumor volume, grading and staging. After review tumor stage and Gleason score were determined. Tumor volume was measured by morphometry as described previously [116]. For tumor staging of radical prostatectomy specimens the 1992 TNM classification for prostate cancer was used [117].
Using the database of the Rotterdam section of the ERSPC the needle biopsies of second round participants with Gleason score ≥4+3 or higher and those with Gleason score 7 (3+4) with more than 30% tumor involvement in their sextant biopsy were identified. Data on clinical stage, therapy and follow-up were retrieved from the ERSPC database.

Statistical analysis
Statistical analysis was done with the SPSS computer program. $P<0.05$ was considered significant. Chi-square tests were used for number of advanced cancers, paired sample T-test for comparing PSA levels of men with PAC at first and second screening round.

Results
Biopsy characteristics of potentially advanced cancers in 2nd screening round
Until March 2003, a total of 503 cancers were detected in the second screening round, including 30 (6.0%) fulfilling the criteria of PAC (Table 1).

Table 1 Frequency of cancers in the first and second screening round

Round	1	2
screened participants	21152	11210
total cancers	1092	503
PAC[1]	214	30
Ratio cancer/PAC	19.6%	6.0%

[1]**PAC** potentially advanced cancers (see text for definition)

During the prevalence screening a total of 1,092 prostate cancers were diagnosed in 21,152 participants, including 214 PAC (19.6%). The reduction of PAC detected in the 2nd round is highly significant ($p<0.001$). Of the 30 PAC found, 11 participants were derived from the PSA doubling time study, and 5 participants from the PSA 2-4 ng/ml side study. Two PAC participants in the PSA doubling time side study had PSA levels <3.0 ng/ml in the 2nd screening round. These participants would not have been invited for sextant biopsy in the main study screening round. There were no participants with PAC in the PSA 2-4 ng/ml side study with PSA <3.0 ng/ml.
In the second screening round 373 participants had Gleason score 6 (3+3) in their sextant biopsies, of these participants a total of 6 had >30% tumor involvement (1.6%). All these tumors were clinically organ confined; their median PSA values as compared to PAC were lower (3.0 vs. 4.7 ng/ml).

The distribution of Gleason scores of PAC in the 2nd screening round is shown in Table 2.

Table 2 Subdivision of potentially advanced cancers on the basis of Gleason score

Gleason score on sextant biopsy	PAC in 2nd round (n)
3+4	8
4+3	4
4+4	12
3+5	2
4+5	1
5+3	1
5+4	1
5+5	1
Total	30

There were 72 cancers with Gleason 3+4 in the second screening round of which 8 (11%) comprised more than 30% tumor of the biopsy length. Four and 15 participants with prostate cancer detected on biopsy had Gleason score 7 (4+3) and 8, respectively. Five out of the 8 participants with a PAC Gleason 3+4 underwent an additional 7th biopsy as a consequence of a TRUS detected hypoechogenic lesion. All these 5 biopsies were positive for tumor. If these hypoechogenic lesions were not biopsied selectively, 4 participants would not have been categorized as PAC.
For convenience, the extent of cancer involvement of the needle biopsies was further broken up according to the following arbitrary system: A = 1 biopsy core positive cancer, B = 2 -3 biopsy cores positive for prostate cancer, C = ≥4 biopsy cores positive for prostate cancer. Distribution of the biopsies according to this categorization is shown in Table 3.

Table 3 Categorization of PAC on the basis of proportion of tumor involvement of the sextant needle biopsies

classification	number of cases
A	8
B	10
C	12
Total	30

In all but 1 participant in category A the sextant biopsies contained less than 10% tumor. If the 8 PAC of participants with Gleason score 7 (3 + 4) and >30% tumor involvement were excluded, 8 participants would be left in category B and 8 participants in category C.

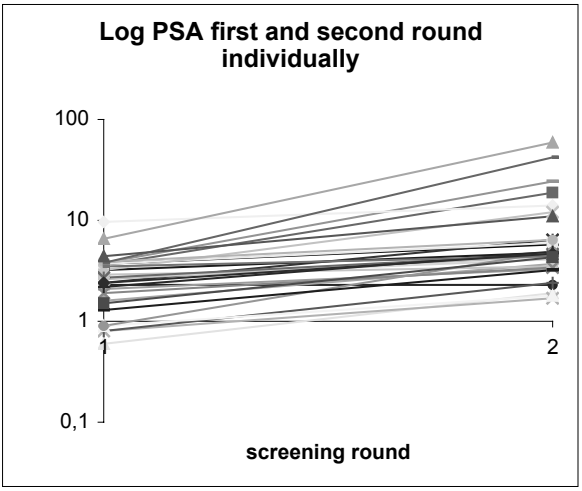
Development of PSA values in PAC patients during 1st and 2nd screening round
The PSA values of participants with PAC in the second round increased significantly from the 1st to the 2nd round (P=0.008) (Table 4), with a median PSA doubling time of 5 years.

Table 4 *S*erum PSA levels at first and second round of men with 2nd round PAC

screening round	PSA if PAC in 2nd round median (range)
1	2.6 (0.8-9.7)
2	4.7 (1.7-59.0)

One patient had a more than 10-fold PSA increase. In Figure 1 the 1st and 2nd round log PSA values of the 30 participants can be compared individually. The median PSA velocity was 0.5 ng/ml per year, range 0.0-12.8 ng/ml per year. In 9 men (30%) the PSA velocity was above 0.75 ng/ml per year suggestive of a rapidly progressive cancer development.

Figure 1 Log PSA change in first and second screening round per individual



History of patients with PAC in 2nd screening round biopsy

In the 2nd screening round a total of 792 men with a previous negative outcome biopsy history during the prevalence screening underwent a sextant biopsy. Forty-nine out of these 792 men (6.2 %) had prostate cancer in the 2nd screening round, including 5 men with PAC. The initial biopsy reports of the 5 men with PAC in the 2nd screening round were negative for prostate cancer and/or high-grade prostatic intraepithelial neoplasia (PIN). After review of these biopsy cores of the prevalence screen, no cancer was found. In one case, however, isolated HG-PIN in a single gland was found. On the whole 2nd screening round a 10-fold increase in PSA level was detected in two men at the 2nd screen. After repeating the PSA-determinations of the two (first round) sera, it was found that serum of one patient was mixed up. This patient actually had a (first round) serum PSA of 6.6 ng/ml instead of 0.8 ng/ml. As a consequence he was not invited for biopsy at the first round.

One participant with a sextant biopsy category C PAC had an abnormal DRE in the first screening round and a PSA of 3.6 ng/ml. He did not show up for biopsy in the first round.

Therapy and follow-up of patients with PAC during 2nd round

Clinical TNM stage and therapy of patients with PAC is shown in Table 5. In 21 patients the prostate cancer was organ confined (70%). Fifteen patients received radiotherapy, of whom 1 received adjuvant hormone therapy. Twelve patients underwent radical prostatectomy.

Table 5 Clinical stage and therapy of patients with 2nd round PAC

clinical T- stage	Prostatec-tomy	Radio-therapy	watchful waiting	endocrine therapy	Radio-therapy and endocrine therapy	number of cases
T1c	4	1	1			6
T2a/b	6	4		2[3]		12
T2c	1	2[1]				3
T3a/b	1	5[2]			1	7
T3c		2				2
total	12	14	1	2	1	30

[1]PIN missed during prevalence screening
[2]Including one man, refusing a biopsy at first round and one man receiving neo-adjuvant endocrine therapy.
[3]Failure to detect elevated PSA during prevalence screening

Patients who received radiotherapy had a higher clinical T-stage compared to radical prostatectomy patients. Two patients received hormone therapy. They had high PSA levels (42.0 and 59.0 ng/ml), cancer in every biopsy core (category C) and one patient developed lymph node metastasis. At a median follow up of 29 months (range 7-73) all PAC patients were alive. PSA values after therapy were known in 20 patients. There was PSA progression (PSA increase >0.2 ng/ml after one measurement) in 7 patients. The patient who was set on watchful waiting had a PSA increase from an initial PSA 5.4 ng/ml to 6.3 ng/ml during a follow up time of 39 months.

Radical prostatectomy findings in PAC patients
In Table 6 the pathologic features of the prostatectomy specimens of 12 patients are given.

Table 6 Needle biopsy findings, PSA levels and prostatectomy findings in patients
with 2nd round potentially advanced cancers.

pros tate	**Gleason score Biopsy**	cate-gory	**Gleason score RP[1]**	PSA-velocity (ng/ml per year)	tumor volume (ml)	clinical T-stage	**Patho-logical T-stage**
1	**4+4**	B	**4+5**	0.39	0.55	T2a	**T2c**
2	**3+4**	C	**4+5**	0.38	7.93	T1c	**T3c**
3	**4+4**	A	**3+4**	0.43	0.11	T2a	**T2a**
4	**4+4**	A	**3+4**	0.34	0.58	T1c	**T2a**
5	**4+4**	A	**4+3**	0.28	0.24	T3a	**T2c**
6	**3+4**	C	**3+4**	0.14	1.55	T1c	**T2c**
7	**4+4**	B	**4+4**	0.36	1.46	T1c	**T2c**
8	**3+4**	B	**3+4**	0.46	5.15	T1c	**T2c**
9	**3+4**	C	**4+5**	0.98	6.91	T2a	**T2c**
10	**3+5**	B	**4+5**	0.21	0.29	T2b	**T2c**
11	**4+3**	A	**3+4**	0.46	0.63	T2a	**T2a**
12	**4+4**	A	**3+5**	1.6	2.08	T2c	**T2c**

Downgrading from Gleason score 8 in the biopsy to Gleason score 7 in the radical prostatectomy was seen in 3 patients, and upgrading from Gleason score 7 (3 + 4) in the biopsy to Gleason score 9 in the prostatectomy occurred in 2 patients. Tumor volumes ranged from 0.11-7.93 ml, with a median value of 1.05 ml. In the 7 prostatectomy specimens matching with Gleason score 7 (4+3) or Gleason score 8 (4+4) in the sextant biopsy most tumors were small (<0,6 ml) except two (1.46 ml and 2.07). The latter included a transition zone carcinoma of Gleason score 4 (2+2) of 1.21 ml and a peripheral zone Gleason score 8 cancer of 0.86 ml. Concerning the 4 patients with Gleason score 7 (3+4) cancer and >30% tumor involvement in their sextant biopsy it was noted that their tumor volumes ranged from 1.55-7.93 ml.
Clinical T stage of all prostatectomy specimens under staged the tumor in 8 patients and over-staged the tumor only in 1 patient. Second round PSA values in men who underwent radical prostatectomy specimens ranged from 2.4-11.0 ng/ml, while a significant correlation of PSA with tumor volume was lacking (p=0.53). Median PSA velocity of PAC treated with radical prostatectomy was 0.5 ng/ml per year, which tended to be lower as compared to PAC not treated with radical prostatectomy (2.2 ng/ml per year p=0.06).

Discussion
Our study shows that PAC, defined by Gleason score ≥4+3 or more than 30% tumor involvement with Gleason score 3+4 in the sextant biopsy, occurs infrequently in the second screening round of the Rotterdam section of the ERSPC. It is known that after onset of the PSA era the incidence of well-differentiated cancers (Gleason score 2-4) decreased [118] and that of moderately

differentiated tumors (Gleason score5-7) increased because more prostate cancers were found through biopsy instead of coincidently by transurethral resection of the prostate [118,119]. When early detection programs were introduced a relative decrease of poorly differentiated cancers (Gleason score 8-10) was noted when comparing prostatectomy specimens before the PSA era and those from participants of the first screening round[62]. Recently, the Rotterdam section of the ERSPC reported a significant reduction in extent of tumor involvement of the biopsies and downgrading in the second screening round [84]. The demonstration on a much larger series of 2nd round cancer containing biopsies of a significantly lower frequency of PAC (i.e. 6.0%) when compared with the first round (19.6%) confirms and extends our previous observation. The calculated long lead-time of prostate cancer, which is 10.3 years at age 55-74 [48], is in line with the observed decrease of PAC in the 2nd screening round.

The employed criteria to define PAC were based on data from literature indicating that amount of cancer in the biopsies and extent of high grade Gleason 4/5 are important predictors of disease outcome [112,115]. Others have questioned the potential to predict organ-confined disease at an individual basis on the basis of biopsy pathology variables of prostate cancer [114]. According to a previous study from the ERSPC, biopsy parameters may well be suitable for an estimation of the proportion of advanced cancers in the context of a large population-based study. In a previously developed categorization model based on a limited number of prevalence screening round prostate biopsies and matched prostatectomy specimens, we demonstrated that about 60% of biopsy defined "advanced" cancers had extra-prostatic extension [84]. It is then remarkable that only one of 12 prostatectomy specimens of men with a 2nd round PAC contained a pT3C cancer. This finding would suggest that employment of the same definition for PAC in the 2nd screening round as in the prevalence screening may rather have led to an overestimation of advanced cancers in the 2nd round. Three of four patients with a small focus Gleason score 8 cancer in one biopsy (category A) proved to have a comparatively low tumor volume in the prostatectomy specimen which was also downgraded to Gleason score 7. In contrast, two of three cases with extensive involvement of needle biopsies (category C) by Gleason score 7 cancer were associated with a large volume prostate cancer Gleason score 9 in the prostatectomy. On the other hand, it cannot be excluded that additional cancers with adverse features may be present among those biopsy-detected cancers, which did not fall within our arbitrary definition of PAC. Indeed, it is likely from previous data that additional cancers with adverse features are present among those biopsy-detected cancers, which did not fall within our arbitrary definition of PAC [84]. When we would include Gleason score 6 (3 + 3) cancers with ≥ 30% biopsy involvement in the 2nd round only 6 cases were identified. Radical prostatectomy was performed in 2 of the latter cases and revealed organ confined cancers with Gleason score 6. Summarizing, it may be inferred from the prostatectomy data that a substantial subset of second round cancers,

defined on the basis of findings in the prostate needle biopsy as PAC were organ confined although a comparatively large proportion of them (6 of 12) were composed of poorly differentiated cancer (Gleason score 8 or 9).

Failure to detect prostate cancer during the prevalence screening as a consequence of a missed abnormality in the biopsy, respectively serum PSA determination occurred in 2 patients. Another failure to detect a PAC during the prevalence screening was patient related (refusal of biopsy). The other 27 PAC in the 2nd screening round had not been detected by the regular prevalence screening. The low tumor volume of 6 of the 12 PAC treated by radical prostatectomy (i.e.< 0.7 ml) may well have precluded their detection 4 years before in the first screening round. On the other hand, a selection bias towards cancers with more favorable features, including small tumor volume may have occurred in those men with PAC in the 2nd round offered a prostatectomy as compared to those given radiotherapy.

The efficacy of our current screening protocol may not only be measured from the presence of PAC in the 2nd screening round, but also on the number of clinically manifest interval carcinomas, occurring during the 4-year screening interval. A recent analysis at the Rotterdam section of the ERSPC revealed 25 interval carcinomas (not patient related) occurring between 1993 and 1996 [120]. More importantly, no advanced cancers (Gleason score 4+3) were found among these interval carcinomas.

If rapidly progressive prostate cancers with short doubling times would comprise a substantial proportion of the PAC, this would be another potential problem related with the current screening protocol with a four-year interval. We demonstrated that the median PSA velocity (PSAV) of the patients with PAC was 0.5 ng/ml per year. In literature a PSAV of 0.75 ng/ml year was considered predictive of the presence of clinical prostate cancer when PSA values were between 4.0 and 10.0 ng/ml [121]. Only 30% of the patients with a second round PAC had a PSAV > 0.75 ng/ml. This indicates that PSAV is not a good predictor for PAC in this subset of patients. Of course it cannot be excluded that a number of potentially highly aggressive prostate cancers with low PSA levels are hidden in our screened population. It is uncertain whether a more frequent PSA testing protocol would be of benefit for their early detection.

The incidence of PAC reported here of about 2.0 per thousand participants in the second screening round seems to be reassuring for the efficacy of our current screening protocol, employing a 4-year interval for PSA testing and sextant biopsy procedure for definite diagnosis of prostate cancer.

Conclusions

A screening protocol employing a 4-year interval for PSA testing and sextant biopsy procedure for definite diagnosis of prostate cancer seems warranted, since 1) the mean lead time of prostate cancer is estimated to be 6-12 years for different age groups [48], 2) the frequency of clinically manifest interval cancers reported so far is negligible [120] and 3) a low incidence of potentially advanced

cancers is detected during the 2nd screening round. Furthermore, the performance of the adopted screening procedure seems to be good, since only 2 of 30 2nd round PAC could be attributed to failure of diagnostic tests at the prevalence screening.

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Chapter 5

Incidence and follow-up of focal prostate cancer in two screening rounds after an interval of 4 years

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Abstract

Objective: Focal cancer detected in needle biopsy is a common finding since PSA based screening is introduced. Clinical-pathologic features of patients with focal cancers, treated with radical prostatectomy (RP) or watchful waiting (WW), were analyzed in order to detect clinical predictors for progression during follow-up.

Methods: Patients were selected from the European Randomized Screening study for Prostate Cancer (ERSPC). Focal cancer on sextant biopsy was defined as ≤3.0 mm involvement by cancer in one biopsy core lacking Gleason pattern 4 or 5. PSA doubling time was used in the WW group as a marker of disease progression.

Results: The proportion of focal prostate cancers increased significantly from 16% in the 1st to 29% in the 2nd screening round, 118 and 108 men were treated with RP and WW. Median tumor volume was 0.13 ml. PSA level and prostate volume predicted tumor volume in multivariate regression. A PSA density cut-off of ≤0.1 ng/ml/cm^3 predicted organ-confined tumor (<0.5 ml) in 94% of the patients. Positive surgical margins were predictive for PSA-recurrence. Four patients in the RP group had PSA recurrence in the follow-up. PSA doubling time < 2, 3, or 4 years was noted in 4.9, 14.6 and 22.0% of patients in the WW-group, respectively.

Conclusions: Median tumor volume was small (0.13 ml). A comparison between PSA recurrence in the RP group and PSA doubling time in the WW group showed a significantly more favorable outcome after RP if a PSA doubling time of <3 or 4 years was chosen as a marker for progression of disease in WW. A WW policy with delayed curative intent may be recommended in patients aged between 55-75 years with focal cancer and PSA density <0.1 ng/ml/cm^3.

Introduction

With frequent serum prostate specific antigen (PSA) testing and systematic biopsy of the prostate, the risk to detect clinically irrelevant prostate cancer increases. A high prevalence of co-morbidity coupled with the relatively slow progression of most prostate cancers [122] implies that competing causes of death may be substantial contributors to mortality in men diagnosed with prostate cancer. Therefore screening for prostate cancer introduces over-detection (defined as: cancers that would not have been diagnosed in the absence of screening) and a lot of men might be over-treated. It was recently calculated that in a screening program with a 4-year interval, the proportion of cancers detected unnecessarily could be as high as 48% [48]. Therefore it is important to make efforts to define clinically insignificant tumor on preoperative variables; wherein it is safe to apply watchful waiting (WW) and to avoid over-treatment associated with side effects, like radical prostatectomy (RP) or radiotherapy.

Many authors have tried to establish a definition of an indolent or clinically insignificant tumor in RP specimens. Stamey et al. proposed that tumors <0.5 ml should be considered as clinically insignificant [123]. This was based on the incidence of prostate cancer in unselected cystoprostatectomy specimens, in which 8% of the largest tumors were considered significant, because the cumulative prostate cancer incidence was calculated at 8%, and all others, which were smaller than 0.5 ml, were considered insignificant. Epstein et al. even proposed a cut-off value for clinical insignificant prostate cancer of 0.2 ml, because occasionally a prostate cancer in the 0.2-0.5 ml tumor volume range was non-organ confined [124]. Other authors elaborated on this cut-off of 0.5 ml, because no other proper definition could be established for an insignificant tumor. In an editorial comment 10 years after his initial publication, Stamey et al. disposed the arbitrary cut-off of 0.5 ml and suggested that even larger tumor volumes and presence of high-grade cancer might be within the definition of insignificant tumors as well. Patient age is the most important predictor to decide whether a tumor might be clinically insignificant [125]. Currently, the tumor volume of 0.5 ml, without Gleason pattern 4 or 5 is the most accepted definition for "insignificant" or minimal cancer.

Increasingly, a small focus of well-differentiated cancer is detected in only one biopsy in men with an elevated PSA. The clinical relevance of such a finding is not entirely clear, since this finding may be the manifestation of either a minimal, moderate or even advanced cancer. Further predictive parameters are required to choose between a WW policy and immediate curative treatment. WW might be an option for those men with a high likelihood of "insignificant" or minimal cancer if close monitoring is possible. PSA measurement at follow-up visit is considered to be a useful monitoring instrument for this treatment. It has been shown that a rapid PSA increase (short PSA doubling time which is the time PSA needs to double) correlates with clinical progression [126,127]. In this report we studied retrospectively if we could predict minimal cancer in RP specimens, based on focal cancer at

sextant biopsy and clinical preoperative variables. We also compared the outcome of patients with focal cancer treated with RP or managed with a WW policy.

Material and methods

Patients and screening strategies

In the Rotterdam section of the European Randomized Screening Study for Prostate Cancer (ERSPC) 42,376, 55-75 years old, were randomized in a screening (n=21,210) and a control arm (n=21,166). The 1st round of screening (prevalence screen) took place from June 1994 until December 1999. Screening for prostate cancer was done by PSA determination, digital rectal examination (DRE) and transrectal ultrasonography (TRUS). Systematic sextant prostate needle biopsy was recommended for participants who had either an elevated PSA level (≥4.0 ng/ml), abnormal DRE or abnormal findings on TRUS in the 1st round. Men with benign diagnoses in the 1st round were re-invited for an early re-screen round, wherein 510 men were biopsied. This policy was discontinued later on, in May 1997, because of the low sensitivity and low positive predictive value of DRE and TRUS in the PSA range <4.0 ng/ml [66]. Also the favorable tumor characteristics in the PSA range 3.0-4.0 ng/ml warranted this protocol change [10]. The protocol was simplified and sextant biopsy was recommended if PSA was ≥3.0 ng/ml, regardless of DRE and/or TRUS. All patients' PSA levels at diagnosis were determined with the Beckman-Coulter Hybritech Tandem E Assay (Hybritech Incorporated, San Diego CA,) that was replaced since January 2000 by the automated version (Beckman-Access, Beckman- Coulter Inc. Fullerton CA, USA).

The 2nd screening round, using the PSA ≥3.0 ng/ml screening protocol started in June 1998 and finished in December 2003. Until November 2003, 11887 men were screened. During this 2nd round of the screening program two side studies were performed [128]. One studied the impact of PSA doubling on the detection of (clinically relevant) prostate cancers. If the PSA level increased twice or more since the 1st round, men were invited for sextant biopsy. The other investigated the incidence of prostate cancer in the PSA 2.0-4.0 ng/ml range. All participants in these side studies were included in our analysis.

Systematic lateralized sextant biopsies were obtained during longitudinal and cross-sectional ultrasonographic scanning of the prostate. A 7th biopsy was taken if a hypoechogenic lesion was visible at TRUS. Prostate biopsy cores were labeled and processed individually. One pathologist (T.H.v.d.K) reviewed all biopsies with cancer, PIN and lesions suspicious for malignancy in order to avoid inter-observer variation. During this review, the number and size of biopsies were recorded as well as proportion of tumor involvement, and proportion of each Gleason pattern. Focal cancer was defined as one single core positive for prostate cancer ≤3mm in sextant biopsy, containing no Gleason pattern 4 or 5.

Pathologic processing

Slides from RP specimens were retrieved from the archives of the pathology laboratories of the Erasmus Medical Center and surrounding hospitals of the Rotterdam region. There was one single protocol for total embedding of the prostate in use in all pathology laboratories allowing accurate measurements of tumor volume, grading and staging [129]. In short, after fixation, RP specimens were inked and serially sectioned at 4 mm intervals and totally embedded in paraffin blocks. After review, pathologic stage and Gleason score were determined. Tumor volume was measured by morphometry as described previously [130]. Fifteen men were excluded in this analysis, because RP specimens could not be retrieved, were not totally embedded, or not embedded according to protocol in 7, 4 and 4 men, respectively. For tumor staging of RP specimens the 1992 TNM classification for prostate cancer was used [117]

Categorization of cancers in prostatectomy specimens and follow-up

The combination of preoperative variables, tumor characteristics and outcome of surgery is predictive of the prognosis after RP [131-133]. We therefore created an arbitrary model that was based on the above-mentioned models. Since we included only men with a diagnosis of focal cancer in the needle biopsy we created two categories. 1) Minimal tumors: tumors <0.5 ml, containing no Gleason pattern 4 or 5, organ confined and negative surgical margins. 2) Moderate to advanced tumors: tumors ≥0.5 ml, or tumors containing any amount of Gleason pattern 4 or 5 or extra-capsular extension or positive surgical margins.

Patients in both therapy groups were seen at 3-month intervals within one year after therapy initiation, thereafter biannual controls were performed in the Erasmus medical center and surrounding hospitals. At each visit serum PSA was obtained and DRE performed. PSA doubling time was calculated in watchful waiting (WW) patients when they had at least 3 PSA values in the follow-up. PSA values obtained by other assays (in surrounding regional hospitals) were corrected for known differences with the Hybritech assay using the regression method of Passing and Bablok [134]. Recurrence of disease was determined as PSA ≥0.2 ng/ml, postoperatively in patients who underwent radical prostatectomy (RP). Median duration of follow-up in the RP and WW group was 45 (n=87) and 30 (n=82) months, respectively.

Statistical analysis

Statistical analysis was done with the SPSS software package (SPSS 11.0 Inc., Chicago, IL). P<0.05 was considered significant. Mann-Whitney tests were used for ordinal variables, T-test for linear variables, i.e. PSA and age. Multiple continuous regression was performed via the backward elimination method. The Kaplan-Meier method was used to calculate PSA-free recurrence curves and significant differences between curves were based on the log-rank statistic.

Results

Prostate biopsies during subsequent screening rounds

Prostate biopsies were obtained in two subsequent rounds and one early re-screening round. In the 1st screening round 1014 prostate cancers were diagnosed, including 157 focal cancers (15.5%). In the early re-screen, which was performed one year after the 1st round in men with an initial benign biopsy outcome, 19 (30.1%) of the prostate cancers were focal cancers. In the 2nd round 546 cancers were detected and 159 (29.1%) of them were focal. Thus, a total of 335 focal cancers were detected in this population based screening program. When patients within the same screening protocol (PSA ≥3.0 ng/ml indication for sextant biopsy) in the 1st and 2nd round were compared, the ratio focal cancer/all cancers increased significantly in the 2nd round from 15.5 to 29.1% (p=0.03). Employing the original screening protocol (PSA ≥4.0) without positive DRE or TRUS as indicator for sextant biopsy, the ratio focal cancer/ all cancer in the 1st and 2nd screening round was 12.8% and 32.8% (p=0.0001). The choice of therapy for patients with focal cancer was RP, WW, radiotherapy or hormonal therapy in 118, 108, 93, and 2 patients, respectively. In 14 men the therapy is as yet unknown.

Radical prostatectomy and watchful waiting policy

The characteristics of patients in the RP and WW groups are shown in Table 1.

Table 1 Characteristics of patients who underwent radical prostatectomy and watchful waiting

variable	RP (n=103)	WW (n=108)	p-value
	median (range)	median (range)	
age (years)	62.8 (55-72)	68.6 (57-77)	<0.0001
PSA at diagnosis (ng/ml)	4.4 (0.9-21.0)	3.7 (1.2-24.8)	0.007
PSAd (ng/ml/cm^3 prostate)	0.10 (0.03-0.48)	0.09 (0.03-0.56)	NS
Gleason score	6 (4-6)	6 (2-6)	NS
prostate volume (ml)	41 (17-154)	40 (14-130)	NS
cT-stage	N (percentage)	N (percentage)	
T1C	65 (63.1%)	63 (58.3%)	
T2A	27 (26.3%)	38 (35.2%)	
T2B	2 (1.9%)	0	
T2C	7 (6.8%)	1 (0.9%)	
T3A	2 (1.9%)	0	
unknown	0	6 (5.6%)	

RP radical prostatectomy
WW watchful waiting
NS not significant

Hundred-three RP specimens suitable for tumor volume measurement were included in our analysis. No patient was treated with adjuvant or neo-adjuvant therapy. Hundred-eight patients with focal cancer were managed with a WW policy. Patients managed with WW were significantly older and had lower PSA values. The co-morbidity, as expressed by ASA score, (Association of Anesthetists-score ranging from class1 (Normal healthy patient) towards class 5 (patient dies without intervention [135]) of men on a WW policy remained equal in two subsequent screening rounds and was rarely (3%) score 3 (patient with severe systemic disease) (data not shown) or above. Impalpable disease in the RP and WW-group was diagnosed in 63.1%, respectively 58.3%, of the patients. Clinically two extra-capsular prostate cancers were diagnosed in the RP-group. Three patients in each group had a 7th positive biopsy core, directed at a hypoechogenic area.

Tumor volume in radical prostatectomy specimens

Median tumor volume in focal prostate cancer was 0.13 ml (range 0.00-8.93 ml). Out of these patients, 61.2% had a tumor volume <0.2 ml and 78.6% <0.5 ml.

Confining the analysis to men screened according to the initial screening protocol (PSA ≥4.0 ng/ml), revealed a tumor volume <0.2 ml in 52.4%. Median tumor volumes in the 1st, early re-screen and 2nd screening round (0.16, 0.18 and 0.07 ml) did not differ significantly. Tumor volume per PSA range can be seen in Table 2.

Table 2 Median tumor volume according to PSA ranges

	tumor volume ml (range)		
PSA range (ng/ml)	1st round (n=56)	Interim screening round (n=7)	2nd round (n=40)
0-2.9	0.09 (0.0-0.4)	0.1 (n=1)	0.07 (0.0-0.6)
3-3.9	0.05 (0.0-0.5)	NA[1]	0.17 (0.0-2.2)
4.0-9.9	0.21 (0.0-2.3)	0.94 (0.0-1.0)	0.04 (0.0-1.1)
≥10.0	0.37 (0.0-0.6)	0.06 (n=1)	1.08 (0.0-8.9)
Total	**0.16 (0.0-2.6)**	**0.18 (0.0-1.0)**	**0.07 (0.0-8.9)**

NA not available

Also after analysis of patients submitted to the same screening algorithm (i.e. PSA ≥3.0 ng/ml) the median tumor volume in the 1st and 2nd round did not differ significantly (0.17 vs. 0.08 ml). Comparison in the 2nd screening round of tumors in patients with PSA <3.0 and those with PSA ≥3.0 revealed no significant difference in median tumor volume (0.07 vs. 0.08 ml). Eighteen patients underwent sextant biopsy in an earlier round. Tumor volume of patients who underwent sextant biopsy in an earlier round had somewhat smaller median tumor volumes (0.08 vs. 0.10 ml, not significant). When all preoperative clinical variables (i.e. Gleason score, clinical stage, length of

tumor in sextant biopsy, PSA, prostate volume and age) were multivariately tested with linear regression, only PSA and prostate volume were significant predictors of tumor volume ($p<0.0001$ for both variables). A larger prostate volume predicted a smaller tumor volume. When all postoperative (RP Gleason score, pathologic stage, positive surgical margins) variables were added to the multivariate analysis, prostate volume and PSA continued to be predictive for tumor volume, but also a positive surgical margin and stage pT4 were significant predictors for tumor volume ($p=0.006$ and $p<0.001$). When we combined PSA and prostate volume and used PSA density ≤0.1 ng/ml/cm^3 (n=48) as a cut-off value, 94% of the 48 patients had a tumor volume of <0.5 ml and organ-confined cancer in the RP specimen. In Table 3, PSA density in relation to tumor volume is shown. Both patients who were converted to RP after 9 and 10 months of WW had Gleason score 5 organ-confined cancers (pT2c). One of these patients had a tumor volume of 0.24 ml.

Table 3 Tumor volume according to PSA density ranges

PSA density range (ng/ml/cm^3)	tumor volume ml (range)
≤0.10	0.08 (0.0-2.2) (n=48)
0.11-0.15	0.10 (0.0-2.2) (n=31)
≥0.16	0.45 (0.0-8.9) (n=24)

Pathological findings in prostatectomy specimens

No patients with focal cancer undergoing RP had tumor positive lymph nodes. In 3 men, it was not possible to find the prostate cancer in the RP specimen, even after additional sectioning and exclusion of mix-up of biopsy material by molecular analysis. Positive surgical margins were present in 15 RP specimens, 4 patients did have positive surgical margins in case of tumor volumes <0.5 ml (Gleason score 6). The pathological stages are listed in Table 4.

Table 4 Pathologic stage of focal prostate cancer, including two patients who were initially managed with watchful waiting

pathologic stage	n	percentage (%)
no prostate cancer	3	2.9
pT2a	40	38.1
pT2b	1	1.0
pT2c	56	53.3
pT3a	3	2.9
pT4	2	1.9
Total	105	100

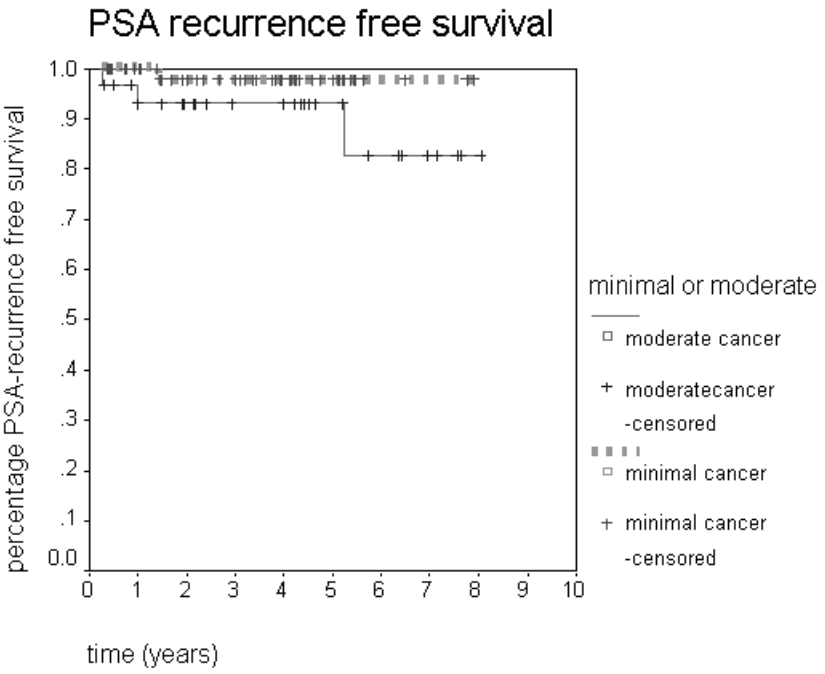
Five patients had extra capsular disease, including 2 with invasion of the bladder neck. One patient with stage pT3a was clinically diagnosed with a cT3a prostate cancer, whereas 3 pT3 patients were clinically diagnosed with cT1c stage. Tumor volume of patients with extra-capsular disease ranged

widely (i.e. from 0.37 to 8.94 ml). Sixteen patients were diagnosed with a Gleason pattern 4 component in their carcinoma (Gleason score 3+4 (n=14), 4+3 (n=1), 4+4 (n=1)) in the RP specimen. In 9 patients Gleason pattern 2 was dominant in the RP specimen. When patients in the RP-group were categorized, 67 (65%) cancers were considered as minimal tumors (<0.5 ml, no Gleason pattern 4 or 5, organ confined and negative surgical margins) and 36 (35%) were considered moderate to advanced tumors (≥0.5 ml, with Gleason pattern 4 or 5, ≥pT3a and or positive surgical margins). The proportion of minimal and moderate to advanced tumors was equal in both rounds. In 6 of 11 patients with a tumor volume >1 ml, more than 50% of the tumor was composed of Gleason pattern 2 carcinoma localized in the transition zone.

Follow-up

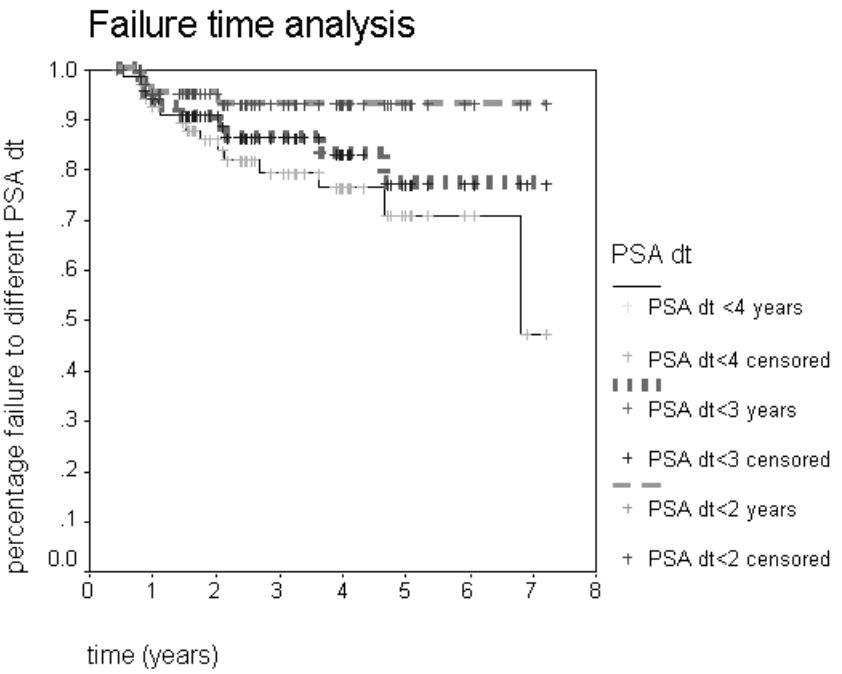
Follow-up of the RP-group was available in 87 men, with a median follow-up of 45 months (range 3-96). Four patients (4.6%) showed PSA recurrence. Despite PSA recurrence, no evidence for local recurrence or (lymph node) metastasis was found. The median time to PSA recurrence was 53 months (4-99 months). PSA levels were stable in 3 patients without adjuvant therapy (0.3, 0.5 and 0.7 ng/ml for 63, 88 and 44 months, respectively). The other patient with PSA recurrence shortly thereafter died of a cancer other than prostate cancer. PSA progression in the RP-group was significantly associated with positive surgical margins (p=0.02) in multivariate analysis. All other variables (i.e. age, Gleason score at sextant biopsy or RP, pathologic stage, PSA, volume, and tumor volume) were not significantly related to PSA progression after RP. The 5-year PSA progression free survival Kaplan Meier curve of minimal and moderate tumors was 98 and 93% (not significant), respectively (Figure 1).

Figure 1 PSA free survival in minimal and moderate tumor in the RP-group



Follow up of patients managed with a WW policy was available in 82 patients, and median follow-up was 30 months (5-86). No patient had a cT-stage increase at follow-up. PSA doubling time can be seen as a marker of progressive disease in the follow-up of WW patients. A PSA doubling time shorter than 2, 3, or 4 years occurred in 4, 12, and 18 patients, respectively. In figure 2 Kaplan Meier curves of "failure" in WW-patients are shown (i.e. using a failure cut-off of 3 different PSA doubling times (<2, 3, or 4 years)). If patients changed therapy they were censored at the time of treatment. The chance of failure to PSA doubling times < 2, 3, and 4 years within 5-year follow-up in WW-patients was 5, 13 and 17%, respectively. Median PSA doubling time was 7.4 years (range: negative (PSA decreased)-63 years).

Figure 2 Different PSA doubling times as marker for progression in watchful waiting patients.



PSA dt PSA doubling time

Negative and PSA doubling time >10 years were seen in 54.3% of the patients. One patient in the WW-group had a Gleason score 8 in his follow-up biopsy associated with a PSA doubling time of 4 months. Follow-up biopsies in other patients were not recorded. Histologically proven lymph node metastasis was seen in one patient, already known with PSA-progression. There was no clinical parameter (PSA, PSA-density, age, Gleason score, age, length of tumor, clinical stage) significantly associated with PSA doubling time <2,3, or 4 years. Four and one deaths have been recorded during the study in the RP and WW group, respectively. However, these patients died of intercurrent diseases and prostate cancer was not a contributing factor.

Conversion from watchful waiting to other therapy

Eighteen patients (21%) changed therapy after a median of 15 months (6-87). The main reason for therapy change was a progressive PSA (PSA doubling time was <4 years in 12 patients). Therapy change to RP was patients wish' in one case and one patient changed to hormonal surgery therapy after lymph node metastasis was diagnosed. In 4 patients reasons for therapy change was unknown. Thirteen, 3 and 2 patients underwent radiotherapy, hormonal therapy, and RP, respectively. These patients showed no disease progression

(defined as: PSA ≥0.2 ng/ml postoperatively or two increasing PSA values after radiotherapy) after therapy change.

Discussion

In this study we show that in a screened population a significant proportion of men was diagnosed with a "focal" prostate cancer in sextant needle biopsies. Its incidence significantly increased to almost 30% of all cancers in the 2nd screening round. This increase could be observed both under the conditions of the initial screening protocol (PSA ≥4.0 ng/ml) and those of the final screening protocol (PSA ≥3.0 ng/ml). This data is similar to findings at the Swedish section of the ERSPC, where the ratio of focal cancer/all cancers increased significantly in subsequent screening rounds at a 2-year interval [114]. In 79% of the men with focal cancer, undergoing RP, a cancer <0.5 ml was found in the RP specimen. Earlier studies demonstrated that the amount of cancer in the needle biopsy couldn't predict accurately the tumor volume in the RP specimen [114,136]. In a similar way, focal cancer in a prostate needle biopsy cannot predict accurately a minimal cancer in the corresponding RP specimen. Allan et al. [137] defined focal cancer as 0.5 mm in one core and no Gleason pattern 4 or 5. They found in a series of 54 patients a minimal cancer in 71% of RP. Furthermore, they reported extra-prostatic extension and positive surgical margins in 3, respectively 5 patients with focal cancer. Apparently, using a more restricted definition of focal cancer (i.e. 0.5 mm instead of 3 mm) does not improve the predictability of minimal cancer organ confined cancer.

Predictive parameters for tumor volume after a diagnosis of focal cancer in our study were PSA level and prostate volume. When PSA and prostate volume were combined as PSA density, using a cut-off value of 0.1 ng/ml/cm^3, 94% of the patients had tumor volumes smaller than 0.5 ml and every tumor was organ confined. After employment of a PSA density cut-off value of 0.15 ng/ml/cm^3, Epstein et al. [124] noted in 86% of patients with focal (stage T1c) cancers a tumor volume <0.5 ml, including two patients with extra-prostatic disease. If exclusively focal T1c carcinomas from our study were considered with PSA density of 0.15 ng/ml/cm^3, only 78% of patients in our study had a tumor volume <0.5 ml and one had extra-prostatic disease. Indeed, addition of clinical staging parameters did not improve the prediction of tumor volume in these focal cancers. Other authors also demonstrated that both PSA-density and PSA level were predictive for tumor volume in case of focal cancers [137,138].

Tumors in RP specimens after a diagnosis of focal cancer tended to be smaller in the 2nd screening round. This is in line with our previous observation that the tumor involvement in needle biopsies during the 1st round was more extensive as compared to those in the 2nd screening round, which would imply that during subsequent screening rounds a downsizing of prostate cancers would occur [84].

Three patients (2.9%) with focal cancer in the 2nd screening round lacked a detectable cancer upon RP (pT0 stage cancer) even after additional

sectioning and exclusion of mix-up of biopsy material by molecular analysis. This considerably higher percentage as compared to the incidence of 0.3% mentioned recently in literature [139] may be attributed to the highly selected group of our patients. Strikingly, also in the latter paper most pT0-stage prostate cancer patients had only one positive needle biopsy core. Obviously, focal cancer increases the risk to encounter a pT0 prostate cancer in a subsequent RP specimen.

Despite the occurrence of 5 deaths in the patients diagnosed with focal cancer, we did not observe disease specific mortality neither in the RP nor WW-group during the 3-year follow-up period. Histologically proven metastasis to the lymph nodes was noted in one patient in the WW-group, but this did not occur in the RP-group. As may be expected, patients with a focal cancer treated by RP rarely showed PSA- progression (4.6%). In this selected group of patients, positive surgical margins were the only variable significantly associated with PSA progression. In the follow-up of focal cancers managed by a WW policy, PSA doubling time is a frequently used variable to monitor prostate cancer. In literature different PSA doubling time cut-offs have been proposed as progression marker in WW patients. McLaren et al. [127] noted clinical progression (defined by increased clinical T-stage) in 80% of patients within 2 years of follow-up with a PSA doubling time of <3 years. Clinical progression was observed in 50% of patients after 2.5 years if PSA doubling time was >3 years. Stephenson et al. [126] reported that clinical progression of WW patients was significantly related to PSA doubling time less than 4 years. On the basis of these studies a cut-off PSA doubling time <4 years may be chosen as the most optimal marker for progressive disease in WW. This somewhat arbitrary cut-off value could be used to compare the frequency of "PSA progression" of patients on WW policy with those treated by RP. Employing PSA doubling time <4 years "PSA progression" was observed significantly more frequently in the WW-group as compared to PSA recurrence in the RP-group: the proportion of patients with a 5-year PSA progression-free interval after RP, respectively the proportion of patients in the WW-group with a PSA doubling time >4 years was 96 and 67% (p=0.0005), respectively. Despite this significant difference a definite conclusion cannot be drawn because no established criteria for "PSA" progression in WW patients are available. If for example, another cut-off value of PSA doubling time <2 years as arbitrarily employed by Choo et al. [140] would be applied, no significant 5-year difference in PSA progression between the RP and WW-group had been observed. Indeed, only 4 patients of our WW series (4.9%) had a PSA doubling time < 2 years. On the other hand, some advocated PSA doubling times even longer than 4 years as a marker for "PSA progression". Stephenson et al.[126] reported that a PSA doubling time <10 years correlated with disease progression on repeat biopsy and DRE, whereas in our screening study the mean PSA doubling time was 6.1 years in men with negative biopsy outcome in the second screening round (average PSA doubling time of 25 years in men who did not undergo biopsy in two

subsequent screening rounds) [141]. No clinical variable available at diagnosis could predict a PSA doubling time <4 years in the follow-up in our study.

In this study, 22% of men on WW policy after a diagnosis of focal cancer changed therapy and 67% of them did so because of PSA progression. Fifteen of the latter patients (83%) were given curative treatment. All converted patients had stable PSA values afterwards. Two patients converted to RP after following a WW policy. They had organ confined disease with Gleason score 5 prostate cancer. Carter et al. [142] saw organ-confined disease in 11 out of 13 patients who underwent RP after a WW policy. It should be noted that these authors defined disease progression as adverse pathological findings in follow-up needle biopsies and their inclusion criteria for watchful waiting were different. The limited proportion of men opting for therapy conversion (i.e. 22%) demonstrates that our follow-up protocol (i.e. biannual PSA testing) for WW is rather acceptable to most men.

A substantial proportion (32.2%) of patients with focal cancer opted for WW. In the entire screened population, the proportion of men opting for a WW policy increased from 10.0% in the 1st round to 24.4% in the 2nd screening round. A possible explanation for this finding might be the significantly older age in the 2nd screening round. The co-morbidity was low in the two subsequent screening rounds and equal in both rounds in our patient population at time of initiation of WW. The increase of focal cancers and lower PSA levels of WW patients compared to men who underwent RP, as shown here, and the less aggressive tumors in the 2nd screening round [85] might also be responsible for the increase in WW-patients.

Conclusions

Patients with focal cancer in their sextant biopsy have a definite, but small risk of PSA progression after RP (4.6%). The positive predictive value of focal cancer for a minimal cancer in the RP was 94% using a PSA density cut-off of ≤0.1 ng/ml/cm^3. PSA progression occurred more frequently in the WW-group (when PSA doubling time of <3 and 4 years was used as a cut-off value for PSA progression) compared to the RP-group. Nevertheless, delayed therapy with curative intent after a WW policy in patients with focal cancer, especially in those patients with low PSA-density levels might be an acceptable option. However, definite criteria for disease progression in WW patients urgently need to be established.

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Part 4

Chapter 6

Tumor features of second screening round detected prostate cancers warrant increased choice for watchful waiting

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Abstract

Objective: To evaluate the features of prostate cancer detected during two subsequent screening rounds in relation to changes in therapy choice.

Methods: Data were retrieved from the European Randomized Study of Screening for Prostate Cancer, section Rotterdam (ERSPC). Men, ages 55-75 years were prostatic specific antigen (PSA) tested at an 4-year interval. A total 1548 sextant biopsies were recorded for Gleason score and tumor extent and 550 radical prostatectomy specimens were evaluated for Gleason score, pathological T-stage and tumor-volume.

Results: Gleason score, involvement of biopsy by tumor, clinical stage and PSA level were more favorable in patients of the second round compared to those of the first round. The number of men choosing for watchful waiting increased from 98 (10%) to 123 (22%) in the first and second round, respectively ($p< 0.0001$). In patients undergoing radical prostatectomy, median tumor-volume in the first and second screening round was 0.65 and 0.45 ml ($p=0.001$). Minimal cancer (cancer <0.5 ml, organ confined, no Gleason pattern 4 or 5) was found in 122 (31.6%) in the first and 60 (42.6%) in the second screening round ($p=0.03$). The 5-year PSA progression free survival after radical prostatectomy was 87%.

Conclusions: In the second screening round, after radical prostatectomy, 42.6% fulfilled the criteria of minimal cancer, reflecting a high level of over-treatment. The 5-year PSA progression free survival in patients with a minimal cancer was high (94%) and suggests that a more conservative approach is warranted in these men. Their proper identification however remains difficult.

Introduction

Prostatic specific antigen (PSA) testing is mainly responsible for the changing incidence in prostate cancer in many countries. Presently, two large randomized clinical trials of screening for prostate cancer are ongoing, one in Europe (European Randomized Study of Screening for Prostate Cancer, ERSPC) and another in the USA (Prostate Lung Colorectal and Ovary cancer, PLCO). The answer with adequate evidence whether screening reduces mortality from these randomized clinical trials will probably not be available before 2008. Until this time the debate on screening for prostate cancer will continue. The goal of the ERSPC is to evaluate whether population based screening reduces mortality from prostate cancer at an acceptable price in terms of quality of life and costs [1]. As a consequence of screening a higher proportion of localized prostate cancer is diagnosed and therefore the opportunity for curative therapy has increased [143]. However, the considerable increase in incidence/mortality ratio in areas where screening is prevalent also led to concerns with regard to the necessity of immediate curative therapy with accompanying severe side effects such as erectile dysfunction or incontinence for urine and faeces. The number of radical prostatectomies and radiotherapy treatments in The Netherlands has increased dramatically over the last decade [144]. Growing awareness of potential over-treatment, that is treatment of men with prostate cancer who may not benefit from therapy, has prompted watchful waiting as an alternative (prior) to immediate curative therapy. Hoedemaeker et al. [84] already reported more favorable tumor characteristics when 25% of men in the second round were screened implying a higher risk of over-treatment. After full completion of the second round of the ERSPC (Rotterdam), clinical staging parameters of detected prostate cancers as well as the histopathological tumor characteristics of sextant biopsies and corresponding radical prostatectomy specimens in two subsequent screening rounds were analyzed and related to therapy choice and follow-up data with regard to PSA failure.

Material and Methods

Patients and screening strategies

In ERSPC, section Rotterdam, 42,376 men, 55-75 years old, were randomized to a screening (n=21,210) and a control arm (n=21,166). Ethical approval of the study was obtained from the Dutch ministry of health (committee on the population screening act, WBO committee nr. 325291). The first screening round (November 1993 - December 1999) was initially done by PSA determination, digital rectal examination (DRE) and transrectal ultrasonography (TRUS). Sextant needle biopsy was recommended for participants who had either an elevated PSA level (≥4.0 ng/ml), abnormal DRE or abnormal findings on TRUS. The protocol was simplified on May 1997, when sextant biopsy was recommended if PSA was ≥3.0 ng/ml, regardless of DRE and/or TRUS findings.

During the second screening round (June 1998 - December 2003), using the PSA ≥3.0 ng/ml protocol, two side studies were performed. One studied

the impact of PSA doubling time on the detection of (clinically relevant) prostate cancer. If PSA doubled and rose above PSA ≥1.0 ng/ml, men were invited for sextant biopsy. The other investigated the incidence of prostate cancer in the PSA 2.0-4.0 ng/ml range and the level of free PSA and human kallikrein 2. All participants in these side studies were included in our analysis.

Prostate biopsy cores were labeled and processed individually. One pathologist (T.H.v.d.K) reviewed all biopsies with cancer, high-grade prostatic intraepithelial neoplasia and lesions suspicious for malignancy in order to avoid inter-observer variability. During this review, the number and size of biopsies were recorded as well as the absolute amount of tumor involvement, and the proportion of each Gleason pattern.

Pathologic processing

Slides from radical prostatectomy specimens were retrieved from the archives of the pathology laboratories of the Erasmus Medical Centre and surrounding hospitals of the Rotterdam region. There was one single protocol for total embedding of the prostate in use in our hospital and in some but not all surrounding pathology laboratories, allowing accurate measurements of tumor-volume, grading, and staging [129]. In short, after fixation, radical prostatectomy specimens were inked and serially sectioned at four mm intervals and totally embedded in paraffin blocks. Two uro pathologists (T.H.v.d.K and G.J.L.H.v.L) determined pathologic stage (tumor, node, metastases (TNM) classification of 1992) [145] and Gleason score [146]. If no prostate cancer could be found, additional sections were made and molecular analysis was performed to exclude mixing up of material. Tumor-volume was measured by morphometry as described previously [130]. Radical prostatectomy in the first and second screening round was performed in 400 and 179 patients respectively. No patients had lymph node metastasis on frozen section material and therefore no radical prostatectomies were abandoned. There was one patient included in this study with a lymph node metastasis on paraffin section, diagnosed as pT4N1M0 stage prostate cancer, and no adjuvant treatment was given. Of 386 (96%) and 164 (92%) patients the radical prostatectomy specimens from the first and second screening round could be retrieved. Tumor-volume was measured in 488 (i.e. 338 from the first and 150 from the second round) radical prostatectomy specimens, which were embedded according to protocol. Gleason score was determined in all, but two radical prostatectomy specimens because of neo-adjuvant endocrine therapy (which falsely induces a higher Gleason score).

Two patients in the first round received neo-adjuvant endocrine therapy for practical reasons (i.e. patients' holiday). No other neo-adjuvant therapies were given. Seven patients received adjuvant radiotherapy within 6 months after radical prostatectomy in the first round, of whom one developed PSA progression.

Categorization of cancers in prostatectomy specimens and follow-up

Tumors were categorized according to a previously reported arbitrary model that was based on tumor characteristics [130], comprising three categories: 1) Minimal tumor: tumor-volume <0.5 ml, containing no Gleason pattern 4 or 5, organ confined, 2) Moderate tumor: tumor-volume ≥0.5 ml, or organ confined tumor containing any amount of Gleason pattern 4 or 5 or tumor with extra-capsular extension without Gleason pattern 4 or 5, and 3) Advanced tumor: Tumors with extra-capsular extension containing Gleason pattern 4 or 5, seminal vesicle invasion or bladder neck invasion.

Biannual controls were performed in the Erasmus Medical Centre or in surrounding hospitals. At each visit serum PSA was determined and DRE performed. Recurrence of disease (PSA failure) was defined as two subsequent PSA levels of ≥0.2 ng/ml, postoperatively. Metastasis was determined by a bone scan.

Statistical analysis

Statistical analysis was done with the SPSS software package (SPSS 11.0 Inc., Chicago, IL). P<0.05 was considered significant. Pearson chi squared tests (two sided) were used for ordinal variables, student's *t* test (Mann Whitney test for non-parametric data) for linear variables. Multinomial regression was used for tumor category. The Kaplan Meier method was used to calculate PSA progression free survival curves in different categories and significant differences between curves were based on the log rank statistic. Patients were censored if follow-up ended or patients died. Univariate and backward multivariate Cox regression analysis was used to create a model for prediction of PSA progression for linear and ordinal variables.

Results

Prostate cancer detection rates and therapy choice

The detection rate of prostate cancer in the first and second screening round was 5.1 and 4.4%, respectively (p=0.0005) (Table 1).

Table 1 Prostate cancer incidence, PSA range, and number of radical prostatectomies performed

Round one PSA (ng/ml)	Number of men screened (n)	Number of biopsies (n)	Cancer (n)	Prostate cancer detection rate (%)	RP performed n (%)
<3.0	15852	918	79	0.5	41
3.0-3.9	1426	791	179	12.6	80
4.0-9.9	2235	2005	526	23.5	229
>10.0	457	403	230	50.3	51
total	19970	4117	1014	5.1	401
round two					
<3.0	10026	693	109	1.1	34
3.0-3.9	949	830	174	18.3	50
4.0-9.9	1362	1215	235	17.3	84
≥10.0	183	166	32	17.5	11
total	12520	2904	550	4.4	179

PSA Prostatic Specific Antigen
RP Radical Prostatectomy

When the analysis was limited to men screened at a cut-off level of PSA ≥3.0 ng/ml, the prostate cancer detection rate in the first and second screening round was 5.0 and 3.0%, respectively ($p<0.0001$). In the first screening round the prostate cancer detection rate increases with higher PSA ranges, which is not the case in the second screening round (Table 1). The proportion of men who underwent radical prostatectomy was 39.4 and 32.5% in the first and second round, respectively ($p=0.002$) (Table 2). The decrease in performed radical prostatectomies in the second round was most prominent in the PSA range ≥4.0 ng/ml. The proportion of men who underwent radiotherapy decreased drastically in the second round (48.5 vs. 35.6%). On the opposite, the proportion of men who were managed by watchful waiting more than doubled from 9.7% in the first to 22.4% in the second round.

Tumor characteristics in two screening rounds

Clinical characteristics are given in Table 2. In all cancers detected, clinically extra-prostatic disease (clinically (c)T3-T4) was significantly less frequent in the second round and the proportion of impalpable (cT1C) cancers increased significantly in the second screening round. Parallel with the clinical stage migration a grade migration was noted as manifested by the reduction of sextant biopsies with Gleason score ≥7 cancers from 35.4% in the first round to 20.7% in the second round ($p<0.0001$). The percentage of patients with a Gleason score ≥7 cancer who underwent radical prostatectomy was 26.3 and 27.7% in the first and second screening round, respectively. In round one, patients who underwent radiotherapy had the worst pre-treatment tumor characteristics, next to patients treated with endocrine therapy. In round two, however, pre-treatment tumor characteristics (Gleason score, cT-stage and

PSA) of patients undergoing radiotherapy were similar to those undergoing radical prostatectomy. The overall percentage of tumor involvement in sextant biopsies was significantly lower in the second round (20.7% vs. 11.1, $p<0.0001$), a phenomenon that also held true for patients who underwent radical prostatectomy in the second screening round (data not shown).

Notably, in men who had a negative biopsy outcome in the first round but a positive outcome in the second round the percentage of tumor involvement in sextant biopsy was smaller as compared to men who were not biopsied before (5.1 vs. 10.4% $p=0.001$).

Table 2 Characteristics of patients in different therapy groups

1st round	all men	RP	WW	RT	ET
biopsy Gleason category n(%)					
<7	647 (63.8)	291 (72.8)	86 (87.8)	265 (53.9)	5 (21.7)
7	278 (27.4)	87 (21.8)	8 (8.2)	170 (34.6)	12 (52.2)
>7	82 (8.1)	18 (4.5)	4 (4.1)	54 (11.0)	6 (21.7)
unknown	7 (0.7)	4 (1.0)	0	3 (0.6)	0
cT-stage n (%)					
T1c	356 (35.3)	167 (41.8)	56 (57.1)	128 (26.0)	5 (21.7)
T2	467 (45.6)	199 (49.8)	34 (34.7)	231 (47.0)	3 (13.0)
T3-4	190 (18.5)	34 (8.5)	8 (8.1)	133 (27.0)	15 (65.2)
age	66.1	63.5	69.4	68.8	69.1
PSA	5.7	5.3	4.4	6.5	41.0
total n (%)	1014	400 (39.4)	98 (9.7)	492 (48.5)	23 (2.3)
unknown therapy 1					

2nd round	all men	RP	WW	RT	ET
biopsy Gleason category n(%)					
<7	436 (79.3)	130 (72.6)	115 (93.5)	149 (76.0)	4 (50.0)
7	96 (17.5)	42 (23.7)	7 (5.7)	39 (19.9)	3 (37.5)
>7	18 (3.2)	7 (4.0)	1 (0.8)	8 (4.1)	1 (12.5)
cT-stage[5] n(%)					
T1c	339 (61.6)	109 (60.9)	95 (77.2)	110 (56.1)	1 (25.0)
T2	191 (34.7)	64 (36.2)	26 (21.1)	77 (39.3)	7 (87.5)
T3-4	20 (0.7)	6 (3.4)	2 (1.6)	9 (4.6)	0
age (median)	67.1	64.9	69.8	68.7	67.8
PSA (ng/ml)	3.9	4.1	3.4	4.1	6.4
total n (%)	550	179 (32.5)	123 (22.4)	196 (35.6)	8 (1.5)
unknown therapy 44					

RP Radical Prostatectomy
WW Watchful Waiting
RT Radiotherapy
ET Endocrine Therapy
cT-stage Clinical T-stage

PSA Prostatic Specific AntigenTumor features in radical prostatectomy specimens
Details are shown in Table 3.

Table 3 Characteristics of radical prostatectomy specimens

	1st round	2nd round	p-value
pT-stage[1]			p=0.07
pT0	1 (0.3)	3 (1.8)	
pT2a	73 (28.9)	41 (25.0)	
pT2b	9 (2.3)	5 (3.0)	
pT2c	211 (54.7)	86 (52.3)	
pT3a	62 (16.1)	20 (12.2)	
pT3b	4 (1.0)	2 (1.2)	
pT3c	8 (2.1)	6 (3.7)	
pT4	17 (4.4)	1 (0.6)	
total	386 (100)	164 (100)	
Gleason score			p=0.11
<7	241 (62.4)	103 (62.8)	
=7	130 (33.7)	49 (29.9)	
>7	13 (3.4)	12 (7.3)	
unknown	2 (0.5)	na	
tumor category			p=0.03
minimal	122 (31.6)	70 (42.6)	
moderate	193 (50.0)	73 (44.5)	
advanced	71 (18.4)	21 (12.8)	
positive surgical resection margins positive	98 (25.6)	36 (22.0)	p=0.39

pT-stage Pathological T-stage

Four radical prostatectomy specimens showed no prostate cancer (pT0). The proportion of radical prostatectomy specimens with extra-prostatic disease decreased from 23.6% in the first to 17.7% in the second round. The proportion of men with organ confined prostate cancer (≤pT2 stage) with positive resection margins was 18 and 15% in the first and second round, respectively. The proportion of radical prostatectomy specimens with Gleason score ≥7 in the first and second round was 37.0 and 37.2% respectively. Of the minimal tumors 9.7% had a positive surgical margin. The proportion of advanced tumors decreased in the second screening round.

Tumor-volume, proportion of minimal tumor in relation to PSA range is shown in Table 4.

Table 4 PSA at diagnosis related to tumor-volume and percentage minimal tumor

PSA range (ng/ml)	median, mean tumor-volume in ml (range) 1st screening round n=342	% minimal tumor	median, mean tumor-volume in ml (range) 2nd screening round n=154	% minimal tumor
<3.0	0.28, 0.32 (0.00-1.09)	67	0.28, 0.38 (0.00-1.80)	56
3.0-3.9	0.58, 0.72 (0.00-3.10)	45	0.43, 0.63 (0.00-2.17)	31
4.0-9.9	0.77, 1.08 (0.00-13.48)	27	0.63, 1.06 (0.01-7.93)	46
≥10.0	1.82, 2.16 (0.00-7.99)	13	1.33, 2.04 (0.00-8.94)	36
total	0.65, 1.06 (0.00-13.48)	33	0.45, 0.86 (0.00-8.94)	43

PSA Prostatic Specific Antigen

Tumors in the second screening round were significantly smaller compared to the first round (p=0.001) with a significant relationship between PSA level at diagnosis and tumor-volume in the first and second screening round ($p<0.0001$ for both rounds, and $R^2 = 0.15$ and 0.12). In the first round the proportion of minimal tumors decreased with increasing PSA range, but not in the second round. A weak correlation was found between increasing age and larger tumor-volumes ($R^2 =0.03$).

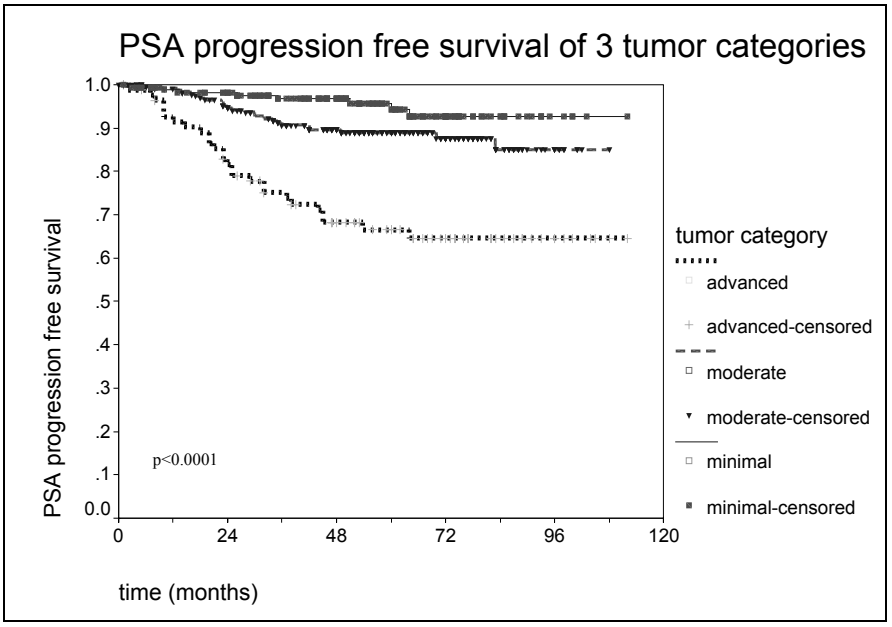
Multinomial regression (pseudo $R^2 = 0.33$) demonstrated PSA range ($p<0.0001$), percentage tumor involvement in sextant biopsy category ($p<0.0001$), and biopsy Gleason category ($p<0.0001$) as preoperative predictors for tumor category (minimal, moderate, and advanced tumors) in the first screening round. Age was univariately associated with tumor category in both rounds. In the second round PSA range was not predictive for tumor category anymore (p=0.11).

Follow-up after radical prostatectomy

The follow-up was known in 383 and 127 patients and median (range) follow-up was 67 (0-112) and 31 (0-67) months in the first and second screening round. PSA progression occurred in 60 patients (10.9%) with a mean time to progression of 52 months (95% CI 50-54 months). Metastases occurred in five patients (four with advanced and one with a moderate tumor) after a median follow-up of 44 months. Univariate Cox regression analysis revealed positive resection margins, pT-stage, age, tumor-volume, radical prostatectomy and biopsy Gleason score, percentage tumor involvement (all $p<0.0001$), number of cancer positive cores (p=0.001), and PSA (p=0.007) as predictors for PSA progression. Clinical T-stage and prostate volume were not predictive in univariate analysis. In multivariate Cox regression, positive resection margins (p=0.001), age (p=0.001), biopsy Gleason score (p=0.001), and pT-stage (p=0.037) significantly predicted PSA progression after radical prostatectomy.

The 5-year PSA progression free survival rates for minimal, moderate, and advanced tumor was 94, 89, and 67% (log rank p<0.0001), respectively (Figure 1).

Figure 1

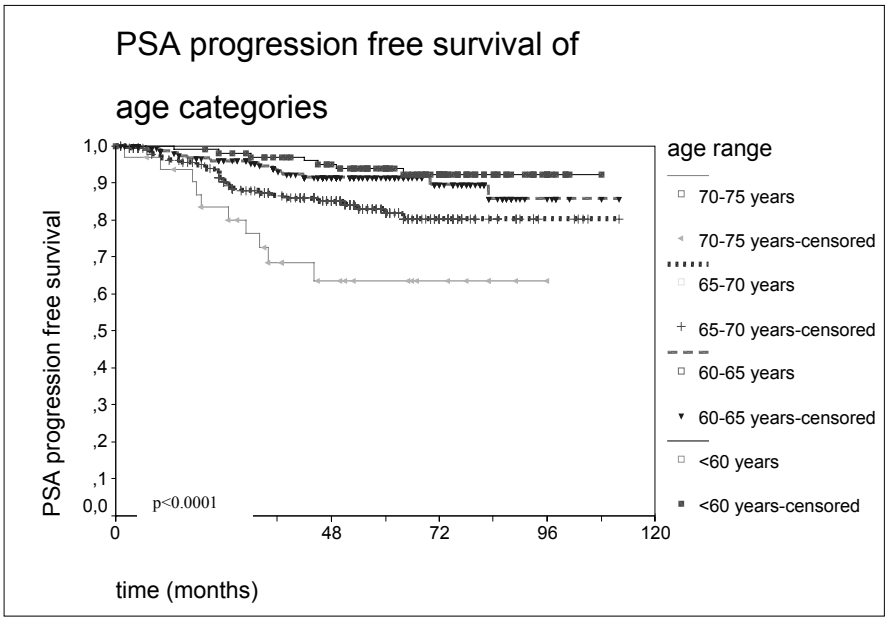


Number of patients at risk						
Minimal	174	152	101	39	4	-
Moderate	244	204	149	66	10	-
Advanced	85	65	45	26	8	-

Of the minimal tumors 10% had a positive resection margin, which was significantly related to PSA progression (p=0.04). Kaplan Meier curves of different age categories and PSA progression free survival is shown in Figure 2.

The category minimal tumor includes tumors <0.5 ml without Gleason pattern 4. Considering a broader definition of minimal cancer, including Gleason patterns ≥4 (n=55), 19% showed positive surgical margins and 9% showed extra-prostatic disease, indicating more aggressive disease. In stage pT3 tumors the 5-year PSA progression free survival was higher in negative surgical margins compared to positive resection margins (93 vs. 75%, log rank p=0.0003).

Figure 2



Number of patients at risk						
<60	165	100	85	49	10	-
60-65	171	142	97	42	7	-
65-70	195	155	101	34	5	-
70-75	31	24	13	6	-	-

Discussion

The observation of more favorable characteristics of cancers detected on biopsies during the second screening round as reported earlier [85] is now further confirmed after comparison of tumor features in radical prostatectomy specimens of first and second screening round patients. Although down-staging of prostate cancers in radical prostatectomy specimens of the second round compared to the first round did not reach significance, both a significant shift to lower tumor category (i.e. increase of minimal cancers) and decreased volume of second round cancers was noted. This category improvement of prostate cancers in radical prostatectomy specimens in the second screening round occurred in spite of the marginal improvement of clinical and biopsy characteristics in the subset of patients undergoing radical prostatectomy as compared to the considerable improvement in pre-treatment variables of all prostate cancers detected in the second round. Thus, during subsequent screening rounds the decrease in volume of cancer already observed in needle biopsies [84], continued.

In the ERSPC, the over-diagnosis was calculated at 48% as a consequence of an estimated lead time of eleven years [48]. If a minimal cancer

is considered as a cancer of which the diagnosis is not of benefit to the patient, over-diagnosis in our screened population would occur in at least three to four out of ten patients, their radical prostatectomy may have been an over-treatment or the treatment has been carried out too early in the development of cancer. This can as yet not be proven. However, we know that the cancer specific survival of untreated highly differentiated prostate cancer is 89 and 72% after 15 and 20 years, respectively [19]. Generally it is felt that prediction of a minimal cancer is not reliably possible at the individual level [114], although Gleason score in the biopsy and percentage of tumor involvement in sextant biopsies by tumor can predict tumor category at a statistically significant level. Importantly, we noted that the PSA range did not predict tumor category anymore during the second round, which can be explained by that larger tumors are detected in an initial screening round, and in a subsequent round tumors are smaller and four years is too short for remaining cancers to re-grow to the same volume and the PSA driven biopsy indication is dominated by non-cancer disorders. Which further supports the notion arising in countries with an intense PSA screening (i.e. USA) that repeat PSA testing is less sensitive for tumor detection [141,147].

In a previous study of focal cancer (≤3 mm of cancer in one biopsy core, without Gleason pattern 4 or 5) detected in participants of the ERSPC, we demonstrated that 66% (n= 74) of men with a focal cancer had a minimal cancer in the radical prostatectomy specimen [15]. In men with a PSA density of ≤0.1 ng/ml per ml prostate volume 94% of the cancers detected in their radical prostatectomy specimen was a minimal cancer[148]. Thus, a combination of focal cancer and PSA density of ≤0.1 ng/ml per ml prostate volume could be used as an indicator of minimal cancer, and in these men watchful waiting could be safely recommended. However, for young men with a potential minimal cancer immediate curative treatment might be preferred in order to avoid the psychological burden of repeated PSA tests and biopsies.

Only a small fraction of men detected with prostate cancer during screening fulfils the combined criterion of focal cancer and low PSA density (7.9 and 18.2% in first and second round respectively). In the current study 35% (n=196) of all tumors detected in radical prostatectomy specimens of first and second round were minimal cancers, and only 70 of them (36%) had a pre-treatment biopsy diagnosis of focal cancer. the majority of minimal cancers thus had been diagnosed with a more extensive cancer than "focal cancer" at biopsy. If the definition was widened to ≤6 mm of tumor in three-biopsy cores still 58% of the tumors were categorized as minimal.

Apart from well know parameters for PSA progression (pT-stage, Gleason score and positive resection margins) age was an independent predictor for PSA progression after radical prostatectomy (Figure 2). For this striking observation we have no explanation. Although older patients have larger tumors and higher PSA levels at diagnosis, if age was excluded in the multivariate Cox regression analysis, no other than the above-mentioned variables were significantly associated with PSA failure. Freedland et al. [149] recently published the same remarkable finding on age related PSA failure

after radical prostatectomy, for which they also could not offer a proper explanation. It remains to be seen whether in these older men, with a higher PSA recurrence rate, the prostate cancer specific mortality will be higher as compared to men whose prostate cancer is detected and treated at a younger age, since other competing causes of death in the older age group may lead to increasing overall mortality. The proportion of men undergoing radical prostatectomy was significantly lower in the second round. The smaller proportion of radical prostatectomy and radiotherapy performed in the second round was due to the more frequent choice for watchful waiting. The increasing choice for watchful waiting treatment (i.e. 22% during the second round) might be encouraged by the more favorable tumor characteristics (lower Gleason score, cT-stage and PSA level) in the second round. Other factors that stimulated the choice of watchful waiting are growing awareness of over-diagnosis by urologists and the aging (although without increasing co-morbidity[150]) of the screened cohort. It remains to be seen how often men choosing for watchful waiting will revert to curative therapy in the course of time. As yet this is rather limited in the ERSPC Rotterdam as only 16% of patients who initially opted for watchful waiting changed therapy (83% with curative intent) [150].

Despite the focus on over-diagnosis in this study, it should be realized that in the second screening round 13% of patients have an advanced tumor (pT3-T4 with Gleason pattern 4 or 5) in their radical prostatectomy specimen. This could among other things indicate that the screening interval of four years employed in this study is too long. In another study with a 6-month screening interval [151], using PSA ≥4.0 ng/ml as a screening tool, 77%, respectively 70% of men with a diagnosis of cancer in the initial, respectively subsequent screening round were treated with radical prostatectomy, but the proportion of advanced cancers (pT3-T4 and pT2 with positive surgical resection margins) decreased only marginally from 33% at the initial to 27% at the subsequent screening round. The proportion of poorly differentiated cancers (Gleason score >8) in radical prostatectomy specimens in the latter study was 11 and 6% at initial and subsequent screening round, respectively. This outcome suggests that even screening at much shorter intervals does not necessarily lead to substantially less advanced cancers. Probably other factors, like biopsy compliance, number and (lateralized) location of needle biopsies also strongly influence the efficacy of screening.

In the current study, the 5-year PSA progression free survival of minimal cancer (after radical prostatectomy) was 94%. We consider failure of surgery (positive resection margins) for the most part responsible for the PSA failures, because of the significant difference in PSA progression free survival in minimal cancer with or without positive resection margins ($p=0.04$). The overall 5-year PSA progression free survival was 87%, which was high compared to other studies, reporting 5-year PSA progression free survival of 80 and 78% [152,153].

Conclusions

After completion of two screening rounds at the Rotterdam section of the ERSPC it is apparent that tumor characteristics are more favorable in the second compared to the first screening round. The number of men choosing for radical prostatectomy or radiotherapy in the second round decreased at the benefit of watchful waiting. Within the current screening protocol about one third of patients treated for prostate cancer with radical prostatectomy had a minimal cancer, with very favourable follow up results. It is questionable whether these patients should have received immediate curative therapy. Observation is likely to reduce over-treatment, especially in older men. Considering predictors for tumor category, urologists might recommend watchful waiting for men with a Gleason score 6 or lower prostate cancer and low tumor involvement, specifically in men with a low PSA density.

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Chapter 7

Tumour features in the control and screening arm of a randomized trial of prostate cancer

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In press European Urology

Abstract

Objective: To compare tumor characteristics at the time of diagnosis of cancers detected in the screening and control arm at the Rotterdam section of the European Randomized study of Screening for Prostate Cancer (ERSPC).

Methods: Data were retrieved from the Rotterdam section of the ERSPC. Men were randomized to the screening arm (n=21,210) or the control arm (n=21,166). Men randomized to screening were offered PSA testing every 4 years. Through linkage with the cancer registry, men randomized to the control arm were detected. The biopsy Gleason score was determined in 1,591 and 373 patients in the screening and control arm, respectively. TURP, radical prostatectomy (RP) and cystoprostatectomy were evaluated for Gleason score, pathological (p)T stage and tumor volume.

Results: More prostate cancers were detected in the screen arm (15.9 vs. 4.2 per 1000 man years, $p<0.0001$). Clinical (c)T stage, biopsy and RP Gleason score distribution were significantly less favorable in the control arm. Proportion of men with advanced disease (i.e. T4/N1/M1), but not their incidence in man years absolute number was significantly ($p< 0.0001$) higher in the control arm (11.0%; 4.6 per 100,000) as compared to the screening arm (3.8%; 6.0 per 100,000). Incidental prostate cancers accounted for 9.3% of all cancers detected in the control arm. The 5-year PSA progression free survival after RP was 68% in the control arm and 89% in the screening arm ($p<0.0001$).

Conclusions: Prognostic features of patients with prostate cancer detected in the control arm are less favorable as compared to those detected in the screening arm. However, in both arms equal number of men present with advanced prostate cancer.

Introduction

The changing incidence in prostate cancer in the Western world is mainly due to PSA driven testing and the increase in life expectancy. PSA screening is thought to be a powerful tool to detect prostate cancer in an early stage, while curable (1). The primary goal of the European Randomized study of Screening for Prostate Cancer (ERSPC) is to evaluate whether population based screening reduces mortality from prostate cancer at an acceptable price in terms of quality of life and costs(2, 3). It is expected that at the end of 2009 conclusive data on the influence of screening on prostate cancer mortality by comparison of both trial arms of the ERSPC will be available.

Comparison of histopathological features of prostatectomies performed on men of the screening arm of the ERSPC with those of a historical control group (4) of the same hospital demonstrated a decreased frequency of lymph node positive disease and a relative increase in proportion of Gleason score 8-10 cancers. However, a comparison of pathological features of all cancers detected in the screening and control arm of the Rotterdam section of the ERSPC was not reported previously.

Incidentally identified prostate cancer can be detected in approximately 10% of trans-urethral resection of the prostate (TURP) specimens. Tumors classified as pT1a diagnosed at TURP are considered as clinically indolent and with low biological potential with favorable follow-up and therefore they are usually managed by watchful waiting (5). Detection of a substantial proportion of pT1a prostate cancers in the control arm may increase the prostate cancer specific survival in the control arm. Incidental prostate cancer may be detected in about 40% of cystoprostatectomy specimens obtained from men treated for a bladder cancer. These prostate cancers mostly have prognostically favourable features (6).

The present analysis of histopathological features of cancers detected within the control and trial arm of the Rotterdam section of the ERSPC was performed to demonstrate whether population based screening for prostate cancer would lead to the increased detection of prostate cancers with favourable characteristics. In addition, this kind of analysis could reveal the proportion of clinically silent advanced cancers in the population.

Material and methods

Patients and screening strategies

In the Rotterdam section of the European Randomized Screening Study for Prostate Cancer (ERSPC) 42,376 men, 55-75 years old, were randomized to a screening (n=21,210) and a control arm (n=21,166). The follow-up of prostate cancer detection in the control arm was complete until the 1st of July 2003. Information on prostate cancer of men in the control arm was obtained through a record linkage with the Dutch cancer registry. Prostate cancer incidence was calculated per 1000 man-years. Man-years were calculated as the interval between dates of randomization to the cut-off date of 1st of July-2003. The cut-off date was overruled if death, prostate cancer diagnosis or the age of 75 occurred before the cut-off date. In the screening arm, 36 prostate

cancers were diagnosed after the age of 75 years (outside screening protocol) and 108 cases were detected within the 4-year screening intervals (interval cancers). In the control arm 107 men were older than 75 years of age at prostate cancer diagnosis. Patients detected as interval cancers and patients diagnosed with cancer older than 75 years of age (in both arms) were excluded in this study. A manuscript describing the features of interval cancers is in preparation.

The details of the screening algorithm of the screened population have been described elsewhere(7). In short, initially men were offered a PSA test, digital rectal examination (DRE) and transrectal ultrasonography (TRUS). Sextant needle biopsy was recommended for participants who had either an elevated PSA level (≥ 4.0 ng/ml), abnormal DRE or abnormal findings on TRUS. The protocol was simplified on May 1997, when sextant biopsy was recommended if PSA was ≥ 3.0 ng/ml, regardless of DRE and/or TRUS findings.

Patients of the screening arm were selected from the 1st, interim (performed one year after the 1st round in men with benign 1st round biopsy outcome), 2nd and 3rd screening round. Both the 2nd and 3rd rounds were not completed at the cut-off date. All diagnoses were based on histological examination. Slides from TURP and radical prostatectomy specimens, Millin prostatectomies, cystoprostatectomies, and prostate biopsies of the ERSPC participants were retrieved from the archives of the pathology laboratories of the Erasmus Medical Center and surrounding hospitals of the Rotterdam region. One single protocol for total embedding of the prostate was in use in all pathology laboratories allowing accurate measurements of tumor volume, grading and staging (8). In case of full compliance with the protocol, the tumor volume was measured. In short, radical prostatectomy specimens were inked and serially sectioned at 4 mm intervals and totally embedded in paraffin blocks. After review, pathologic stage (TNM 1992 classification)(9) and Gleason score(10) were determined by two uro-pathologists (T.H.v/d K. and G.J.L.H.v L.). Tumor volume was measured by morphometry as described previously(9). Tumor volume could be determined in 44 and 470 radical prostatectomy specimens of the control and screening arm, respectively. Ten radical prostatectomy specimens and 4 TURP specimens of patients in the control arm could not be retrieved. Five men initially diagnosed with prostate cancer in the control arm did not have cancer after review. Even after immunohistochemistry was performed on these slides, prostate cancer could not be diagnosed. High-grade prostatic intra-epithelial neoplasia (PIN) and a lesion suspicious for prostate cancer was diagnosed in two and one man, respectively. These cases were excluded from analysis. Treatment decisions were not under the control of the ERSPC trial.

Statistical analysis was done with the SPSS software package (SPSS 11.0 Inc., Chicago, IL). $P<0.05$ was considered significant. Student T-test was used for linear variables, i.e. PSA and age. The Mann-Whitney U test was used for non-parametric data (tumor volume). The Kruskall-Wallis test was used for ordinal data. The Kaplan-Meier method was used to calculate PSA-

progression free survival curves and significant differences between curves were based on the log-rank statistic.

Results

Prostate cancer incidence

Until July 2003, a total of 1,596 and 464 of prostate cancers were diagnosed, which corresponded to a cumulative incidence of 7.5% and 2.2% in the screening and control arm, respectively. In man-years, the incidence in the screen and the control arm was 15.9 and 4.2 per 1000 men years, respectively ($p<0.0001$).

Pretreatment tumor characteristics

Patients in the control arm had significantly higher PSA levels at prostate cancer diagnosis and a lower proportion of clinical T1c prostate cancer, compared to the screening arm (Table 1).

Table 1 Patient, pre-treatment and tumor characteristics

	Screening arm n (%) n=1596	Control arm n (%) n=464	p-value
Age (years)	66.5	67.9	p<0.0001
PSA (ng/ml) mean, median (range)	8.6, 4.9 (0.3-315.7)	57.2, 11.0 (0.3-1500.0)	p<0.0001
clinical T stage n (%)			
T1c	685 (42.9)	117 (25.2)	
T2	530 (33.2)	112 (24.1)	p<0.0001
T3	196 (12.3)	71 (15.3)	
T4/N1/M1	36 (2.3)	47(10.1)	
Unknown/ other (TURP detected)	149 (9.3)	88/29 (19.0)/(6.2)	
Biopsy Gleason score * n (%)			
<7	1111 (69.6)	153 (41.0)	
=7	378 (23.7)	126 (33.8)	p<0.0001
>7	102 (6.4)	93 (5.2)	
Total	1591 (100)	373 (100)	
Unknown	5	45	

*Does not include TURP and cystoprostatectomy in the control arm (n=54)

The distribution of Gleason scores of sextant biopsies in the control arm showed a significantly higher proportion of Gleason score >7 cancer compared to the screening arm. The absolute number of (clinically) advanced disease (T4N0M0/TXN1M0/TXN0M1) was higher in the control arm (47 vs 36).

Therapy and pathological tumor characteristics

Table 2 lists the therapy choices of patients of the screening and control arm.

Table 2 Therapy choices of patients from the screening and control arm

	Screening arm n (%)	Control arm n (%)	p-value
radical prostatectomy	595 (37.3)	84 (18.1)	P<0.0001
Radiotherapy	713* (44.7)	171* (36.9)	
watchful waiting	231 (14.5)	63 (13.6)	
endocrine therapy	33 (2.1)	71 (15.3)	
Other	1 (0.1)	2 (0.4)	
Unknown	23 (1.4)	73 (15.7)	
Total	1596 (100)	464 (100)	

*one patient in both screening and control arm received palliative treatment.

Curative therapy (i.e. radiotherapy and radical prostatectomy) was offered to 81.9 and 54.7% of patients in the screening and control arm, respectively. Radical prostatectomy was performed in 37.3 and 18.1% of the patients in the screeing and control arm, respectively. Radical prostatectomy was abandoned because frozen sections on lymph nodes were positive for prostate cancer in 3 and 7 patients in the screening and control arm, respectively. Apart from advanced disease diagnosed by clinical examination (36 in the screening arm and 47 in the control arm), an additional 24 and 4 patients in the screening and control arm were diagnosed pathologically with stage T4 disease (invasion of the bladder wall), lymph node-or distant metastases. The total number of patients with advanced disease was 60 in the screening and 51 in the control arm (6..0 vs. 4.6 per 100,000 man years). The proportion of advanced disease (pathological (pT)stage T4/N1) in the radical prostatectomies was only slightly higher in the control arm (4.9 vs. 4.2%).

In radical prostatectomy specimens of the control arm 53.5% of the cancers were Gleason score ≥7, a significantly higher proportion as compared to the 34.6% of cancers in the screening arm (Table 3).

Table 3 Tumor characteristics of radical prostatectomy specimens

radical prostatectomy Gleason score n (%)	Screening arm n (%) n=595	Control arm n (%) n=84	p-value
<7	355 (46.5)	37 (45.7)	p=0.001
=7	182 (32.4)	34 (42.0)	
>7	24 (4.3)	10 (12.3)	
Total	561 (100)	81 (100)	
unknown	34	3	
Pathological tumor stage n (%)			
pT2#	438 (77.4)	53 (65.4)	p=0.07
pT3a/pT3b	93 (16.4)	19 (23.5)	
pT3c	11 (1.9)	5 (6.8)	
pT4/N1	24 (4.2)	4 (4.9)	
unknown	29	3	

Median tumor volumes determined in 40 radical prostatectomy specimens in the control arm were significantly larger (3.9 ml) compared to those found in prostatectomies of the screening arm (1.0 ml) ($p<0.0001$). Of 27.0% of the prostatectomy specimens of men of the screening arm the prostate cancers were stage pT2, Gleason score 6 with a volume < 0.5 ml as compared to 11.4% of those of the control arm ($p < 0.01$).
TURP specimens with pT1a cancer and the cystoprostatectomy specimens with cancer (except one) were Gleason score 6 or lower. The cancers found in the cystoprostatectomy specimens as well as the prostatectomies performed according to Millen for BPH were organ confined.

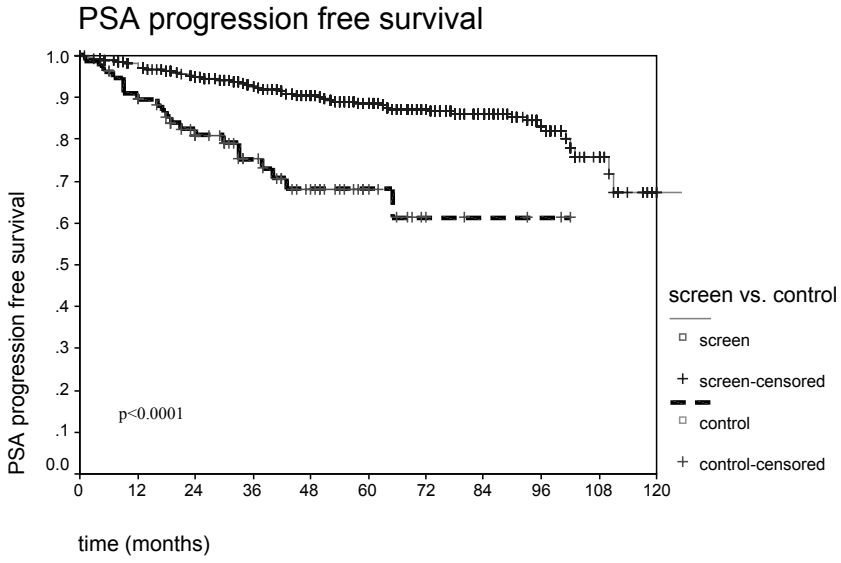
Follow up
Follow up data in the screening and control arm were known for 97% and 93% of patients who underwent radical prostatectomy. Median follow-up after radical prostatectomy or prostate cancer diagnosis in case of TURP or cystoprostatectomy in the screening and control arm was 61 and 42 months, respectively (range 0-126). PSA progression occurred in 71 resp. 21 (12 and 25%) and distant metastasis in 3 resp. 6 (3.8% and 1.1%) of patients in the screening and control arm after radical prostatectomy, respectively. The 5-year PSA progression free survival was 89 and 68% in the screening and control arm, respectively (log rank $p<0.0001$). However, a log rank test from randomization date to PSA progression showed no statistically significant difference ($p=0.13$). Three patients with an incidental diagnosis of prostate cancer (1 pT1a, and 2 pT1b), developed metastatic disease. One of 8 patients who underwent a cystoprostatectomy developed PSA progression.

Discussion

The significantly higher prostate cancer detection rate in the screening arm (15.9 per 1000 man years) as compared to the control arm (4.2 per 1000 men years) is associated with a shift in clinical stage and Gleason score distribution of detected cancers. Our data of the diagnostic samples (needle biopsies, TURP) of both arms of the trial now provide direct evidence that the cancers detected in the screening arm have more favorable characteristics as compared to those in the control arm. A similar stage and grade shift during subsequent screening rounds for prostate cancer screening was previously reported by different ERSPC centers(11-13). Indeed, the difference in prognostic factors between screening and control arm seems to increase further with subsequent screening rounds. In the 1st screening round the proportion of men with a lymph node metastasis was 1.7% of all cancers detected, whereas in the 2nd round this was reduced to 0.2% (data not shown).

The observation of more favorable tumor characteristics in the screening arm as compared to the control arm was also shown in the radical prostatectomy specimens, although treatment bias may have reduced the differences. Patients in the control arm showed a significantly reduced PSA progression free survival after radical prostatectomy, as compared to patients treated in the screening arm (p<0.0001) (Figure 1).

Figure 1 PSA progression free survival screen versus control arm after radical prostatectomy



Number of patients at risk						
screen	572	492	367	195	74	4
control	77	53	23	4	2	0

However, when comparing PSA failure rates from randomization date, instead of from date of surgery, no statistical significant difference was found (p=0.13). The latter analysis would compensate for lead-time bias (time between screen-detected and clinically detected prostate cancer). Since lead-time bias in our study was calculated to be 10 years (120 months) (14) and the mean time from randomization to prostate cancer diagnosis in this study was 49, respectively 101 months in the screening, respectively control arm, this would imply a lead-time of only 52 months. This shorter lead-time may arise from opportunistic screening and incidentally diagnosed prostate cancers in the control arm. Therefore analyzing the data from randomization date only in part corrects for lead-time bias.

If the control arm would include a large number of patients offered curative treatment for a clinically significant, but not advanced prostate cancer this could jeopardize the demonstration of a difference in prostate cancer survival between the control and screening arm. Although the proportion of incidental prostate cancers detected in the control arm is rather high (9.3%) they also include advanced cancers (that is: 6 TURP's with a Gleason score >7, 1 patient with pT1a with distant metastasis). Information of opportunistic screening is not yet complete, but more information is underway. In an earlier report on PSA testing in the control arm of the ERSPC until 1997, it was shown that a large proportion of men in the control arm had a PSA test (20.2%)(15). However, only 6% of that total proportion of men underwent prostate biopsy and finally 3.0% of these men in the control arm were diagnosed with prostate cancer as a consequence of their increased PSA (effective contamination).

The proportion of men with clinically advanced cancers, likely to be beyond reach of curative treatment (T4/N1/M1) was higher in the screening arm compared to the control arm (6.0 vs 4.6 per 100,000 man years). This unexpected difference might be explained by two reasons 1) more prostate cancers are diagnosed in the screening arm, which automatically leads to a more intense search for metastases. 2) More radical prostatectomies are performed in the screening arm, which in Rotterdam and surrounding hospitals is always preceded by a bilateral lymph node dissection for investigation of metastases increasing the chance of detecting (lymph node) metastases. In addition, clinical T-stage of prostate cancer is frequently underestimated, and therefore the pathological stage might increase after radical prostatectomy (28% of tumors staged as cT3 became pT4/N1/M1 after radical prostatectomy). Importantly, our observations show that about half of the asymptomatic advanced prostate cancers could be detected by clinical examination (36 of 60). Since about an equal number of advanced prostate cancers was found in the screening and control arm, one must assume that a large proportion of advanced prostate cancer remains clinically latent for a longer period of time.

The pilot trials performed in the area of Rotterdam suggested a reduction of prostatic cancer specific mortality in the screening arm (16). We cannot confirm this in the current study, since follow-up is short (61 and 42

months in screening and control arm), in contrast to the data of the pilot trials (10 years). I.e. Bill-Axelson et al.(17) showed that in clinically detected prostate cancer there was no difference in incidence of metastases at a 5-year follow-up period between men treated with radical prostatectomy and watchful waiting. In addition, the median time for PSA recurrence after radical prostatectomy in the screening and control arm was 3 and 5 years, respectively. In a previous report it was stated that at biochemical recurrence after radical prostatectomy, the median time to metastasis was 8 years, and from metastasis to death was 5 years (18). This implies that the mean age of a man diagnosed in our study at the time of prostate cancer death would be 82 or 81, (mean age at radical prostatectomy in screening and control arm is 64 and 65 years of age) and therefore the chance of dying from a competing cause of dead is high and correspondingly, the chance of dying from prostate cancer after treatment with radical prostatectomy would be small.

Conclusions

The prostate cancer detection rate in the control arm is significantly lower compared to its rate in the screening arm. Tumors in the screening arm do have favorable tumor characteristics compared to the control arm. Advanced disease is almost equally divided between the two arms. Patients treated with radical prostatectomy in the control arm have significantly worse tumor characteristics and accordingly a lower PSA progression free survival rate. In the control arm 9.3% of the cancers were detected incidentally

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Part 4

Chapter 8

Gleason score, age and screening: modeling dedifferentiation in prostate cancer

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Abstract
Objective: Tumor differentiation as measured by the Gleason score is highly predictive of the course of prostatic cancer after diagnosis. Since the introduction of the prostate-specific antigen (PSA) test tumors are diagnosed with a favorable tumor stage and differentiation grade. Does screening with PSA just detect more tumors with favorable characteristics or is dedifferentiation actually being prevented by early detection and consequent treatment? We used data from the Rotterdam section of the European Randomized study of prostate cancer screening (ERSPC) and a simulation modeling to answer this question.
Methods: We analyzed the clinical stage and Gleason score of 2041 tumors diagnosed in the ERSPC-Rotterdam trial in relation to age at diagnosis. We fitted two MISCAN simulation models to the observed data: one model where dedifferentiation occurs before the tumor becomes screen-detectable and another where dedifferentiation occurs during the screen-detectable pre-clinical phase. The hypothesis that dedifferentiation occurs during the screen-detectable phase was tested by comparing the goodness of fit of both models.
Results: High Gleason scores were significantly associated with age in the first screening round. The percentage of Gleason scores less than 7 decreased from 76% in men aged 55-59 to 57% in men aged 70-74; Gleason scores greater than 7 increased from 9% to 32%. In the second round and control arm no significant association between Gleason scores and age was found. Only the MISCAN model where dedifferentiation occurs during the screen-detectable phase reproduced the relation between Gleason score and age.
Conclusions: This study provides epidemiological evidence of dedifferentiation as a major mechanism of tumor progression in prostate cancer. Furthermore dedifferentiation occurs during the screen-detectable phase and consequently may be prevented by screening with PSA.

Introduction

In the western world the incidence of prostate cancer is increasing due to general availability of serum tests for prostate specific antigen (PSA) and aging of the population [5]. Early detection by PSA testing and curative treatment of prostate cancer may result in a decrease in mortality from prostate cancer [159], but definite evidence for this effect is not yet given. Nevertheless, trial results show that TNM stage and Gleason score of screen-detected tumors compare favorably to those of clinically diagnosed tumors in the control arms of the trials [84,157]. It is tempting to deduce that detection by screening and consequent treatment prevent tumor growth and dedifferentiation. However, the very favorable characteristics of screen-detected cancers might be due to length bias sampling: tumors with favorable characteristics probably grow more slowly and have more chance of being detected; moreover these tumors might never give rise to clinical symptoms and therefore not show up in unscreened/non-screened populations [48]. In the following we concentrate on Gleason score, as it is highly predictive of treatment success (progression free survival) and prostate-specific survival after diagnosis [19].

Screening can only prevent dedifferentiation if it occurs in cancers that are detectable by the screening test: screening cannot affect dedifferentiation that has taken place before the tumor has become screen-detectable. Evidence on the natural history of prostate cancer is scarce. Dedifferentiation in the screen-detectable phase is supported by a recent publication of Johansson et al. [19] who followed-up over 200 prostate cancer patients managed by watchful waiting. Some of these patients do have a higher Gleason score after repeat biopsy several years after watchful waiting was initiated. Epstein et al.[161] also noted dedifferentiation in watchful waiting, but because of the small number of cases, and the short time between successive biopsies the authors attribute the observed change of Gleason score to biopsy variability. In contrast, Thompson et al. reported a detection rate of prostate cancer of 15% in men with PSA levels below a cut-off point of 4 ng/ml. In these men 15% Gleason scores ≥7 were found, suggesting that high Gleason scores already exists before a tumor becomes detectable by the PSA screening. Also the widespread screening with PSA in the US has caused a shift to earlier-stage disease, and a markedly lower incidence of distant disease, but it has (not yet?) lead to a shift to lower grade disease [162].

This study aims at modeling the natural history and dedifferentiation of prostate cancer using detailed information from 2041 cancers diagnosed in ERPSC-Rotterdam. We investigated whether our date show a relation between Gleason score, clinical T-stage and age at diagnosis as reported in [163,164]. We used the findings to discriminate between two MISCAN simulation models: a model where dedifferentiation occurs only before the screen-detectable phase and a model where dedifferentiation occurs also during the screen-detectable phase. A significantly better fit to observed data of the second model is a strong indication of dedifferentiation that could be prevented by early detection and treatment.

Patients and methods

In ERSPC, section Rotterdam, 42,376 men, 55-75 years old, were randomized to a screening (n=21,210) and a control arm (n=21,166). Ethical approval of the study was obtained from the Dutch ministry of health (committee on the population screening act, WBO committee nr. 325291). The first screening round (November 1993 - December 1999) was initially done by PSA determination, digital rectal examination (DRE) and transrectal ultrasonography (TRUS). Sextant needle biopsy was recommended for participants who had either an elevated PSA level (≥4.0 ng/ml), abnormal DRE or abnormal findings on TRUS. Men with a benign biopsy result in the first round were invited for a recall visit after 1 year (interim screening round). The protocol was simplified on May 1997, when sextant biopsy was recommended if PSA was ≥3.0 ng/ml, regardless of digital rectal examination (DRE) and transrectal ultrasonography (TRUS). During the second screening round (June 1998 - December 2003), performed 4-years after the first screening round, the PSA ≥3.0 ng/ml protocol was used. Information on the diagnosis of prostate cancer in men randomized to the control arm and of cancers diagnosed outside the screening program in the screen arm was obtained through a record linkage with the Dutch cancer registry. The registry and subsequent linkage leads to a delay of 1 to 2 years in the reporting of these tumors. In this study we used a cut-off date of 1-July-2002 for inclusion of cancers diagnosed outside the screening program, i.e., cancers in the control arm and interval cancers in the screening arm.

Pathologic processing

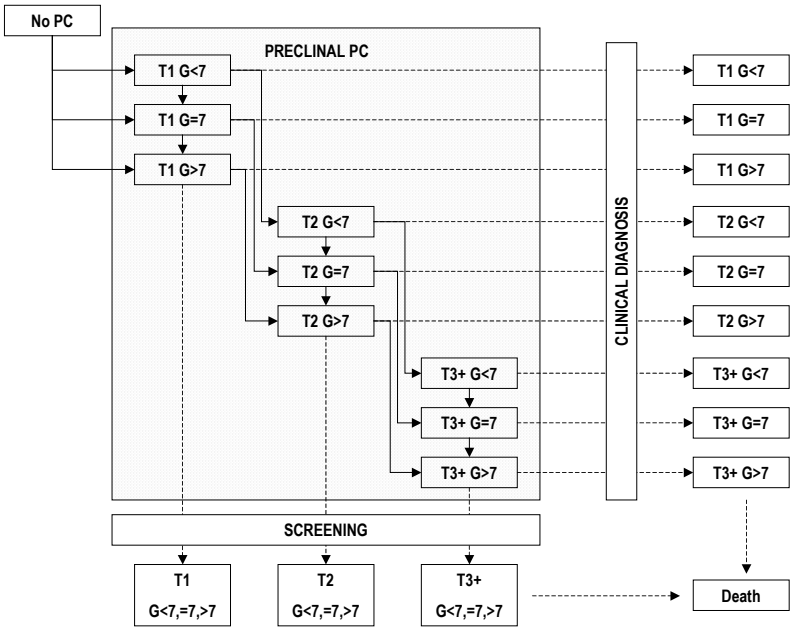
Slides from prostate biopsies, transurethral resection of the prostate (TURP) specimens and cystoprostatectomy specimens were retrieved from the archives of the pathology laboratories of the Erasmus Medical Center and surrounding hospitals of the Rotterdam region. Biopsies from the surrounding hospitals varied from 2 to 12 cores per biopsy. One uro-pathologist (T.H.v.d K) reviewed all biopsies with cancer, PIN and lesions suspicious for malignancy in order to avoid inter-observer variation. The grading of prostate cancer used in the ERSPC is the Gleason score system [17], which is based on growth patterns present in the tumor, classified from pattern 1 to 5. The Gleason score system combines the two most prominent patterns. Therefore Gleason scores range from 2-10, with score 2 indicating the best differentiated tumors and score 10 the poorest. Tumors with Gleason score 2-6 are considered well differentiated, with 7 moderately and with Gleason score 8-10 poorly differentiated. Internationally it is agreed that a needle biopsy is not graded under Gleason score 6 [18]. In addition to Gleason score, the number and size of biopsies were recorded as well as proportion of tumor involvement (only in the screened population). During this review, the number and size of biopsies were recorded as well as proportion of tumor involvement (only in the screened population), and proportion of each Gleason pattern. In the first screening round and in the control arm Gleason

scores are missing for 4 and 77 patients, respectively. As control arm biopsies were reviewed at random, no bias due to missing scores is to be expected.

MISCAN modeling and statistical analysis

The MISCAN prostate model has been described earlier [48]. In short MISCAN, an acronym for micro-simulation screening analysis, is a simulation model that uses a semi Markov process to generate the transitions from one tumor stage to the next in individual men. The natural history model used in this study is shown in Figure 1.

Figure 1 The MISCAN prostate cancer model. Prostate cancer develops from no cancer via one or more screen-detectable pre-clinical stages to a clinically diagnosed tumor. In each pre-clinical stage a tumor may grow to the next T-stage, dedifferentiate to a higher Gleason score or give rise to symptoms and be clinically diagnosed. Screening may detect tumors earlier in one of the pre-clinical stages. In model I all dedifferentiation occurs before the pre-clinical detectable phase (as indicated by the arrows on the left); in Model II dedifferentiation occurs also in the pre-clinical phase.



Tumors are characterized by T-stage (T1 impalpable, T2 palpable, confined to the prostate and T3+ palpable, with extensions beyond the prostatic capsule) and differentiation grade (Gleason score < 7, 7 and > 7). Parameters in the model are transition probabilities and dwelling times. These are estimated by numerical minimization of the deviance between observed data and corresponding model predictions. We constructed two MISCAN models for

the trial data: in Model I we assumed that Gleason score is determined before entering the pre-clinical phase, and does not change after the time the tumor has become screen-detectable; in Model II we assumed that dedifferentiation may take place in the screen-detectable phase. Predictions from both models were compared to: baseline incidence in 1990 (before the introduction of PSA screening in the Netherlands); baseline cT-stage distribution (Rotterdam cancer registry data 1992); cancer incidence, stage and Gleason score distribution in the control arm of the ERSPC Rotterdam; 1st and 2nd screening round detection rates and cT-stage and Gleason score distribution of screen detected cancers; incidence, cT- stage and Gleason score distribution of the interval cancers (cancers diagnosed in the screening arm after a negative screening test). For modeling purposes, cases detected in the interim screening round were counted as being detected in the 1st screening round. For cancers in the control arm and interval cancers we used a cut-off of July 2, 2002 for inclusion and calculating men-years. Goodness of fit of both models was based on the calculated deviance of observed from predicted age specific incidence and detection rates, and age specific distributions of cT-stage and Gleason scores; a likelihood ratio test was used to decide whether goodness of fit of Model II was significantly better than that of Model I.
Ordinal regression was used to test the significance of the association between Gleason score and age at diagnosis and clinical T-stage (cT-stage)[165].

Results

Prostate cancer incidence

In ERSPC Rotterdam, 19970 men were screened in the first round and 1013 cancers detected (detection rate 5.1%); if we include the 65 cancers found in the interim screening round, in total 1078 cancers were found in the first round (detection rate 5.4%) In the second round 12529 men were screened and 550 cancers detected (detection rate 4.5%), (of which 393 before July 2002). Up to July 2002 473 men were diagnosed with cancer in the control arm, and 128 men were diagnosed in the screening arm outside the screening program. Cumulative incidence in July 2002 after a mean follow-up of 5 years was 7.5% in the screened arm and 2.2% in the control arm.

Gleason scores

The Gleason score distribution by age group, study arm and mode of diagnosis is given in Table 1 and illustrated in Figure 2.

Tables

Table 1 Gleason scores by age of diagnosis of cancers diagnosed in ERSPC Rotterdam

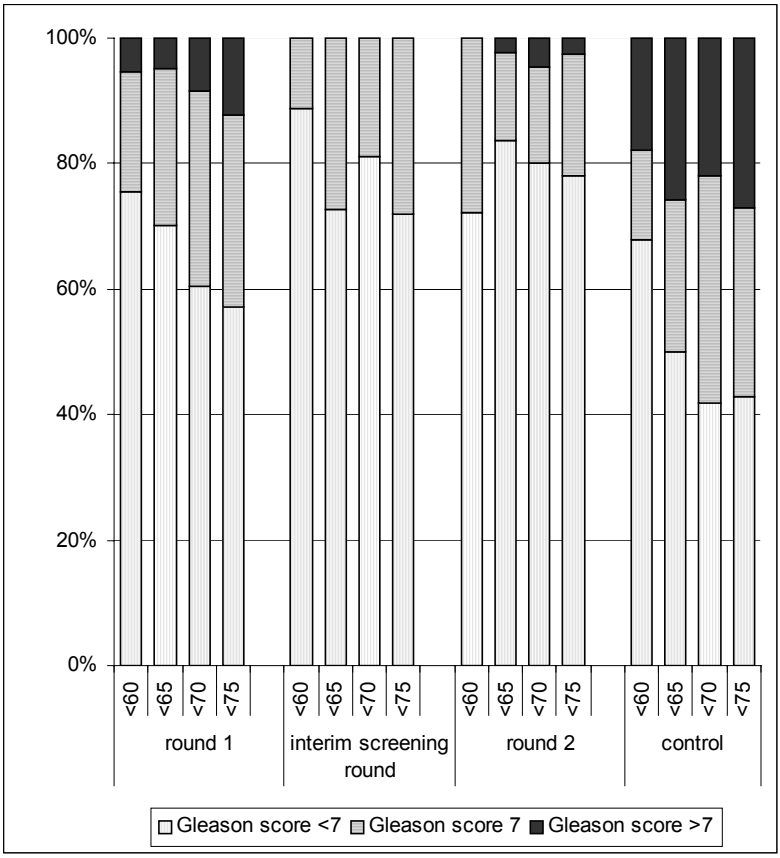
study group	age category	Gleason score								total	rate
		<7		=7		>7		unknown			
		n	%[3]	n	%[3]	n	%[3]	n	%[4]	n	
control arm[1,2]											incidence per 10^3 men-years
	55-59	19	67.9	4	14.3	5	17.9	1	3.6	29	1.7
	60-64	37	50.7	17	23.3	19	26.0	9	12.3	82	2.7
	65-69	53	41.7	46	36.2	28	22.0	10	7.9	137	5.1
	70-74	57	42.9	40	30.1	36	27.1	20	15.0	153	6.7
	75+	26	46.4	17	30.4	13	23.2	16	28.6	72	8.3
	total	192	46.0	124	29.7	101	24.2	56	13.4	473	4.5
screening arm											detection per 10^3 tests
round 1											
	55-59	125	75.8	31	18.8	9	5.5	1	.6	166	28[b]
	60-64	161	70.6	56	24.6	11	4.8	0	.0	228	45
	65-69	212	60.9	107	30.7	29	8.3	2	.6	350	77
	70-74	148	56.5	82	31.3	32	12.2	0	.0	262	87
	75+	4	66.7	1	16.7	1	16.7	1	16.7	7	167
	total	650	64.4	277	27.5	82	8.1	4	.4	1013	54
interim round											
	55-59	8	80.0	1	10.0	1	10.0			10	
	60-64	8	72.7	3	27.3	0	.0			11	
	65-69	13	81.3	3	18.8	0	.0			16	
	70-74	18	72.0	7	28.0	0	.0			25	
	75+	3	100.0	0	.0	0	.0			3	
	total	50	76.9	14	21.5	1	1.5			65	
round 2											
	55-59	15	75.0	5	25.0	0	.0			20	21
	60-64	139	81.3	27	15.8	5	2.9			171	29
	65-69	146	77.2	36	19.0	7	3.7			189	49
	70-74	135	79.4	30	17.6	5	2.9			170	72
	total	435	79.1	98	17.8	17	3.1			550	44

[1]including Gleason scores of 40 TURP specimens, 6 cystoprostatectomy specimens and 2 Millin prostatectomies

[2]Follow-up up to July 2002

[3]% of cases with Gleason score known, [4]% of total cases

Figure 2 Proportion of Gleason score category per age category



Gleason score distribution of screen-detected cancers (70% Gleason score less than 7) is more favorable than that of cancers diagnosed in the control arm (46% less than 7) and more favorable in the second round (79% less than 7) than in the first round of screening (65% less than 7). In the control arm the Gleason scores of TURP, Millin and cystoprostatectomy specimens are included. When we excluded these specimens and take only biopsy Gleason score, the proportion of Gleason score <7 was 43%.

Interestingly, Gleason score is related to age at diagnosis (Table 1 and Figure 1). In the first screening round and in the control arm older men have higher Gleason scores. Univariate ordinal regression showed that the association is significant (p=0.011) in the first screening round, but not in the control arm. A multivariate analysis, correcting for clinical T-stage, showed that both age and T-stage were predictive for the Gleason score. There was no significant relation between clinical T-stage and age, neither in the screening arm nor in the control arm (data not shown).

Modeling

Table 2 compares the observed cT-stage and Gleason score distributions with the predictions from Model I and II.

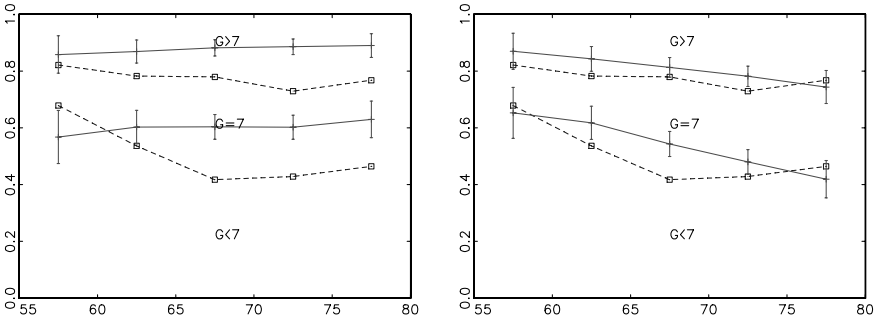
Table 2 Percentage distribution of clinical T-stage and Gleason score in ERSPC Rotterdam as observed and predicted by MISCAN Model I (no dedifferentiation in detectable pre-clinical phase) and Model II (dedifferentiation in pre-clinical phase).

	clinical T-Stage			Gleason score		
Population	T1	T2	T3+	G<7	G=7	G>7
Observed						
baseline 1991	0.14	0.59	0.27			
control arm	0.42	0.31	0.28	0.46	0.30	0.23
screening arm round 1	0.36	0.46	0.18	0.65	0.27	0.08
screening arm round 2	0.62	0.35	0.04	0.79	0.18	0.03
Screening arm interval cancers	0.59	0.26	0.15	0.72	0.16	0.13
model I						
baseline 1991	0.18	0.53	0.28			
control arm	0.27	0.50	0.23	0.60	0.28	0.12
screening arm round 1	0.40	0.44	0.16	0.67	0.24	0.08
screening arm round 2	0.59	0.34	0.07	0.70	0.23	0.07
Screening arm interval cancers	0.35	0.53	0.12	0.65	0.24	0.11
Model II						
baseline 1991	0.20	0.52	0.28			
control arm	0.28	0.49	0.23	0.53	0.28	0.20
screening arm round 1	0.39	0.45	0.16	0.65	0.27	0.07
screening arm round 2	0.61	0.34	0.05	0.79	0.18	0.03
Screening arm interval cancers	0.43	0.45	0.12	0.60	0.21	0.19

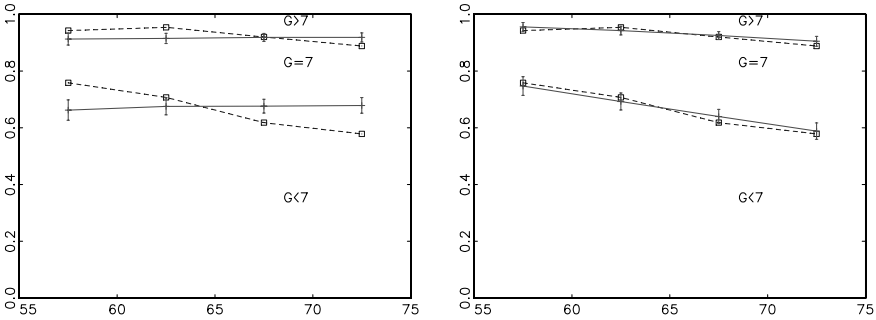
Both models accurately predict the clinical stage distribution of tumors detected by screening in the first and second round. However the models predict a more favorable distribution than observed predictions at baseline, and a less favorable distribution than observed in the control arm and interval cancers. With respect to Gleason scores both models reproduce the distribution observed in tumors detected the first round of screening, but Model II reproduces the observed differences between the various study groups more closely than Model I. Figure 3 compare the predictions from both models with the observed age-specific Gleason score distributions. Model I predicts only a modest difference between the study groups and detection modes, and no association between Gleason score and age; Model II predictions fit the observed data significantly better. The difference in goodness of fit is statistically significant (Difference in deviance of 100 with 6 extra parameters; $p<0.0001$). Still it is interesting to note that Model I predicts a more favorable distribution of Gleason scores in screen-detected cancers.

Figure 3 Gleason score by age group and study population as observed and predicted by a model without (Model I) dedifferentiation in the screen-detectable phase and a model with (Model II) dedifferentiation. The graphs indicate the cumulative proportions of Gleason scores less than 7 and less than or equal to 7. The dashed lines indicate the observed distribution, and the solid lines indicate the predicted distribution. Error bars indicate the standard error of the predicted proportions.

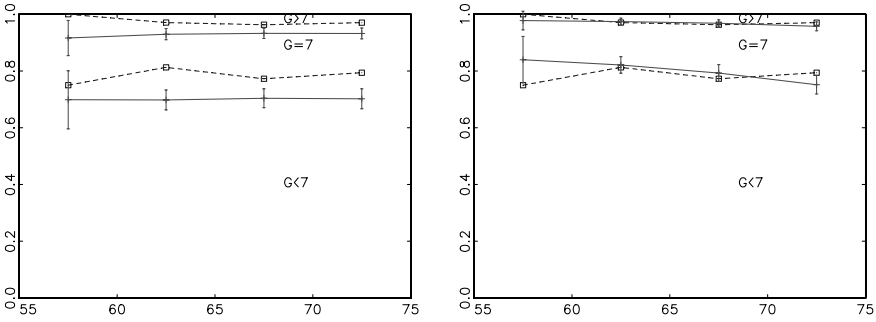
Model I Model II



Control arm



1st Screening round



2nd Screening round

Discussion

In this report we studied tumor development and dedifferentiation by examining the association between clinical T-stage, Gleason score and age of

diagnosis in ERSPC Rotterdam. We did find a significant relation between Gleason score and age in the 1st screening round. The relation is also present, but not significantly, in the tumors found in the control arm, but not in the second round of screening. The results are compatible with the MISCAN simulation model that assumes that dedifferentiation occurs for a large part during the phase in which the tumor is screen-detectable, and not with the model that assumes that the differentiation grade is already determined when the tumor has become detectable by screening.

Prostate cancer is a heterogeneous and often multi-focal cancer, which is marked by different Gleason patterns in one tumor. Two mechanisms can explain the presence of high-grade and low-grade components in prostatic tumors: 1) during their development prostatic tumors dedifferentiate, and progress from well differentiated (low Gleason score) to poorly differentiated (high Gleason scores) or 2) the combination of low and high grade evolves from pluri-potent stem cells that shed throughout the prostate and grow in different sites of the prostate to form a heterogeneous cancer [166]. In the second case Gleason score is determined early in the development of the tumor. Both mechanisms might also occur at the same time.

The findings in this study give support to the first mechanism: tumors progressively dedifferentiate during their development, which results in the association between age and high Gleason score observed in the data, most clearly in the first screening round. This might be explained that tumors in older patients had a longer time of development and had more chance to dedifferentiate. In the second round, most tumors are newly developed and consequently have lower Gleason scores and no relation with age can be seen. The fact that 80% of the tumors had a Gleason score less than 7 in the second screening round, indicates that a 4-year screening interval is not sufficient for dedifferentiation towards higher-grade for most tumors. In the control arm diagnosis is delayed in comparison with the screening arm and tumors had more time to dedifferentiate. This results in a greater proportion of high Gleason scores in the control arm.

The finding that older men have more poorly differentiated prostate cancers than younger men has been reported before [163,164]. Dedifferentiation has been studied by comparing the differentiation of metastases to that of the primary tumor. Cheng et al. [167] examined 242 men with regional lymph node metastases who underwent radical prostatectomy and bilateral lymphadenectomy. In 45% of the cases, Gleason score of lymph node metastases was higher than the primary tumor Gleason score, and in only 12% the Gleason score of the primary tumor was higher. They concluded that there is a trend toward histological dedifferentiation when prostate carcinoma metastasizes to regional lymph nodes. Similarly, Brawn et al. [168] reported that in the majority of patients with widespread metastasis the metastases tended to be more poorly differentiated than the primary tumors. It could be argued however, that these observations are the consequence of a higher potential for metastasis of poorly differentiated tumor cells, rather than dedifferentiation.

Repeated samples in time from individual patients are the most direct way to study dedifferentiation. An early study of Brawn in 1983 [169] reported dedifferentiation between two subsequent transurethral resection of the prostate (TURP) specimens with an interval of 3 to 11 years, in 65% of 54 patients. Another 33% showed equally well or only little less differentiated prostate cancer (Anderson score was used). In this study, the majority of patients received hormone therapy, which artificially produces the appearance of a higher Gleason score [21]. Another study reported dedifferentiation in 68% of tumors not treated with hormones between two subsequent TURP procedures with a mean interval of 2.4 years [170]. Wheeler et al. [171] analyzed 49 patients who were initially treated with radiotherapy. After local recurrence of prostate cancer, a statistically significant shift to higher grades was seen. Multiple logistic regression analysis revealed that the only predicting factor for dedifferentiation was time since diagnosis. The above-mentioned studies related to a very select group of patients. Only few men will undergo a repeat TURP, and patients selected for repeat TURP might have worse prostate cancer characteristics than men who are sufficiently treated by a single TURP. Similarly, the patients who had recurrence after radiotherapy (3.9%) in the study of Wheeler et al. are a very select group. Not every patient in this study underwent repeat biopsy and dedifferentiation could only be studied in patients with poor tumor characteristics (local recurrence, urinary obstruction).

Several investigators have studied dedifferentiation in repeat biopsies in patients choosing for watchful waiting instead of curative treatment. Tumor characteristics in these patients might be more representative of the favorable characteristics of tumors found in screened populations. Adolfsson and Tribukait [172] studied cytological differentiation in 78 patients with at least 2 fine needle aspiration biopsies taken at an interval of 2 years more. Progression to less differentiated tumors was seen in 18 patients (23%). Johansson et al. [19] reported dedifferentiation in 17% of 178 prostate cancer patients with localized disease managed by watchful waiting who underwent repeat fine needle biopsy. Epstein et al. [161] reported about patients who were managed by watchful waiting, selected according to the Epstein criteria [124] for insignificant cancer: a Gleason score less than 6; less than 3 cores positive for prostate cancer, not occupied by more than 50% of cancer; and a PSA density less than 0.15 ng/ml/cm^3. The protocol indicated yearly prostate biopsy. Seventy patients underwent at least two subsequent biopsies, of whom only 9 (13%) showed an increase in Gleason score from less than 7 to 7 or more. But in only one 1 case the increase in Gleason score was observed in a repeat biopsy taken after an interval of more than 24 months. The authors conclude that the higher score might be the result of sampling a higher-grade component initially not sampled.

Not only tumor grade is related with age of diagnosis. In an earlier study (Postma, submitted) we found that age of diagnosis is a predictor for biochemical recurrence, independent Gleason score, pathological stage and

surgical margin status. Older age seems to be associated with poor prognosis with respect to prostate cancer treatment.

Conclusions

Our results, based on 2101 tumors diagnosed in the ERSPC trial, section Rotterdam support the hypothesis of dedifferentiation during the screen-detectable, pre-clinical phase of prostatic tumors. This means that dedifferentiation may be prevented by screening and subsequent curative treatment. Because of the prognostic value of Gleason score, this information is crucial and an indication of the potential of screening with PSA to reduce prostate cancer mortality. Final proof of the latter point may require more follow-up than five years to show up, as by July 2002 the cumulative incidence of high grade tumors of Gleason scores greater than 7 is equal in both arms: 101 per 21,166 participants in the control arm, and 101 per 21,210 in the screening arm.

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Chapter 9

Array-based genomic analysis of screen detected Gleason score 6 and 7 prostatic adenocarcinomas

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In press anticancer research

Abstract

Objective: Prostate cancer is known for its highly heterogeneous histological appearance, which is predominantly seen by the pathologist as well differentiated and moderately differentiated adenocarcinoma, i.e. Gleason grade 3 or 4. The heterogeneous aspect may arise from dedifferentiation or from different stem cells. It is presently not clear, whether the histological heterogeneity is also reflected in the genomic composition of a tumor.

Methods: Patients were selected from the screening arm of the European Randomized Study of Screening for Prostate Cancer section Rotterdam (ERSPC), a PSA based screening program for prostate cancer. Tumors with volumes 1.0-1.5 ml and a Gleason score of 3+3 or 3+4 were selected. The cancer DNA's were retrieved from formalin-fixed and paraffin-embedded tissues allowing optimal recognition and selection of target cells. Comparative genomic hybridization with a 3500-element BAC array was used to detect differences in the genetic content of Gleason patterns 3 and 4.

Results: 17 Gleason patterns (10x G3, 7x G4), derived from 11 radical prostatectomies, were investigated. A total of 1155 gains and 583 losses were discriminated in 10 G3 areas, 768 gains and 497 losses were detected in 7 G4 regions. Frequent losses included chromosome arms 6q, 8p and 13q, frequent gains were seen on 7q and 8q. There were no significant differences between Gleason patterns 3 and 4, or between Gleason grades within one cancer.

Conclusions: Histological heterogeneity, defined by Gleason grades 3 and 4, does not have a genomic counterpart, as measured by array-based CGH. Furthermore, these asymptomatic screen detected prostate carcinomas have genetic signatures comparable with those commonly seen in symptomatic cancers.

Introduction

Prostate cancer is the most common cancer in males in the western world and the second most leading cause of cancer-related death in the Western world [4]. Whether screening for prostate cancer is of benefit for the male population has not yet been established by randomized controlled trials. The European Randomized Study of Screening for Prostate Cancer (ERSPC) investigates whether PSA based screening reduces prostate cancer related death. Although preliminary results are promising considering a stage and grade shift towards more favorable prostate cancers, final results will not be here until 2008 or later [84]. Concern has raised about over diagnosis in prostate cancer screening [48]. It is difficult, however, to distinguish which cancer is clinically significant or not. Histopathologically, prostate cancer is marked by heterogeneity and multi-focality . The heterogeneity and multi-focality might arise from dedifferentiation of cancer cells. For example, a prostate cancer with Gleason score 6 (3+3) might become a Gleason score 7 (3+4) tumor through clonal evolution, in which part of the Gleason pattern 3 dedifferentiates into Gleason pattern 4. Another hypothesis that might explain the histological heterogeneity is based on the concept of different cancer stem cells, which grow individually and collide to one tumor in time. The multi-focal appearance of prostate cancer would be in favor of the stem cell concept. The first hypothesis, however, is supported by the fact that older patients do have higher Gleason scores at diagnosis . In addition, patients followed by watchful waiting have a higher Gleason score after repeat biopsy several years later [19]. Patients with Gleason score 7 prostate cancers have higher therapy failure than Gleason score 6 patients [173]. It is therefore important to investigate, whether dedifferentiation occurs in prostate cancer. If so, screening might prevent dedifferentiation towards poorly differentiated tumors with a poor prognosis. Ruijter et al.[174] demonstrated genetic heterogeneity in some but not all focal tumor lesions, indicating a multi-focal origin is one mechanism for prostate cancer origin, but there could be several other mechanisms that are likely to operate. However, a comparative genomic hybridization (CGH) based investigation did not reveal genetic heterogeneity. A FISH study of a prostate cancer tissue micro-array revealed significant differences in *HER2/neu* amplification or chromosomal gains between Gleason grades 3 and 4 .

In this study, radical prostatectomy specimens of patients with Gleason score 6 and 7 were examined with array-based CGH. The following questions were addressed: 1) Is there a difference between Gleason patterns 3 or 4 in general? 2) Are there genomic differences between Gleason patterns within the same tumor? 3) What is the genetic "signature" of screen detected asymptomatic prostate cancers?

Material and Methods

Patient selection

Patients were derived from the screen arm of the European Randomized Study of Screening for Prostate Cancer (ERSPC). In the ERSPC, section

Rotterdam, 42,376 men, 55-75 years old, were randomized to a screening (n=21,210) and a control arm (n=21,166). There was a 4-year screening interval. The study started on November 1993. Sextant needle biopsy was recommended for participants who had an elevated PSA level (≥4.0 ng/ml), abnormal DRE or abnormal findings on TRUS. The protocol was simplified on May 1997, when sextant biopsy was recommended if PSA was ≥3.0 ng/ml, regardless of DRE and/or TRUS findings.

Tissue specimens

Patients detected with prostate cancer in the screen arm who underwent radical prostatectomy were selected. One pathologist (T.H.v/d K.) reviewed all the prostatectomy specimens, in order to determine pathological T stage, surgical margin status and Gleason score. Radical prostatectomy specimens were embedded according to a protocol described earlier [129], allowing accurate measurements of tumor volume, exact location of the tumor, grading, and staging. In short, after fixation, radical prostatectomy specimens were inked and serially sectioned at four mm intervals and totally embedded in paraffin blocks. Tumors with a Gleason score of 6 or 7 and a total volume between 1.0 and 1.5 ml were collected for DNA extraction. The radical prostatectomy specimens suitable for analysis are shown in Table 1.

Table 1 clinico-pathological data

patient	Gleason score		surgical margin	biochemical progression
1	3x	4x	+	+
2	3x	4x	+	+
3	3	4x	-	+
4	3x	4x	+	-
5	3x	3	-	-
6	3x	3x	-	+
7	3x	3	-	-
8	3x	4x	-	-
9	3x	4x	+	+
10	3x	4	+	-
11	3	4x	-	-

X Gleason pattern included in the analysis

The area of interest was marked on the glass slide and taken over on the paraffin block and punched out. After punching a new slide was made for control of the right area for tumor sampling. Isolation of DNA from the formalin-fixed, paraffin-embedded material was performed using the Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN) in accordance with the manufacturer's instructions.

Array-based CGH

The array-based CGH procedure was performed as previously described . Slides containing triplicates of ~3500 large insert clones spaced at density over the full genome were produced in the Leiden University Medical Center. The particular BAC set used to produce these arrays is distributed to academic institutions by the Welcome Trust Sanger Institute (UK) at no cost, and contains targets spaced at ~1 Mb density over the full genome, a set of subtelomeric sequences for each chromosome arm, and a few hundred probes selected for their involvement in oncogenesis. Information regarding the full set is available in the ''Cytoview'' window of the Sanger Center mapping database site, Ensembl . Insert clones were isolated from the bacteria, using the Wizard SV 96 Plasmid DNA Purification System (Promega, Leiden, the Netherlands) in combination with the Biomek 2000 Laboratory Automation Workstation (Leiden Genomic Technology Center facilities - LGTC, the Netherlands). DNA amplification, spotting on the slides and hybridization procedures were based on protocols previously described . We used commercially available female genomic DNAs (Promega, Leiden, the Netherlands) as test DNA. Test and reference DNAs were labeled with Cy3- and Cy5-dCTPs (Amersham Bioscience, Roosendaal, the Netherlands), respectively. After hybridization, the slides were scanned with a ScanArray Express HT (Perkin Elmer Life Sciences, Boston, MA, USA) to collect 16-bit TIF images through Cy3 and Cy5 filter sets. The spot intensities were measured by means of the GenePix Pro 5.0 software (Axon Instruments, Leusden, the Netherlands). Further analyses were performed using Microsoft Excel 2000. Spots outside the 20% confidence interval of the average of the replicates were excluded from the analyses.

Log2 ratios of chromosomal gains and losses were listed by an algorithm using flexible thresholds based on the standard deviation (SD) of the data sets of the specimens. SD's over windows of five consecutive BACs were averaged, sliding along the chromosomes one BAC at a time. Thresholds for gains and losses were defined empirically at +/- 2.5 x SD. This procedure resulted in a sample-dependent detection of genomic alterations with less interference of "noise" from deparaffinized formalin-fixed DNA samples. In addition, sex-mismatch with male or female reference DNA was used as an internal CGH control. Only samples showing a clear sex-mismatch were included in the evaluation. Subsequently, X- and Y-mapped BAC clones were excluded from the frequency overviews of the (sub)groups. Frequency plots were smoothed for better visualization of gains and losses by a moving average over windows of five consecutive BAC's, chromosome by chromosome. Critical areas in CGH of paraffin-derived DNA's, i.e. (sub)telomeric and pericentromeric regions, and distal chromosome 1p and 9q were excluded from analysis. BAC clones with aberration frequencies above or below 15% were listed as gains and losses, respectively. Single BAC clone alterations were removed, as they are likely to represent artefacts.

Statistical analysis

Fisher's Exact Test of unsmoothed data was applied for comparison of gains and losses between tumor sub-groups using SPSS 11.0 software (SPSS Inc., Chicago, IL). A p-value of <0.05 (two-sided) was considered as statistically significant.

Results

Eleven radical prostatectomies were taken from the screening arm of the European Randomized Study of Screening for Prostate Cancer section Rotterdam (ERSPC). Adenocarcinomas with a tumor volume of 1.0-1.5 ml and a Gleason score of 6 (3+3; n=3) or 7 (3+4; n=8) were selected. The cancer DNA's were retrieved from formalin-fixed and paraffin-embedded tissues improving recognition and selection of target cells. Comparative genomic hybridization with a 3500-element BAC array (aCGH) was applied to detect differences in the genetic content of 17 Gleason patterns (10x G3, 7x G4) from the 11 patients. A total of 1155 gains and 583 losses were discriminated in 10 G3 areas (116 and 58 per area, resp.), 768 gains and 497 losses were detected in 7 G4 regions (110 and 71 per area, resp.). Frequent losses included chromosome arms 6q, 8p, and 13q, frequent gains could be seen on 7q and 8q. Overviews of the alterations can be seen in Figure 1 and Table 2.

Table 2 Genetic data in chromosomal order (overlapping alterations in **bold**).

Gleason 3 pattern	**Gleason 4 pattern**
Loss	
4q24-q25	
	6q14.1-q14.2, 6q15-q16.1
8p12-p21.2, **8p22-p23.2**	8p11.21, **8p12-p23.3**
	10q23.31
11q22.1	**11q22.1**
13q14.13-q14.2	**13q13.2-q32.1**, 13q32.3-q34
Gain	
1q32.1	
3q21.2-q21.3	3p21.31
7q21.11, 7q21.13, 7q22.1, **7q33-q34**	**7q33-q24**
	8q11.23-q12.1, 8q22.1
	9p21.32-p21.33
10q22.1-q22.2	
11q13.3-q13.4	11q12.3, **11q13.2-q13.5**
17q21.2-q21.31	**17q21.2-q21.31**
22q12.1-q12.2	**22q12.1-q12.2**

There were no significant differences between Gleason patterns 3 and 4. Neither were differences observed between the two dominant Gleason grades within one cancer (Figures 2 and 3).

Figure 1 Overview of gains and losses of Gleason patterns 3 and 4. The top panel shows the combined data, the middle panel G3, and the bottom panel G4.

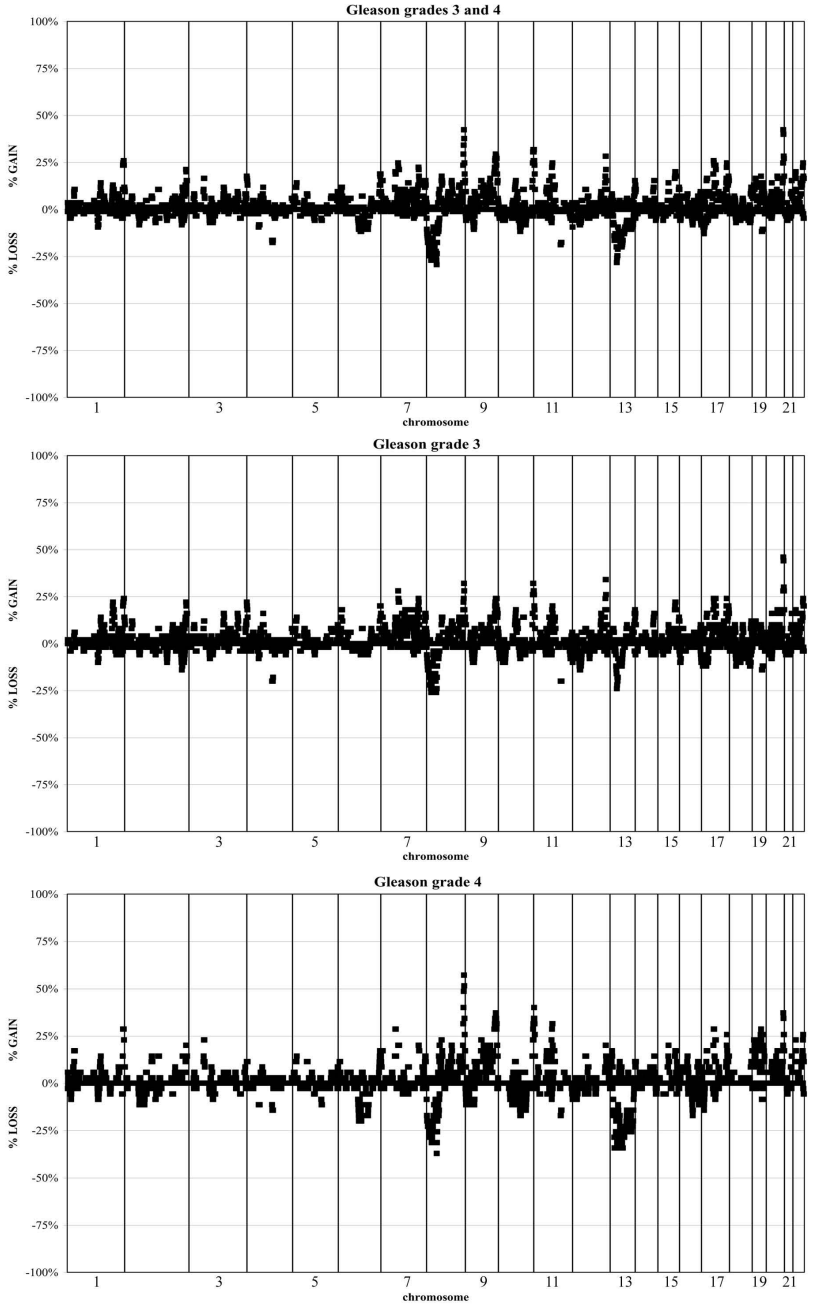


Figure 2 Log_2ratios along the genome illustrating genomic imbalances (raw data) of the two G3 patterns of within one tumor.

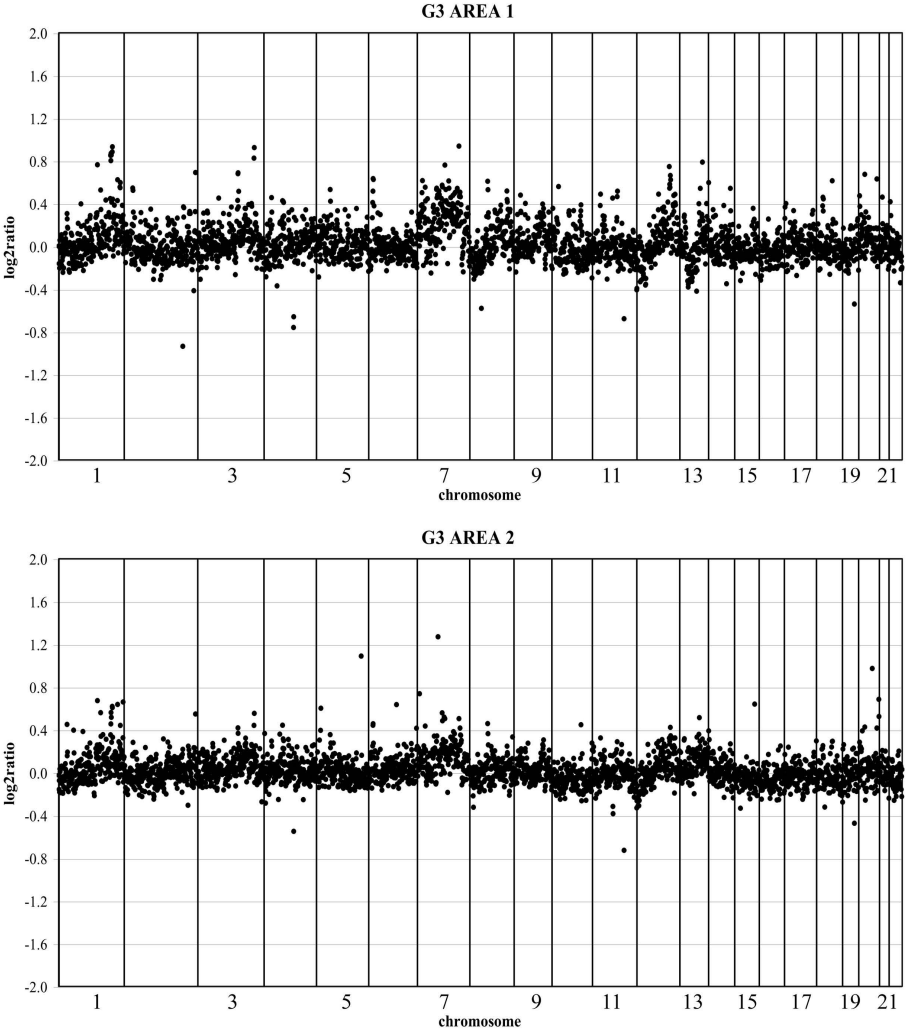
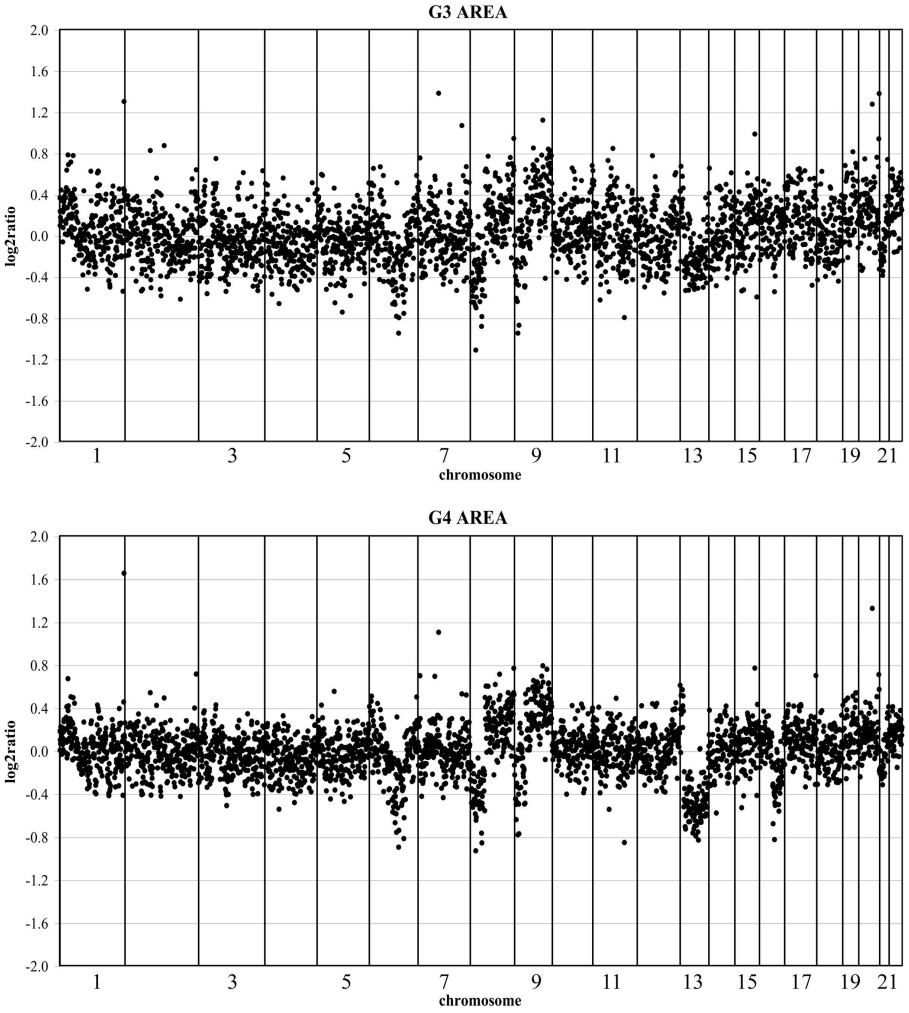


Figure 3 Log_2ratios along the genome depicting genomic imbalances (raw data) of G3 (upper) and G4 (lower) patterns within tumor 1.



Discussion

In this study we compared prostate cancers composed of well-differentiated (Gleason score 6; grades 3+3) and moderately differentiated (Gleason score 7; grades 3+4) tumors. We did not observe significant differences between Gleason patterns 3 and 4, nor did we see in Gleason patterns 3 of Gleason score 6 and 7 tumors. Thus, we cannot support the hypotheses of dedifferentiation or multiple cancer stem cells in prostate cancer. It should be mentioned that our number of samples is limited, which might have some

impact on the results. However, the spectrum of gains and losses were in accordance with those reported in the literature.

Loss of heterozygosis (LOH) analyses have shown frequent loss on chromosome arms 3p, 6q, 7q, 8p, 9p, 10pq, 13q, 16q, 17q, and 18q . CGH analysis applied to a panel of both primary and recurrent tumors revealed losses of 8p and 13q in over 30% of cases, whereas recurrent tumors showed gains of 8q and of chromosomes 7 and X, as well as loss of 8p in over half of cases [175]. A CGH study of a panel of lymph node metastases showed loss of 8p, 10q, 13q, 16q, and 17p, as well as gain of 1q, 3q, 8q and 11p sequences in 50% or more of tumors . Some of these alterations could already be distinguished in early stages of prostatic cancer, measuring <0.5 ml. In these tumors, abnormalities with chromosomal losses were most frequently seen in 13q (31%), 6q (23%) and Y (15%), and gains on 20q (observed in 15% of the tumors) . These abnormalities were also seen in our set of tumors, with even more extended abnormalities. Paris et al. performed aCGH on a primary and metastatic tumors to investigate, which markers present in primary tumors might be predictive of tumor progression. Specific loss at 8p23.2 was associated with advanced stage disease, and gain at 11q13.1 was found to be predictive of postoperative recurrence independent of stage and grade . FISH revealed numerical alterations of chromosomes 7, 8, 10, 16, 17, 18, X, and Y , as well as deletions and amplifications of specific chromosomal regions, e.g. 8p22 and the MYC region on 8q24 .

A limited amount of data is available of (cyto)genetic heterogeneity of prostate cancers. Flow cytometry revealed significant degrees of variation in DNA ploidy within individual cases . Another flow cytometry study showed that foci with different ploidy were infrequent in early prostatic carcinomas . Intra-tumoral heterogeneity was distinguished by LOH analyses of multifocal cancers . Different patterns of allelic imbalance were also discerned between multiple foci of preneoplastic lesions in the prostate . Furthermore, mutation analysis of *TP53* showed heterogeneity in intratumor distribution of primary cancers , whereas the *PTEN* gene displayed mutational heterogeneity among different metastatic sites . In situ hybridization with centromeric DNA probes showed considerable heterogeneity within cases of prostatic adenocarcinoma . However, a drawback of all these investigations is the low number of genetic targets making it difficult to differentiate between a monoclonal or polyclonal origin of tumors. We applied a genome-wide approach to answer questions concerning genetic variation in prostate cancer. In a previous study using aCGH we did not find significant differences between Gleason 3 and 4 areas in symptomatic prostatic carcinomas . This is in accordance with the present study comprising asymptomatic cancers with a tumor volume of 1.0-1.5 ml.

The prostate carcinomas in this study were screen detected cancers. Tumors detected by PSA screening mostly do have more favorable tumor characteristics compared to tumors diagnosed after symptoms have developed [176]. Further, one third of the cancers diagnosed in subsequent screening arms of the Rotterdam screening study were of focal nature [148]. Although asymptomatic our 11 cancers had tumor volumes between 1.0-1.5

ml, which is relatively large, and nearly half of the patients showed biochemical recurrence during follow-up. Therefore, we conclude that a subgroup of screen detected prostate cancers with a genotype, commonly found in prostatic adenocarcinomas, might have an aggressive phenotype.

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Chapter 10

Evaluation of apoptosis-related proteins in asymptomatic prostate cancers from a screening study

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Abstract
Objective: Asymptomatic prostate cancer and early stages of prostate cancer are encountered frequently nowadays due to surveillance and screening programs. For these cancers watchful waiting and biochemical treatment modalities, e.g. COX-2 inhibitors, might be good alternatives for radical surgery.
Methods: A tissue micro-array was constructed comprising asymptomatic prostate cancers from the Rotterdam screening study of 100 patients (mean age 63.7 years, range 55-73 years) with varying tumor volumes (mean 1.0 ml, range 0.2-4.5 ml) and 10 men who underwent trans urethral resection of the prostate (TURP) without prostate cancer. Proliferation was defined by Ki67 protein immunohistochemistry, anti-apoptotic features were determined of COX-2, Bcl-2, Bcl-xl and iNOS, and pro-apoptotic activity was measured by Caspase-3 immunostaining.
Results: In general, low or virtually absent apoptosis-related protein expression was detected in luminal epithelium of both cancers and normal controls. Only Ki67 and iNOS showed significant higher expression in the carcinomas ($p=0.03$ and 0.001, respectively) compared to benign prostate tissue. No significant associations were found between proliferative and apoptotic activity of all our markers and tumor volume, Gleason score or pathological T-stage.
Conclusions: Anti-apoptotic activity in screen detected prostatic carcinomas is low. It suggests that the use of apoptosis modulators would be of limited value in the treatment of asymptomatic prostate cancers detected in screening programs.

Introduction

Prostate cancer is the most common non-cutaneous cancer in males in the Western world. Since the introduction of Prostate Specific Antigen (PSA) testing in the last decade, prostate cancer incidence increased dramatically [1]. Due to PSA testing a lot of cancers are diagnosed which would never be detected clinically [2]. Consequently, prostate cancer prevention programs have been subject to discussion. In the Prostate Cancer Prevention Trial, finasteride, which induces lower intraprostatic dihydrotestosteron levels, caused a 25% reduction in prostate cancer prevalence compared to men who were randomized in the placebo group [3]. This was the first indication that prostate cancer might be prevented.

Apoptosis, or programmed cell death, is involved in normal tissue in order to keep normal homeostasis through the elimination of redundant or potentially deleterious cells [4]. Genetic lesions in cells may lead to diminished apoptosis and play a general role in tumorigenesis. Cells with DNA damage might escape cell cycle checkpoints, in which they otherwise would have gone into apoptosis. Now these cells can not be cleared, divide freely and a tumor may develop. The execution of apoptosis involves the activation of caspases by pro-apoptotic signals released from damaged mitochondria. In this respect the Bcl-2 family, which plays a role in maintaining mitochondrial integrity, is important. It is assumed that deficiencies in these mechanisms that regulate the apoptotic process may in another way contribute to tumor development. For example, when Bcl-2 is highly expressed cells do not go into apoptosis, but stay alive and might proliferate [4-6].

Androgen dependent prostate cancer cells undergo apoptosis in response to androgen withdrawal. Prostate cancer cells develop multiple apoptosis blocking strategies during progression from normal epithelial cells to androgen independent cancer cells [7,8]. It has been reported that loss or alterations of apoptotic regulatory genes (which induce programmed cell death) play a role in prostate cancer progression. For instance, loss or inactivation of tumor suppressor genes such as p53 and PTEN, or over-expression of anti-apoptotic proteins Bcl-2, COX-2 and Bcl-xl contribute to progression of prostate cancer [9]. Following therapy with androgen ablation, p53 and Bcl-2 expression change and the apoptotic index increases in a large proportion of cases. Failure of apoptotic response as measured by the apoptotic index correlates with relapse [10]. A chemoprevention trial targeted at induction of apoptosis has been set up (ViP) with rofecoxib (a COX-2 inhibitor) to prevent prostate cancer [11]. COX-2 inhibition has been shown to induce apoptosis in several prostate cancer cell lines [12]. However, rofecoxib was withdrawn of the market because of cardiovascular side effects [13].

In this study a tissue micro-array was constructed containing 100 asymptomatic screen detected prostate cancers with tumor volumes ranging from 0.2 to 4.5 ml. Ki67, COX-2, Bcl-xl, Bcl-2, Caspase-3, and iNOS protein expression patterns were tested by means of immunohistochemistry. The following questions were addressed: 1) What is the level of apoptosis in screen detected prostate cancer? 2) Does the level of apoptosis warrant

treatment with medication that induces apoptosis? 3) Is there a relation between tumor volume, Gleason score and pathologic stage and the apoptotic profile?

Patients and Methods

Patients and screening strategies

Data were retrieved from the Rotterdam section of the European Randomized Study of Screening for Prostate Cancer (ERSPC). Men, 55-75 years old, were randomized to a screening (n=21,210) and a control arm (n=21,166). They underwent screening every four years (starting from November 1993) until the age of 75. Screening was performed by PSA determination, and systematic sextant prostate needle biopsy was recommended for participants who had an elevated PSA level (≥3.0 ng/ml). Once diagnosed treatment was based on advise of the urologist and patients wish. For this study patients diagnosed in the screen arm and treated with radical prostatectomy were selected. Radical prostatectomy specimens were completely paraffin embedded and sliced according to protocol allowing accurate measurements of tumor volume, histological grading (Gleason score), and pathologic staging [14].

Construction of the tissue micro-array

Hundred radical prostatectomy specimens were collected for the tissue micro-array (TMA). Ten trans-urethral resection of the prostate (TURP) specimens, wherein prostate cancer was not detected after thorough sectioning, were used as normal controls. The tissue micro-array was constructed as described by Kononen et al. [15]. Six cylindrical tissue cores were taken of each of the 10 control samples. Of the 100 radical prostatectomy specimens a total of 534 tissue cores were collected in the TMA. Of each patient 4 to 10 core biopsies were inserted in the TMA depending on the number of different Gleason grade areas (range 2-4). Total tumor volume categories included < 0.5 ml (n=25), 0.5-1.0 ml (n=25), 1.0-2.0 ml (n=25), > 2.0 ml (n=25). The pathological characteristics of the prostate cancer specimens within our tissue micro-array are listed in Table 1.

Tables

Table 1 Characteristics of the 100 prostatic cancers.

tumor volume (mean; ml)	1.0 (range 0.2-4.5)
pathological stage (pT)	
pT2	83
pT3	15
pT4	2
Gleason score	
<7	62
7	37
>7	1

Immunohistochemistry

Immunohistochemistry was performed on 4-µm-thick tissue sections of the TMA adhered to aminoacetylsilane (AAS) coated slides (Starfrost, Berlin, Germany). Immunostaining was performed using the ChemMate Envision kit (DAKO, Zoetermeer, The Netherlands). After de-paraffinization microwave (700 W) pretreatment was performed for 15 minutes using citrate buffer (10mM citric acid monohydrate, pH 6.0), except for PSA. The following antibodies, diluted in phosphate-buffered saline/5% were used: Ki67 antigen (Immunotech, Miami, FL; diluted 1/200), COX-2(Vector, Burlingame, CA; diluted 1/200), Bcl-xl (Zymed laboratories Inc, San Francisco, CA; diluted 1/200), Bcl-2 (DAKO; diluted 1/100), Caspase-3 (DAKO; diluted 1/400), and iNOS (BD-transduction lab; diluted 1:800). As a positive control PSA antibody (DAKO; diluted 1/1600) was used, as a negative control the primary antibody was omitted.

Scoring and statistical Analysis

The immunostaining results of the TMA were scored per tissue core by two independent investigators. Moderate or intense brown nuclear/cytoplasmic staining was considered positive. The percentage of brown stained cells was determined in at least 50 prostate epithelial cells by each investigator. For the statistical analysis SAS-8 and Statistical Package for Social Sciences software 11.0 were used (SPSS, Chicago, IL). A p-value <0.05 was considered significant.

Results

A tissue micro-array was constructed comprising asymptomatic screen detected prostate cancers of 100 patients (mean age 63.7 years, range 55-73 years) with varying Gleason scores (4-9) and tumor volume (0.2 -4.5 ml; see Table 1)). The majority of these cancers was low-grade, i.e. Gleason score <7 (62%), and/or low-stage, i.e. pT2 (83%). Protein immunohistochemistry was used to evaluate proliferative and apoptotic activity in the luminal epithelial compartment. Proliferation was defined by Ki67, anti-apoptotic features were determined of COX-2, Bcl-2, Bcl-xl and iNOS, and pro-apoptotic activity was measured by Caspase-3 (examples in Figure 1). The protein expression patterns were scored and statistically analyzed. Proliferation marker Ki67 showed a significantly higher protein expression in the carcinomas as compared to normal prostate tissues ($p=0.03$). The pro-apoptotic activity, as measured by Caspase-3 did not show a significant difference between tumor and normal prostate tissue. For the anti-apoptotic markers low or absent protein expression levels were observed in (luminal) epithelium of both carcinomas and normal controls. However, positive immunostaining was seen for these markers in other (expected) cell types, such as basal epithelial cells, lymphocytes or fibromuscular stromal cells (Figure 1). Only iNOS showed a significant higher staining profile in the prostatic cancers ($p=0.001$). (table 2) . No significant associations were found between proliferative and

apoptotic activity of all our markers and tumor volume, Gleason score, and pathologic stage.

Figure 1 *a-c* Immunostaining of cellular proliferation with Ki67 showing occasional positive cell nuclei in normal basal cells *(a),* whereas (luminal) cancer cells display low *(b)* and high *(c)* frequencies of labeling. *d* Pro-apoptotic marker Caspase-3 revealing positive staining in normal luminal cells (gland at right), but not in adenocarcinoma cells. *e-h* Anti-apoptotic activity illustrated by iNOS, Bcl-xl, Bcl-2 and COX-2. Note cytoplasmic positivity for iNOS *(e)* in tumor cells; in contrast, the normal gland (at right) is negative. F shows weak cytoplasmic staining of cancer cells with Bcl-xl, whereas Bcl-2 *(g)* reveals negative tumor cells, but positive labeling of basal cells in normal glands (at right). COX-2 *(h)* is negative in (normal) prostatic epithelium, whereas myofibroblasts are clearly positive.

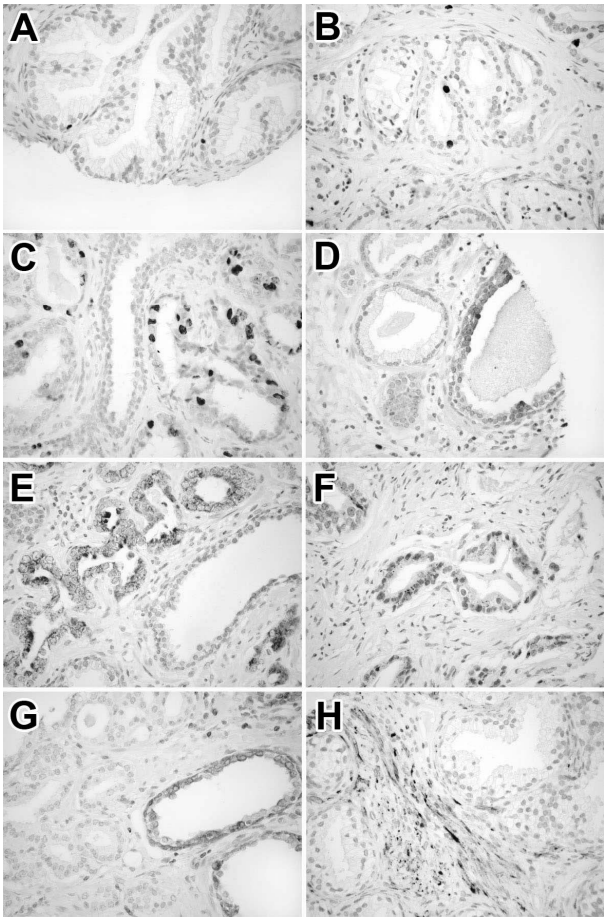

Table 2 differences between tumor and nomal prostate tissue

	% postivity in normal	% postivity in tumor	P
Ki-67	1.8	2.8	0.03
COX—2	4,3	10,4	Ns
Bcl-xl	2.3	7.5	ns
Bcl-2	-	56,9	ns
iNOS	0.1	4,1	<0.0001

Discussion

We investigated markers related to apoptotic activity in asymptomatic prostate cancers, detected by a population based screening. Of all the markers only iNOS was significantly different between the prostate cancer specimens and normal prostate tissues. This low spectrum of apoptosis might be explained by the great proportion of low-stage (83%) and well-differentiated tumors (62%) in our series. In the literature, a correlation between Ki67 expression and increasing Gleason score has been reported [16]. We found a significant difference in prostate cancer versus normal prostate tissue, but no association was present between the Ki-67 defined proliferation index and cancer grade or stage. This can be attributed to the fact that only one high-grade prostate carcinoma (Gleason score >7) was present in our series of asymptomatic cancers.

iNOS (inducible nitric oxide synthases) conducts the cytotoxicity of macrophages and tumor-induced immunosuppression [17]. Positive iNOS immunostaining was described in BPH, PIN and prostatic carcinoma [18]. Furthermore, prostate cancer samples showed increased staining, when compared to BPH samples [18]. However, a higher Gleason score was not correlated with increased iNOS expression. In another immunohistochemistry study benign hyperplasia stained negative, but prostate cancer cells were highly positive for iNOS [19]. Also a marked variation of iNOS mRNA levels in prostate cancer was reported [20]. All these findings are in line with the results of our study. Finally, strong iNOS protein expression was found to be a predictor of poor survival in univariate analysis, but appeared inferior to established prognostic factors in multivariate analysis [21].

Recent studies suggest that induction of apoptosis may be a potential mechanism for prostate cancer prevention, especially by using COX-2 inhibitors [9]. Grupta et al. described over expression of COX-2 in prostate cancer [22]. They showed that prostate carcinoma cells stained intense as compared to mild staining in benign prostatic epithelium. Other investigators described similar results. However, when COX-2 expression according to pathological stage was compared, no significant difference was found between pT1/pT2 tumors and BPH samples [23]. Immunohistochemical analysis of prostate cancer also showed that there was no consistent over expression of COX-2 in cancer or high-grade PIN versus adjacent normal prostate tissue [24]. Positive staining was seen only in scattered cells (<1%) in both tumor and normal tissue. Thus, no consistent line of literature seems present concerning

COX-2 expression in the successive stages of prostate cancer. This is supported by a recent study describing a lack of involvement of COX-2 in prostate cancer development [25].
The Bcl-2 protein is predominantly expressed in prostate basal cells in benign prostate epithelium, is absent in most low to intermediate grade carcinomas and is highly expressed in androgen independent prostate cancers [26]. Another study reported that almost all androgen independent prostate cancers stained positive for Bcl-2, whereas androgen dependent prostate cancers stained negative and weak in 2/3 and 1/3 of cases [27]. It is suggested that Bcl-2 over expression might be a useful prognosticator in more advanced tumors, because the expression increased with stage and grade [28]. In a recent tissue micro-array study immunostaining of the TMA was positive for Bcl-2 in only 24% of the cores, whereas the corresponding radical prostatectomy specimen stained positive in 66% [29]. The investigators concluded that the heterogeneity of immunohistochemical protein expression of this marker requires a sufficient number of tissue for adequate TMA evaluation. In our study a large number of cancer cores was present in the TMA. We therefore attribute the overall negative expression profile of Bcl-2 to the predominance of low-grade and low–stage cancers in our asymptomatic screen detected series of prostate cancers. A likewise scenario seems to be present for Bcl-xl, a gene closely related to Bcl-2 [30].

Caspase-3 protein expression by immunohistochemistry as a measure for apoptotic activity has been demonstrated before [31]. Winter et al. showed a significantly reduced staining pattern in prostate cancer cells, as compared with normal tissues [32]. Further, a relation between increasing Gleason score and apoptotic index was seen. This was, however, not confirmed in another investigation [33].

Conclusions

In literature there is no consistent line of evidence to firmly support the use of apoptosis modulators, such as COX-2 inhibitors, for prevention or treatment of prostate cancer. In this study we used a tissue micro-array that comprised of 100 asymptomatic prostatic cancers, detected by a PSA-based screening study, to assess the spectrum of apoptosis. In general, a low anti-apoptotic activity was detected. This could in part be explained by the large proportion of low-stage and well-differentiated tumors. However, based on our results we can not recommend COX-2 inhibitors as medication for the treatment of asymptomatic prostate cancer.

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General discussion

Introduction of the large scale PSA testing for prostate cancer without prior evidence for its efficacy has now created a social economic health problem in the Western world. Its' unintended use, on large numbers of aging men, led to a considerable claim on health budgets at the expense of other health related issues. It was calculated that the costs of a nation wide screening program (screening interval of 3 years) in Sweden with almost 9 million people would cost 244 million Swedish crones (27 million euros) per year [1]. The costs would be much higher in countries with a larger population. The clinical complexity of prostate cancer still remains a challenge. While much is known about the risk factors and biology of other common cancers such as breast and lung cancer, prostate cancer is surrounded by uncertainties.

What has become clear in the last decade is that PSA screening and to a lesser extent, aging of the population has brought about enormous changes in the epidemiology of prostate cancer. Since the recommendation to test men 50 years or older for PSA in 1993 in the USA, and after introduction of two randomized clinical trials (ERSPC and PLCO) more than 10 years ago, it is as yet unknown whether PSA screening reduces prostate cancer mortality, let alone overall mortality.

One of the goals of PSA screening is to detect prostate cancer at an early stage, reducing the number of patients with advanced disease, which are likely to be beyond of cure. Indeed the efforts to detect prostate cancer at an early stage seemed to be successful since the enormous gap between clinical incidence (8–15% lifetime risk) [2] and autopsy-based prevalence (80% by age 80 years was reduced [3]. As a consequence of the tremendous increase in prostate cancer incidence (e.g. in the USA the prostate cancer detection rate increased twofold since the introduction of PSA [2], a great proportion of the prostate cancers detected by PSA screening might now be considered as over diagnosis. In the ERSPC, the over diagnosis was calculated to be as high as 48% on the basis of computer modeling [4]. Others even find that this percentage would be 84% with respect to prostate cancer mortality [5]. One of the important challenges of PSA screening is not to detect as many prostate cancers as possible, but to determine which men after a diagnosis of prostate cancer are at greatest risk of developing advanced disease. To these men, an appropriate curative treatment might be offered in order to reduce the risk of disease recurrence and progress to metastasis. In men with favorable tumor characteristics, initially watchful waiting might be considered in order to reduce over treatment.

At present, the age-adjusted prostate cancer mortality per 100,000 men is declining in the USA [6]. This is probably attributed at least partly to PSA screening. These results might be influenced by lead time bias (clinical tumors are detected later without screening and therefore the life span is longer in a screen detected cancer) and length time bias (detection of tumors that would not have been detected without screening and would not otherwise pose a threat on the life expectancy of the host.

Unfortunately, the final outcome of the ERSPC is not to be expected until the end of 2008 or later [7]. The studies outlined in this thesis describe the intermediate endpoints, concerning stage and grade of prostate cancer in subsequent screening rounds and the forthcoming therapy choices, the efficiency of the screening protocol employed at the Rotterdam section of the ERSPC and the natural biology of prostate cancer and its' possible premalignant lesions.

Importance of lesions with potentially increased risk for subsequent detection of prostate cancer

The conclusions of chapter 2 are that the prostate cancer incidence after repeat biopsy in men with a benign biopsy in the 1st round was 12.1%, a percentage not significantly different from the prostate cancer incidence after repeat biopsy for PIN (13.3%) neither in the 1st nor in the 2nd screening round. Before completion of the 1st screening round of the ERSPC, Vis et al. [8] already reported that the risk for subsequent detection of prostate cancer was not significantly increased. The data of chapter 2 include the longer follow-up after completion of the 1st and 2nd screening round. A yearly linkage with the cancer registry allowed us to detect prostate cancers which were diagnosed outside the ERSPC trial. Even after a long follow-up, including the recognition of cancers diagnosed outside the trial, the prostate cancer incidence in men previously diagnosed with isolated PIN proved to be limited. This finding was discrepant with several previous data from other studies. Reports from opportunistic screening studies demonstrated that prostate cancer was diagnosed in 22-100% of men that underwent repeat biopsy after an intial diagnosis of PIN [9-15,16-18]. Our finding that PIN is not associated with increased risk of detecting prostate cancer was confirmed be a few other studies, confirming that the risk for detecion of prostate cancer at repeat biopsy after a PIN diagnosis is not increased [19,20].

The incidence of PIN in a screening based population might be considered as a reflection of that in the general male population. Although the PIN incidence increases in the 2nd screening round towards 2.5%, it continues to be low compared to literature, where a PIN incidence of 3.7-29% was reported on needle biopsies [21]. The incidence of PIN in autopsy specimens in benign prostates was reported to be as high as 43% [22]. At radical cystoprostatectomy specimens with concurrent cancer, the PIN incidence was even higher, 85-100% [21]. It must be concluded that the limited amount of tissue sampled by prostate needle biopsies (sampling bias) explains the comparatively low incidence of PIN detected in our screened population. Other explanations for the discrepancies in incidence and subsequent prostate cancer detection at repeat biopsy are for the main part due to differences in study populations, but also indications for biopsy, inter-observer variation of pathologists and differences in the biopsy protocol might influence the observed incidence of PIN [23]. Both the high incidence of PIN in autopsy series in men dying of other causes than prostate cancer [22] and the low risk of subsequent prostate cancer detection would-to our opinion- imply a diagnosis

of isolated PIN by itself does not require repeat biopsies. A repeat biopsy also is not favored, because, despite physical side effecs, it might induce psychological stress and therefore reduce the quality of life. In addition repeat biopsies have an impact on the costs associated with prostate cancer screening, which is another argument, to restrict repeat biopsies in men with isolated PIN.

In contrast to our finding regarding PIN lesions, the incidence of LSPC lesions (not premalignant lesions) and the subsequent detection of prostate cancer in repeat biopsy in the ERSPC screening study were comparable to literature [24-26]. Based on these findings, we do advise continuation of performing repeat biopsies in men with a LSPC, even though the risk of a subseqeunt cancer is much lower in the 2nd screening round.

New immunohistochemical markers as basal cell marker p63 and α-methylacyl CoA racemase (AMACR) have been introduced to more easily distinguish between prostate cancers and their benign mimics, which may raise suspicion for a cancer. The marker p63 is generally used for distinguishing benign small acinar proliferations from Gleason pattern 2 and 3 prostate cancers; it is a basal cell marker that is absent in prostate cancer. The AMACR marker gives a particular, intense staining in prostate cancer and PIN lesions and is of diagnostic use in resolving ASAP or LSPC foci in prostate biopsies. Because the markers distinguish between prostate cancer and foci suspicious for malignancy, more suspicious cases will be definatively diagnosed and a decrease would be expected in the incidence of LSPC lesions. However, as a consequence of this, there is a risk that suspicious foci might be more often diagnosed as PIN lesions because morphologically unremarkable prostate glands are occasionally positive for AMACR and basal cell markers (p63, 34ße12) (ref).

In chapter 3, the incidence of different variants of atrophy and the subsequent prostate cancer incidence is reported. Some authors suggested that atrophy might be a precursor lesion for prostate cancer. If so, a more close surveilance of men with atrophy in their biiopsy might be warranted. The incidence of atrophy was not reported before on prostate needle biopsies. If atrophy would carry an increased risk for prostate cancer, it would be important to have an estimate of the incidence of atrophy in a general screening population [28]. After reviewing 202 benign prostate needle biopsies with a follow-up of 8 years, 94% of the biopsies contained some form of atrophy. After expanding follow-up until 2005 4.4% of men with atrophy in their biopsy had a prostate cancer in the follow-up, which is sligthly lower than the prostate cancer detection rate in the general screening population (5.1%). More importantly, the extent of atrophy in the biopsies was also not relevant to subsequent cancer risk. Although a small proportion of these patients underwent repeat biopy in the 4-year screening interval, the limited number of interval cancers, detected through linkage with the cancer registry, supports the view that in the general population atrophy diagnosed on prostate biopsy, does not increase the risk of having a prostate cancer diagnosed within at least 8 years.

Therefore, due to the extremely high incidence of atrophy in sextant biopsies and the low cancer detection rate, we do not consider this lesion as a premalignant lesion and will not encourage to do repeat biopsies on these men. We can not exclude however, that atrophy, as a consequence of chronic inflammation, with an onset at a young age and persisting for e.g. 10 years or more will not eventually progress to prostate cancer.

Intermediate endpoints to determine the efficacy of the screening protocol
We tried to shed a light on the efficacy of the screening protocol used in the Rotterdam section of the ERSPC. Optimalization of the screening protocol may involve parameters such as DRE, TRUS, different PSA cut-offs for indication of biopsy, employment of sub forms of PSA (e.g. pro-PSA, free PSA), number of prostate biopsy cores, site of punction and age category.
The ERSPC was heavily criticized for the adoption of the long interval of 4 years between two screening rounds employed by most of the centers [29]. It was hypothesized that 77% of the prostate cancer diagnoses would be delayed if a 4-year screening interval was used [29]. However, it was not investigated whether this delay might lead to increased morbidity or mortality. In addition, in the ERSPC Rotterdam, lateral sextant biopsies are used, which are now considered as old fashioned because the chance to miss prostate cancer is as high as 20-30% [30,31].

The efficacy of the screening protocol in the Rotterdam section of the ERSPC was investigated by determination of the incidence of potentially advanced and focal cancers in 1st and 2nd screening round. In addition, pathological stage and therapy choices were examined during the two screening rounds and in the control arm. Since biopsy Gleason score is one of the best predictive factors for prostate cancer recurrence and is correlated with pathological tumor stage, [32] we used this parameter for the assessment of the aggressiveness of prostate cancer. The incidence of potentially advanced cancer, defined as Gleason score 7 (4+3, or 3+4 with a >30% cancer involvement) or Gleason score 8-10 carcinoma in sextant biopsies decreased from 20% in the 1st to 6.0% in the 2nd round, respectively. This implies that the screened population was effectively depleted of aggressive cancers during the 1st round, an effect lasting for at least 4 years. The incidence of focal cancer, defined as ≤3.0 mm involvement by cancer in one biopsy core lacking Gleason pattern 4 or 5 on sextant biopsy, increased significantly from 16% in the 1st to 29% in the 2nd screening round. This observation is consistent with a screening protocol, detecting cancers at an early stage. The dramatic increase of focal cancers in the 2nd round would suggest that the 4-year interval does not allow most tumors to grow to a large size. The latter could be confirmed by findings reported in chapter 6.

In chapter 6, features of prostate cancer detected during two subsequent screening rounds in relation to changes in therapy choice were reported. Gleason score, involvement of biopsy by tumor, clinical stage and PSA level were more favorable in patients of the 2nd round compared to those of the 1st

round. It was already reported that there was a stage and grade shift when a limited proportion of men were screened in the 2nd screening round [33]. The number of men chosing for watchful waiting increased from 10% in the first to 22% in the second round. In patients undergoing radical prostatectomy, median tumor-volume in the first, respectively second screening round was 0.65 resp. 0.45 ml. Minimal cancer (cancer <0.5 ml, organ confined, no Gleason pattern 4 or 5) was found in 31.6% in the first and 42.6% in the second screening round, a significant increase. Independent Prognostic factors for PSA failure were positive resection margins, age, biopsy Gleason score, and pT-stage. Thus the biopsy findings in chapter 5 could be confirmed by the observation made on radical prostatectomy. However, it should be realized that only a proportion of men underwent surgery for their cancer. This may have biased the less dramatic findings in radical prostatectomy specimens.

The prostate cancer incidence rate decreased from 5.1% in the 1st towards 4.4% in the 2nd round. This observation should be considered with caution, because of protocol changes and side studies in the 2nd screening round with lower cut-off levels for biopsy indication. It must be realized that the prostate cancer incidence in Rotterdam was the highest among the other European centers (other centers reported incidences varied between 1.7 and 2.7%). Thus the observations made of the Rotterdam section of the ERSPC may not necessarily be extrapolated to the other screening centers [34].

The incidence of high-grade cancer (Gleason score 8-10) not only decreased in the Rotterdam section, but also in Finland. Sweden had already a very low incidence of high grade prostate cancers in the 1st round, making it drop below that initial incidence of high grade prostate cancer very unlikely. To some extent, the overall cancer detection rate as well as the high-grade cancer detection rate was related to the biopsy compliance [34]. The fact that prostate cancer incidence is dependent on biopsy compliance was also supported by a recent publication of the PLCO study. They reported in the same age categories a biopsy compliance of 31.5%, which resulted in an overall prostate cancer detection rate of 1.4% as compared to the cancer detection rate of 5.1% in the ERSPC Rotterdam [35].

The median tumor volume in our series of radical prostatectomy specimens was small. As a manifestation of the efficacy of screening, one would also expect that tumors detected in the screening arm of the ERSPC are smaller compared to tumors detected by clinical symptoms. The median tumor volume reported in another screening study by Humphrey et al was 1.0 ml [36]. Ohori et al reported a median tumor volume of 2.23 ml, which was considerably larger compared to our data[37]. A few explanations for the discrepant findings in volume between studies may concern technical aspects of tumor volume measurements. In the ERSPC Rotterdam tumor volume is calculated after the radical prostatectomy was submitted in toto and cut serially in slices of 4 mm. The tumor volume area was measured by morphometry and multiplied by 4. Prior experiments had shown that no correction factor was required for the alleged shrinking effects of fixation and

paraffin embedding. Some other authors, however used a correction factor of 1.4 or 1.3 for alleged shrinkage [38].

Tumor volume was an independent predictive factor in the 1st screening round for biochemical recurrence [39]. After completion of the 2nd round, tumor volume lost its' predictive value additional to Gleason score pathological stage and resection margin status. This might probably explained by the limited variation in size of the tumors. Because of the small tumor size, other factors such as Gleason score and pathological stage have become more important, compared to tumor volume.

On the basis of the above mentioned results, and the observation that the number of interval carcinomas was limited, while displaying favorable tumor characteristics [40], we must conclude that the ERSPC protocol conducted in Rotterdam, i.e. PSA cut-off ≥3 ng/ml, employment of sextant needle biopsy and a screening interval of 4 years is highly effective at an early stage, and in preventing the development of advanced cancers.

As yet, no proper definition of insignificant cancer, also designated as: minimal cancer or cancer that does no harm, or cancer that should be considered as over diagnosis, can be given. The parameters to define a minimal cancer in a radical prostatectomy specimen are somewhat arbitrary and it remains unproven that minimal cancers are indeed harmless cancers on the long term. On the basis of our results, we would argue that a focal cancer on sextant biopsy, or a minimal cancer in a radical prostatectomy specimen might be considered as an insignificant cancer. Their detection could be considered as over diagnosis, and indeed these cancers were shown to have a good prognosis in the ERSPC Rotterdam.

Our analysis of needle biopsy and prostatectomy specimens of men diagnosed with prostate cancer in the screening arm, was an attempt to confirm whether the over diagnosis of 48% based on computer modeling would be an appropriate calculation. The incidence of focal cancers on sextant biopsies increased to almost 30% in the 2nd screening round brings us close to the 48% calculation. We could confirm this by investigating radical prostatectomy specimens in the 2nd screening round as we detected a 43% incidence of minimal cancers. It should be mentioned that some focal cancers in sextant needle biopsies are just the tip of the iceberg. Five percent of the focal cancers were upstaged to a pT3 or higher after radical prostatectomy. Therefore it is hard to predict, at the individual level, which patient would really harbor an indolent cancer.

A marker that detects specifically the aggressive or indolent cancers is urgently needed to solve the problem of over diagnosis. However, for over 15 years researchers have been investigating this issue, with as yet no result that may lead to a change in prostate cancer detection. Even in this era of cDNA arrays and proteomics no really exciting markers have been discovered at present, so more practical solutions need to be put in the practice at this time. A less intensive screening protocol might also decrease over diagnosis but at the cost of missing cancers that are beyond of cure. Another practical way to avoid over diagnosis or rather over treatment is to defer treatment, i.e.

managing men with favorable pre-operative features (i.e. Gleason score ≤6, limited tumor involvement, low PSA or PSA density) with watchful waiting. The proportion of men on watchful waiting in the Dutch part of the ERSPC has now doubled in the 2nd screening round, which we consider as a good development, bearing in mind the detection of a comparatively large proportion of focal cancers in the 2nd screening round. To date there has been one randomized clinical trial comparing radical prostatectomy and watchful waiting. In this trial men treated with radical prostatectomy showed significantly increased prostate cancer specific survival as well as overall survival compared to men managed by watchful waiting [41]. However, patients with presence of Gleason pattern 4 (<25%) and 5 (<5%) cancers identified ar=t radical prostatectomy were also included in this trial. As mentioned before, patients with a high grade Gleason pattern in their biopsy do worse, compared to patients without a high grade component [42]. In contrast, the natural history studies of Johansson en Albertson [43,44] show a prostate cancer specific survival rate of 72% and 73% without curative treatment after a follow-up of 20-years in men with a well-differentiated prostate cancer. The latter studies support the initiation of watchful waiting in men with favorable tumor characteristics. We do hearten the initial management of these men with watchful waiting. A delayed treatment can always be given in these men, with a very small risk of metastasis or death from prostate cancer. Furthermore, it was shown that their delayed treatment is almost always curative (manuscript in preparation).

Significantly more prostate cancers were detected in the screening arm vs. the control arm (15.9 vs. 4.2 per 1000 man years, p<0.0001)(chapter 7). Clinical (c)T stage, biopsy and radical prostatectomy Gleason score distribution were significantly less favorable in the control arm. The 5-year biochemical free survival after radical prostatectomy in both rounds of 87% was high and there was no significant difference between the two rounds. In two other large series, a somewhat lower 5-year biochemical progression free survival rate of 78 and 80% was reported [45,46]. This difference is explained by the fact that the men in the latter studies were not part of a screening study, which is in line with the presumed potential of screening to detect most cancers at a stage that they are curable. However, with a 50% of T1c tumors in the study by Roehl et al., one might speculate that a great proportion of their cancers detected on biopsy are due to increased PSA (opportunistic screening). We expect that in the future biochemical recurrence free survival will increase if the radical treatment of low-grade low stage cancers continues. The 5-year PSA progression free survival after radical prostatectomy was 68% in the control arm and 89% in the screening arm (p<0.0001). Advanced disease (T4/N1/M1) was significantly more common in the control arm (11.0%) as compared to the screening arm (3.8%) (p< 0.0001). All these features increase the likelihood of a reduction of prostate cancer mortality in the end. A recent publication on the pilot studies prior to the start of the ERSPC showed data on prostate cancer mortality with 12 and 3 prostate cancer deaths in the control and

screening arm, respectively [47]. As these numbers of patients in this study are very small, no statistical calculations could be performed and the follow-up time (median 5 years) is still too short to encourage the view that screening for prostate cancer might reduce prostate specific mortality in the end.

The power of the ERSPC might be undermined if a large number of prostate cancers in the control arm would be detected incidentally or by opportunistic screening. Only a limited proportion of cancers in the control arm was detected incidentally. Incidentally detected cancers are most frequently low stage, low-grade tumors and they are found because of treatment for another disease than prostate cancer, e.g. bladder cancer or BPH. Since the incidence of these tumors was 9.3% of all tumors detected in the control arm, we don't think their prevalence might have a strong impact of the power of study. Another effect that probably will have an impact of the power of the study is the opportunistic screening in the control arm. The opportunistic screening in the control arm was found to be 3,0% per year [48]. However, the proportion of T1c cancers in the control arm has risen to 25% nowadays, which for a great part might be due to opportunistic PSA screening. To avoid that the opportunistic screening of the control arm affects the power of the study a Cuzick analysis is planned [49]. This method was originally developed to adjust for non-compliers in the screening arm while respect to randomization and the prevention of bias were conserved. In this analysis adjustments for contamination (use of the treatment and screening by individuals in the control arm) were also developed, which might be useful in the ERSPC, since due to awareness of screening for prostate cancer in the population an increasing proportion of men (in the control arm) undergo PSA testing.

Natural behavior of screen detected prostate cancers

Prostate cancer is a heterogeneous and often multi-focal cancer, and frequently different Gleason patterns may be noted within one tumor. Two opposing hypotheses may explain the presence of both high-grade and low-grade components in a prostatic cancer: 1) During their development a prostatic cancer starts to grow and to dedifferentiate from a well differentiated (low Gleason score) to a poorly differentiated (high Gleason score) tumor, or 2) the combination of low and high Gleason grade cancer evolves from multiple pluri-potent stem cells that form a heterogeneous single cancer focus after the collision of the low-and high-grade cancer. Both mechanisms might also occur at the same time. If prostate cancer would be high grade at the onset of disease, the effect of screening might be limited as at an early stage the prostate cancer cells may have already acquired the capacity to metastasize.

In chapter 8 it is shown that a high Gleason score is significantly associated with age in the first screening round. The percentage of cancers with Gleason scores less than 7 decreased from 76% in men aged 55-59 to 57% in men aged 70-74; Those with Gleason scores more than 7 increased from 9% to 32% in the same age categories. Notably, in the 2nd screening round and in the control

arm no significant association between Gleason scores and age was found. To examine whether these findings could provide a clue to the underlying mechanism of prostate cancer heterogeneity, MISCAN modeling was used. Two models were developed and tested for best fit with the actual findings in the screening study: 1) A model wherein the Gleason score is allowed to progress towards a higher score in time and 2) a model wherein the Gleason score would be fixed at the time of detection by screening. The 1st model fitted best to the first and second screening round findings in the ERSPC-Rotterdam. These results support the view that dedifferentiation is the most important mechanism to explain the heterogeneity of prostate cancers. Moreover, they indicate that detection of prostate cancer by screening is early enough to intercept this dedifferentiation process. This is the first time that epidemiological evidence is found for the view that dedifferentiation represents a common pathway and occurs early in the prostate carcinogenesis.

Another striking result as identified in chapter 6 was that age, independent of Gleason score, was a significant predictor of biochemical recurrence of prostate cancer after radical prostatectomy. This finding was reported only by one article before [50]. For this finding we have no proper explanation. It might be that the generally small size, low grade cancers diagnosed in the screening arm of the ERSPC brings about a reduction in the predictive power for the other well known prognostic factors, allowing the aging effect to be a more significant parameter. We hypothesized that it could be that in older men micro metastasis occurs more frequently because of a diminished functionality of the immune system or because of yet unknown genetic alterations or increase genetic instability of tumor cells that would occur more frequently in older men. Such factors might lead to an enhanced metastatic potential of prostate cancer cells in older as compared to younger men.

In chapter 9 we tried to obt additional evidence for the dedifferentiation hypothesis at the genomic level. Array comparative genomic hybridization was performed on tumor areas with Gleason pattern 3 and 4 within the same geographically defined tumor. However, there were no significant genetic differences neither between Gleason pattern 3 and 4 in the same tumor, nor between Gleason patterns 3 of Gleason score 6 and 7 tumors. Thus, we could not obtain genetic evidence for the view that dedifferentiation actually would occur in these tumors. A previous paper from our group was also not able to find genetic differences between Gleason pattern 3 and 4 [51]. However, in the latter study, the tumor areas with different Gleason patterns were not taken from the same geographically defined tumor [51]. The study reported in this thesis has a few limitations: 1) The number of samples was limited and as a consequence it was hard to detect BAC clones with significant differences between two Gleason groups, 2) The tissue samples that were suitable for analysis had a considerable “noise” reducing the sensitivity to identify significantly different BAC clones, because an increased or decreased log2 ratio (gain or loss), compared to baseline level, pointing at a

real gain or loss that might be masked by the noise and 3) the DNA was extracted from tissue obtained by punching of the paraffin blocks. Although an H&E slide was performed to control whether the correct part of the tumor was punched out, mixing of different tumor parts and normal tissue might have occurred, as we could not check the deepest part of the tissue core that was punched from the paraffin block. Therefore it is possible that admixed normal cells may have diluted the Log2 ratios. Nevertheless, we were able to demonstrate that tumors in the 1.0-1.5 ml range with a Gleason score 6 or 7 do have genetic abnormalities that are typically found in advanced prostate cancers. This observation would suggest that this subset of tumors might be considered clinically significant tumors on genetic grounds. This was confirmed by the occurrence of biochemical failure in almost half of these tumors during follow-up compared to 13% of biochemical failure when considering all men who underwent radical prostatectomy.

In chapter 10, an attempt was made to investigate the apoptotic activity and the presence of some apoptosis-related molecules in screen-detected cancers with different Gleason patterns and volumes. Prospective cohort studies showed a significant reduction in prostate cancer detection rate in men regularly using aspirin (NSAID). Potential mechanisms include inhibition of cyclooxygenase 2 (COX-2) by aspirin which induces apoptosis [52]. Apoptosis may play an important role in the natural behavior of prostate cancer [53]. Some reports claim that prostate cancer show an increased expression of COX-2 [54]. It was therefore hypothesized that prostate cancer might be prevented or progression of the tumor might be delayed, when COX-2 inhibitors were taken. In our study, we did not see a significant difference in apoptosis level in COX-2 between prostate cancer and control samples, let alone there was a significant difference between the different Gleason patterns and tumor volumes. This was in line with another report in literature, that failed to observe a significant difference in COX-2 expression between benign prostatic hyperplasia and low stage prostate cancers [55]. Despite a significantly lower incidence of prostate cancer in aspirin users, we cannot attribute this effect to COX-2 inhibition and we consider it more likely that some other mechanism explains the lower prostate cancer incidence in aspirin users.

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Summary

The studies in this thesis were aimed at the histopathologic characteristics and therapy choices of prostate cancer detected in the Rotterdam section of the ERSPC in the screening and control arm. The studies were performed to get insight in 1) intermediate endpoints, concerning stage and grade of prostate cancer in subsequent screening rounds and the forthcoming therapy choices, 2) the efficiency of the screening protocol employed at the Rotterdam section of the ERSPC and 3) the natural biology of prostate cancer and its' possible pre-malignant lesions.

Since the outcome of the final analysis of the ERSPC is not expected until the end of 2008, intermediate endpoints were studied. In the screening arm of the ERSPC, the proportion of men diagnosed with low stage and low-grade disease was significantly higher compared to the control arm. In addition, the number of men diagnosed with low stage and low-grade disease increased further from the 1st to the 2nd screening round of the ERSPC. These proportional differences were also reflected in therapy choices. The number of men managed by watchful waiting more than doubled from the 1st to the 2nd screening round. Accompanying the increase in low stage and low-grade cancers (focal cancer on sextant biopsy and minimal cancer in the radical prostatectomy) the incidence of advanced cancers decreased. We stated arbitrarily that minimal cancers were probably indolent cancers, because of their good follow-up results. We therefore considered minimal cancers as over diagnosis. The proportion of over diagnosis in the ERSPC Rotterdam was estimated to be 48% of all detected cancers on the basis of epidemiological data. The actual incidence of minimal cancers found in prostatectomy specimens proved to be 43% in the 2nd screening round, which is in line with the estimated incidence. It is difficult to predict on the basis of prostate needle biopsy findings which cancer should be considered as clinically indolent. However, if a patient was diagnosed with a focal cancer on sextant biopsy combined with a PSA density of ≤ 0.1 ng/ml/cm^3 there was a 94% chance that the cancer was minimal in radical prostatectomy specimen. This was an attempt to predict the risk of over diagnosis pre-operatively.

In combination with the limited amount of interval carcinomas detected within the 4-year screening interval, the small proportion of advanced cancers and the increasing incidence of low stage and low grade cancers in the 2nd screening round, the conclusion may be drawn that a 4 year screening interval is short enough to restrain the development of advanced tumors.

In the screening arm of the ERSPC Rotterdam, the incidence of isolated PIN diagnosed on sextant needle biopsies was low. Importantly, the follow-up of PIN did not reveal a significantly increased risk of a subsequent prostate cancer after this diagnosis. Thus, this small subset of men would not require extensive repeat biopsies in order to demonstrate a cancer. Since atrophy is considered by some as a potential preneoplastic lesion or condition we determined the incidence of different variants in prostate biopsies and we analyzed the follow-up. The incidence of atrophy was very high, and men

with atrophy in their biopsies were not at an increased risk for a prostate cancer diagnosis at repeat biopsy or at follow-up. We conclude that men with (extensive) atrophy in their prostate biopsies do not require increased surveillance. In contrast, lesions suspicious for cancer in a biopsy were often followed by a prostate cancer diagnosis in subsequent biopsies, particularly during the first screening round. Although in the 2nd screening round these suspicious lesions predicted much less the presence of a carcinoma during follow-up we still consider it wise to continue to perform a repeat biopsy after this diagnosis.

Older men were diagnosed with higher Gleason scores in the 1st round of the ERSPC, which supports the hypothesis that prostate cancer might dedifferentiate in time from a well-differentiated tumor to a less differentiated tumor. With MISCAN modeling we were able to support this hypothesis of dedifferentiation. Furthermore, we showed that the Rotterdam screening protocol allowed the interception of carcinomas at an early enough stage to prevent dedifferentiation. Although, as a consequence of "dedifferentiation" prostate cancers are morphologically heterogeneous, this morphological heterogeneity was not reflected by genetic heterogeneity. On the other hand, screen detected prostate cancers of intermediate size (volume 1.0- 1.5 ml) displayed genetic changes that are associated with more aggressive disease. This underlines that these moderate cancers are a good target for curative therapy. If it would be possible with simple means to inhibit the growth of minimal prostate cancers by enhancing apoptosis, this would be a potential chemo preventive measure. Unfortunately, our study on apoptosis related molecules showed that this therapeutic approach is unlikely to be of benefit.
The studies performed in this thesis point without exceptions show favorable characteristics of screen detected cancers. However, they do not provide evidence that men with screen-detected cancers show decreased prostate cancer mortality.

Samenvatting

Prostaatkanker is de meest voorkomende kanker bij mannen na huidkanker en is, na longkanker, de meest frequente doodsoorzaak aan kanker in de Westerse wereld.
In Nederland zijn momenteel twee bevolkingsonderzoeken: landelijk onderzoek naar baarmoederhalskanker en naar borstkanker. Een screeningsprogramma Er komt een bevolkingsonderzoek als is vastgesteld dat een bepaalde soort kanker een aanzienlijk risico op ziekte of sterfte geeft; deze soort kanker met een test in een vroeg stadium kan worden opgespoord; de test de ziektespecifieke mortaliteit verlaagt en, de test betrouwbaar, 'betaalbaar', eenvoudig en weinig belastend is. Voordat besloten wordt of een bevolkingsonderzoek naar prostaatkanker zinvol is, moet bekend zijn of prostaatkanker met een screeningstest daadwerkelijk in een vroeg stadium kan worden ontdekt of door vroegere ontdekking van prostaatkanker minder mannen aan deze ziekte overlijden en welke test men zou moeten toepassen. Er zijn voor prostaatkanker diverse screeningstest beschikbaar. De meest gebruikte test is het prostaat specifieke antigen (PSA). PSA is een eiwit dat door de prostaat gemaakt wordt en dat in het bloed gemeten kan worden gemeten. Het PSA is verhoogd in mannen die prostaatkanker hebben. Echter, ook mannen met prostaatziekten zoals benigne prostaat vergroting en prostaatontsteking hebben ook een verhoogd PSA. Daarom is bij mannen met een verhoogd PSA nader onderzoek nodig om prostaatkanker aan te tonen. Het rectaal toucher (DRE) is een andere screeningstest net als een rectale echografie (TRUS). Om prostaatkanker vast te stellen moet men een prostaatbiopsie ondergaan. In het prostaatweefsel kan dan prostaatkanker worden aangetoond, danwel uitgesloten.

In 1994 is vanuit Nederland begonnen met een Europees onderzoek naar prostaatkanker screening (ERSPC). Het onderzoek wordt uitgevoerd in 7 Europese landen. Het doel van de ERSPC is de prostaatkanker specifieke mortaliteit te verlagen met 20%. Het betreft een gerandomiseerde studie waarbij in de regio Rotterdam ongeveer tweeënveertig duizend mannen tussen de 55 en 74 jaar zijn gerandomiseerd. Zij zijn gerandomiseerd in een screening en controle groep. De mannen in de screeningsgroep worden iedere vier jaar onderzocht d.m.v. een PSA test. Indien deze test 3,0 ng/ml of hoger is dan wordt een prostaatbiopsie verricht. De PSA test in positief in ongeveer 20% van de onderzochte mannen. Uiteindelijk wordt bij 5% van de mannen prostaatkanker vastgesteld. De mannen in de controlegroep ondergaan geen tests. Een jaarlijkse koppeling met de kankerregistratie identificeert mannen met kanker. De uitkomst tussen van deze studie wordt op zijn vroegst pas verwacht aan het eind van 2008. Omdat de uitkomst van de ERPSC nog niet bekend is, zijn in dit proefschrift tussentijdse uitkomsten onderzocht. In de screenigsgroep van de ERSPC is het deel mannen met een laaggradig en kanker in een vroeg stadium significant hoger dan in de controle groep. Tevens neemt het percentage mannen met laaggradige kankers in een vroeg stadium significant toe, in een tweede screeningsronde. Dit was onder meer

ook te zien in therapie keuzes. Het aantal mannen wat nu werd behandeld met watchful waiting (afwachtend beleid) verdubbelde in de tweede ronde. Samengaande met de toename van laaggradige kankers in een vroeg stadium, nam het aantal vergevorderde agressieve kankers af. We hebben arbitrair gesteld dat minimale tumoren (<0.5 ml, laaggradig, vroeg stadium) waarschijnlijk tumoren zijn waar een man niet aan zal overlijden, omdat deze tumoren goede follow-up resultaten geven. Daarom hebben we deze tumoren beschouwd als overdiagnose. Het berekende percentage overdiagnose in de Rotterdamse ERSPC was 48%, gebaseerd op epidemiologische data. De incidentie van minimale tumoren in radicale prostatectomie preparaten in de tweede screenings ronde was 43%, welke in de buurt komt van de berekende 48%. Het is moeilijk om vooraf te voorspellen op basis van prostaatnaaldbiopten aan welke tumoren een man niet zou overlijden. Maar als een man gediagnosticeerd is met een focaal carcinoom (≤3 mm in 1 biopt) in combinatie met een PSA dichtheid van ≤0.1 ng/ml/cm3, was de kans op een minimale tumor 94% in het radicale prostatectomie preparaat. Dit was een poging om preoperatief het risico op overdiagnose vast te stellen.

In combinatie met een beperkt aantal interval kankers die in het 4-jaar interval ontdekt zijn in de mannen van de screening groep, een klein deel vergevorderde agressieve kankers en een steeds groter wordende groep langdradige kankers in een vroeg stadium in de tweede screenings ronde, kunnen we concluderen dat een screeningsinterval van vier jaar is kort genoeg om de ontwikkeling van agressieve kankers tegen te gaan. De incidentie van PIN in the screenings groep van de Rotterdamse ERSPC was laag. Belangrijker is dat in de follow-up van deze mannen PIN geen significant verhoogd risico geeft op het ontdekken van prostaatkanker. Een herhalingsbiopsie (om de kanker beter te kunnen vinden) in deze kleine groep mannen is dus niet geïndiceerd. Prostaat atrofie wordt sinds kort ook beschouwd als een voorloper laesie van prostaatkanker. Daarom hebben we de incidentie van (verschillende soorten) atrofie onderzocht in prostaatnaaldbiopten, omdat dit nog niet bekend was, en het risico tot het ontwikkelen van prostaatkanker in de follow-up. Het bleek dat atrofie ontzettend veel voorkwam in prostaatnaaldbiopten van mannen zonder kanker. Mannen met atrofie in hun prostaatnaaldbiopsie hadden geen verhoogd risico tot het ontwikkelen van kanker in een follow-up periode van 8 jaar. Hieruit kunnen we concluderen dat ook mannen met atrofie geen herhalingsbiopsie hoeven ondergaan. Mannen met Laesies die verdacht zijn voor prostaatkanker, maar waarop niet met zekerheid de diagnose prostaatkanker op kan worden vastgesteld, hadden in tegenstelling tot mannen met PIN en atrofie wel een verhoogde risico op een prostaatkanker diagnose in een herhalingsbiopsie, met name in de eerste ronde. In de tweede ronde was het risico op prostaatkanker niet significant hoger vergeleken bij een herhalingsbiopsie in een prostaatnaaldbiopt zonder afwijkingen maar de kans was nog steeds verhoogd, daarom adviseren we nog steeds een herhalingsbiopt in mannen met een laesie die verdacht is voor

prostaatkanker. Oudere mannen hadden een significant hogere Gleason score in de eerste screenings ronde. Deze bevinding ondersteund de hypothese dat prostaatkanker kan dedifferentieren van een goed gedifferentieerde tumor tot een slecht gedifferentieerde tumor in de loop van de tijd. Een computer model (MISCAN) ondersteunde deze hypothese. Verder konden we laten zien dat het screenings programma van Rotterdam tumoren kan ontdekken in een stadium voordat dedifferentiatie optreedt. Hoewel prostaatkanker (o.a. door dedifferentiatie) morfologisch een heterogene tumor is, kon dit niet worden aangetoond in het genetische profiel van tumoren. Aan de andere kant, de tumoren van gemiddelede grootte (1.0-1.5 ml) lieten wel genetische afwijkingen zien, die ook in agressieve vergevorderde tumoren voorkomen. Dit onderstreept het feit dat een tumor van gemiddelde grootte een goede kandidaat is voor curatieve therapie. Als het mogelijk zou zijn om minimale tumoren in hun groei te kunnen remmen door middel van het bevorderen van apoptose, dan zou dit een goede chemopreventieve therapie zijn. Alleen, hoofdstuk 10 van dit proefschrift laat zien dat deze therapie waarschijnlijk geen nut heeft. De studies de in dit proefschrift beschreven staan laten zonder uitzondering allemaal gunstige tumorkarakteristieken zien van mannen die in de screenings groep gedetecteerd zijn. Ondanks deze gunstige tumoren, kunnen we nog geen uitspraken doen over een eventuele mortaliteitsreductie in de screenings groep.

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Curriculum Vitae

Ik ben geboren op 10 februari 1978 te Bolsward. In 1995 heb ik het HAVO diploma gehaald aan het Titus Brandsma college te Bolsward. Hierna heb ik de propadeuse voor biotechnologie gedaan op de Noordelijke Hoge school Leeuwarden. Met dit diploma kon ik meeloten voor geneeskunde. Omdat geneeskunde toch altijd in mijn achterhoofd bleef zitten. Ik werd gelukkig ingeloot en ben ik in Groningen geneeskunde gaan studeren. Tijdens mijn studie heb ik een keuze co-schap chirurgie gedaan. Verder heb ik onderzoek verrricht naar levertransplantatie en per/post-operatieve complicaties op een chirurgie addeling. Na het behalen van mijn doctoraal en arts-examen ben ik in 2003 begonnen bij de afdeling urologie en pathologie met het onderzoek dat de basis vormt voot dit proefschrift. Mijn onderzoek werd geleid door prof. Dr. Theo van der Kwast en prof. Dr. F.H. Schröder. Sinds april 2005 ben ik in opleiding tot patholoog. Mijn opleider is dr. Michael den Bakker samen met prof. Dr. J.W. Oosterhuis.

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Nu ik in opleiding ben, wil ik ook mijn collega arts-assistenten (AIOS tegenwoordig) bedanken. En mijn collega's in Dordrecht waar ik nu tijdelijk verblijf, voor de collegialiteit en het geduld om alles uit te leggen.

Leave heit, mem, en Djo, tige tank foar untelbarre kwestjes

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