Prostate Specific Antigen and ultrasonography in detection and follow-up of prostate carcinoma

PROSTATE SPECIFIC ANTIGEN AND ULTRASONOGRAPHY IN DETECTION AND FOLLOW-UP OF PROSTATE CARCINOMA

PROSTAAT SPECIFIEK ANTIGEEN EN ECHOGRAFIE VOOR DE DETECTIE EN HET VOLGEN VAN PATIËNTEN MET PROSTAAT CARCINOOM

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'Het begrijpen is mij te wonderbaar,
te verheven, ik kan er niet bij.'

Psalm 139 : 6

CONTENTS

PART 6 THE VALUE OF FREE TO TOTAL PSA RATIO IN SCREENING ADENOCARCINOMA OF THE PROSTATE

LIST OF ABBREVIATIONS

ACT	α-1-antichymotrypsin
BPH	benign prostatic hyperplasia
Bx/PCa	ratio between the number of men in whom prostate biopsies were taken, and the number of these men in whom a prostate carcinoma was histologically confirmed in the biopsy
DRE	digital rectal examination
F/T ratio	free to total serum PSA ratio
ng/ml	nanogram per milliliter (units of serum PSA, normally used as such in american orientated literature, equivalent to microgram per liter)
PCa	prostate carcinoma
PSA	prostate specific antigen
PSAD	prostate specific antigen density = PSA / prostate volume
PSADT	prostate specific antigen doubling time
PSAT	prostate specific antigen density of the transition zone = PSA / volume of the transition zone
PSAV	prostate specific antigen velocity
TRUS	transrectal ultrasonography

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Introduction

For detection of localised carcinoma of the prostate (PCa) various tools are available. During the last decade transrectal ultrasonography (TRUS), Prostate Specific Antigen in serum (PSA), and ultrasound guided transrectal biopsy have been added to digital rectal examination (DRE), while use of the transperineal route for prostate biopsy has been reduced, and determination of Prostate Acidic Phosphatase in serum has been abandonned. The new methods have improved the clinical detection of PCa, but continuously undergo changes to increase their combined sensitivity and specificity. Concerning the high mortality of PCa in western countries, improvement of early detection and treatment seems to be the only practical way forward [1].

The aim of the various studies is firstly to improve serum PSA and TRUS for carcinoma detection and follow-up, and secondly to define the restrictions for the clinical application of these tests. The ultimate goal of the studies is the use of serum PSA and TRUS for screening prostate carcinoma in the general population. Furthermore the role of PSA in certain therapeutic decisions, and during treatment is illustrated. TRUS is discussed regarding its role as a tool for prostatic volumetry, and the relation between prostate volume and PSA production.
PSA is a protein mainly produced in the prostatic epithelium. Increased values of PSA in serum are related to pathology of the prostate gland, but are not cancer specific. There is an important overlap in PSA levels between benign and malignant disease. Detection of serum PSA is complicated, especially because the variable binding of the molecule to carrier proteins in serum, and the various antigens used to bind the epitopes of the molecule in the detection reactions. Because of this overlap between benign and malignant disease, and because of the variability of the serum PSA determination, various factors have been analysed to improve the specificity of serum PSA to detect PCa, without decreasing its sensitivity.
In chapter 1 an overview regarding PSA in clinical use and in screening for prostate cancer is given. Several issues mentioned in this article [2] will be subject to the chapters following. Some overlap of background information among the separate chapters, which have been published as articles, is therefore inevitable.
Chapter 2 gives backround information on the determination of serum PSA by immunoassays. It describes the fundamental problems to come to a uniform PSA assay. Chapter 2.4 illustrates the variability of PSA determinations by several assays, and the confusing dilemmas with which the Dutch physician may be confronted [3].

Part 2 discusses transrectal ultrasonography as a tool for volumetry of the total prostate gland and its inner adenomatous zone. In chapter 3 planimetric and prolate spheroid (caliper) volumes are tested for in-vivo intra- and inter-observer reproducibility of total gland and inner zone volume [4]. It also presents data concerning volumetric changes which may be relevant for observations made in individuals over time. Selecting the most reproducible method, this is step section planimetry, various errors of this method are assessed in chapter 4 in a computer model of the prostate. Results are compared with observations of in-vivo volumetry in a screening population [5]. This section illustrates the theoretical limits of planimetric volumetry. In chapter 5 various other forms of transrectal ultrasonic volumetry are compared to planimetry as a standard. Planimetric, semi-planimetric, and mathematical methods using caliper measurements are applied to a screening population. Their use for clinical volumetry is evaluated [6].

Part 3 discusses the use of PSA values adjusted for prostatic volume to discriminate between benign and malignant prostatic disease in a screening population. It concentrates on that part of the population in which low and intermediate serum PSA values are found, as in this group there is a need to clarify the indication for prostate biopsy. The relation between PSA, age, prostatic volume, and symptom score in a screening population excluding PCa has been described by Bosch et al. [7,8]. There was an obvious correlation between prostate volume and serum PSA in these men. Increased serum PSA values in men with enlarged prostate glands may interfere with the detection of prostate cancer. Several mathematical methods to adjust PSA for total gland and inner zone volume are shown, using the various methods of volumetry [9].

In Part 4 a different way to study serum PSA determinations for detection of PCa is shown. Instead of single values of PSA serial measurements are analysed. Chapter 8 discusses the backrounds of single and serial PSA determinations for detection and for follow-up of PCa [10]. The question whether an elevation of serum PSA parallels or even preceeds clinical tumour progression in groups of patients and in individual cases stays central. Davidson et al analysed patients with untreated lymphnode positive prostate cancer in the Academic Hospital Rotterdam, and showed that PSA increases preceeded metastatic progression [11]. Conform this study, chapter 9 discusses the relations found between clinical observations, and serum PSA together with prostate volumetry [12].

In Part 5 a study on the relation between serum PSA and the staging of prostate carcinoma is described. It concerns a practical question whether the relatively time consuming procedure of peroperative frozen section during a radical prostatectomy can be eliminated [13].

Part 6 illustrates the development of new PSA assays during the period of the previously mentioned studies (1993-1995), and their application to a community based population. Serum PSA was found to be complexed to various serum proteins, and the ratio between free and total measurable serum PSA offered a way to increase the specificity of PSA to detect PCa in selected groups of men. Chapter 11 describes the determination of the free to total PSA ratio (F/T ratio) in a community based population [14]. In chapter 12 the value of various screening tests is evaluated in a sample of 1726 men of the general population [15]. The same subject is discussed in terms of the daily practice of the urologist by means of a simulated case

selection for biopsy in this population; the value of the F/T ratio is compared to that of PSA density and age-related PSA reference ranges in chapter 13 [16].
In chapter 14 the relation between the F/T PSA ratio, serum PSA, tumour stage and grade is shown for a selected group of patients with various stages of untreated prostate carcinoma. The clinical value of the F/T ratio is furthermore illustrated by serial measurements in men after radical prostatectomy, and in men treated by watchful waiting [17].

In the Summary the main results of the studies are discussed and combined. Their meaning for screening prostate cancer and in general practice is mentioned.

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Part 1

Prostate specific antigen Assays, and Sonography of the prostate

1

PROSTATE SPECIFIC ANTIGEN: ITS CLINICAL USE AND APPLICATION IN SCREENING FOR PROSTATE CANCER*

Chris H. Bangma, Bert G. Blijenberg, Fritz H. Schröder

ABSTRACT

Prostate cancer in most European countries is the second most frequent cancer in males and the second most frequent cause of cancer death. Prostate specific antigen is an important marker, which relates to many aspects of this disease. It has been shown that PSA is helpful in the early diagnosis of prostate cancer and in this respect is superior to the other available tests like rectal examination and transrectal ultrasonography. PSA is also helpful in staging of locally confined disease. It can be used to identify or exclude local extension of disease, if combined with T category and grade of differentiation determined on biopsy. The same parameters also give an indication of the presence of lymph node metastases, which may prevent unnecessary and invasive staging procedures in certain groups of patients with favourable prognostic factors and a low PSA value. PSA is less suitable as a marker for metastatic disease. Progression of untreated prostate cancer in various stages can be monitored by PSA. The true value of the marker in this respect is still underexplored. It may be possible that PSA will be shown to differentiate effectively between aggressive and non-progressive disease. In this respect, it could become an essential tool to identify those patients that may not require treatment at all. PSA is also a useful marker for therapy response. An elevation of PSA after radical prostatectomy indicates local or metastatic progression, which will occur within 1-2 years. PSA is an androgen dependent enzyme and decreases under endocrine treatment. It is unexplained why, in spite of its endocrine dependent character, PSA rises with endocrine independent progression of prostate cancer.

INTRODUCTION

Prostate specific antigen (PSA) is a proteinase which is produced mainly in the epithelial cells of the prostate. Although PSA is not tumour specific, it may be considered as one of the most useful tumour markers in present time medicine. This review will concentrate on the general clinical usefulness of PSA.

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PSA production

PSA is a 34,000 Dalton serine protease which can be detected in semen and male serum [1]. This single chain aminoglycoside has strong molecular similarities with kallikrein. Immunohistochemical staining shows PSA production in epithelial cells of the prostate, especially in adenomatous and in prostate cancer tissue [2]. It can also be detected in other male and female Wolffian duct derivates like the seminal vesicles, the bladder, the urethra, and the glands of Skene in minimal amounts. Other nonprostatic sources have been found, and may have clinical importance in the near future [3]. In nowadays clinical practice however, PSA can be regarded as 'prostate-specific'.

The PSA concentration in semen is more than 10^6 ng/ml. Its physiological function is liquefaction of the seminal coagulum. Up to 70 % of the secreted PSA purified from semen has been estimated to have enzymatic activity. It remains uncertain whether the partial inactivity is a manifestation of the zymogen, due to degradation by internal bond cleavages in the PSA structure, or due to inhibition by one of the serpins, the family of extracellular serine protease inhibitors which include Protein C Inhibitor and a-1-antichimotrypsin (ACT) in serum. The 'purified' seminal PSA forms the base of standards in several PSA assays.

A small fraction of PSA leaks to the blood, and can be detected in serum in a free form, and in several protein bound forms. The free form is most likely the inactive (internally clipped) form of PSA with the same molecular weight as the free active seminal form. The main complex formation occurs with the abundantly available ACT in up to 95 % of the detected serum PSA [4]. In contrast to the unbound form, this complex of ca. 90.000 Dalton cannot normally be cleared by the kidney. A serpin complex receptor in the liver has been identified which may be involved in degradation of the complex. The serum half-time value of PSA has been determined to be between 2.2 and 3.2 days [5,6]. It is likely that clearance of the free form of PSA is much faster than the clearance of the complexed form predominantly circulating in serum.

At -20 degrees Celsius the PSA molecule is stable for a long period, and can therefore be kept for research purposes [7,8]. It is customary to freeze sera at -80 degrees Celsius. The individual PSA concentration in serum shows a fluctuation during the day between 7.2 and 17.6 %, independent of prostate pathology, and for various PSA assays [9,10]. As no diurnal rhythm has been found, it is well possible that this fluctuation is caused by other factors. Physical stress does not appear to be one of these [11]. At least in young men the PSA seems to have a maximum during the spring, most likely due to hormonal influences [12].

Almost all PSA assays which determine total serum PSA, detect free and bound forms simultaneously, but not necessarily in an equimolar fashion. The fraction of free PSA in selected groups of patients with prostate cancer is smaller than in BPH [13,14]. Recent reviews on the molecular structure and immunochemical detection are available [15,16,17].

The PSA shed per gram of benign tissue has been estimated from resection of adenomatous tissue during prostatectomy [18,19]. A variation between 0.1 and 0.3 ng/ml/gram has been found. These values however do not correlate with the epithelial fraction of the resected tissue [20]. In malignant tissue a value of approximately ten times higher has been reported [21]. Tumour volume therefore is the most important determinant of the serum PSA value in patients with PCa. Tumour differentiation contributes further to the variability of the PSA production [22]. High grade intraepithelial neoplasia in itself does not account for elevated serum PSA levels, but because of its association with carcinoma elsewhere in the prostate, an increase of serum PSA is usually found [23].

PSA in benign conditions

The serum PSA concentration is influenced by manipulation and various benign diseases of the prostate. Any trauma to the prostate may increase the serum PSA [24]. Diagnostic procedures like digital rectal examination (DRE), cystoscopy, transrectal ultrasonography (TRUS), and prostate biopsy also give reversible PSA elevations. Only in 4 % of patients undergoing DRE or cystoscopy the increased PSA values were reported to be clinically relevant [25,26]. The maximum PSA levels are measured ca. one hour after the procedure [27], and return to a stable level may take up to 30 days [26]. Prostatic infarction [28, 29] and prostatitis [30] show reversible PSA increases during their clinical course. Immunohistochemical study showed a negative staining for PSA antigen in cells with a histologically active inflammation. This supports the concept of PSA leakage from the epithelial cells to the circulation [31]. After transurethral resections of the prostate the serum PSA is stabilized after approximately 6 weeks.

In benign prostatic glands the main sole parameter influencing serum PSA concentration is prostatic volume, followed by patient age. The coefficient of correlation (r) between total prostatic volume, as determined by TRUS, and serum PSA in community based populations varies between 0.55 an 0.58. [32,33,34]. The ultrasonic volume of the adenoma in benign prostatic hyperplasia (BPH) correlated similarly with serum PSA (r = 0.58) [34]. The correlation to age was much weaker, varying between 0.43 [32] and 0.25 [34].

PSA IN EARLY DETECTION OF PROSTATE CARCINOMA

Screening modalities

The identification of PSA has contributed to the methods available for early detection of PCa. Various clinical studies indicate that elevation of PSA may be due to prostate malignancy, but a large overlap with benign conditions exists. In early studies of PCa detection a cut-off value of 4.0 ng/ml in serum was arbitrarily chosen as the upper limit of 'normal' [35] to restrict the numbers of participants undergoing further investigations like DRE and TRUS.

In a recent multicenter study [36] rectal examination revealed a suspicious finding in 481 of 5.647 men with a PSA value below 4.0 ng/ml. 1 in 10 of these 481 men, who underwent biopsies, was shown to have prostate cancer. In total, the rate of prostate cancer diagnosed in this protocol in men with a PSA below 4.0 ng/ml was 48 of the total number of cancers detected (18.2 %). These results are at variance with those data resulting from the NPCDP project [37]. In this study consisting of 2.450 men, 33 % of the prostate cancers were detected in the group having a serum PSA value below 4.0 ng/ml. This finding is confirmed in the population based study of Labrie [38]. It is also confirmed by the fact, that in a very large entry representative series of prostate cancer patients treated by radical prostatectomy [39], about 1/3 of the patients who presented with clinical prostate cancer had a PSA value below 4.0 ng/ml.

Because of these findings the cut-off value of a serum PSA level of 4.0 ng/ml as an indicator for suspicion of prostate cancer seems rather arbitrary. Labrie et al. [38] have made a major effort to redefine this level of suspicion. From their data (the ROC curve is shown in FIGURE 1) they conclude that a PSA value of 3.0 ng/ml is a more suitable cut-off level for further investigation. With this cut off level about 20 % of prostate cancers would be missed.

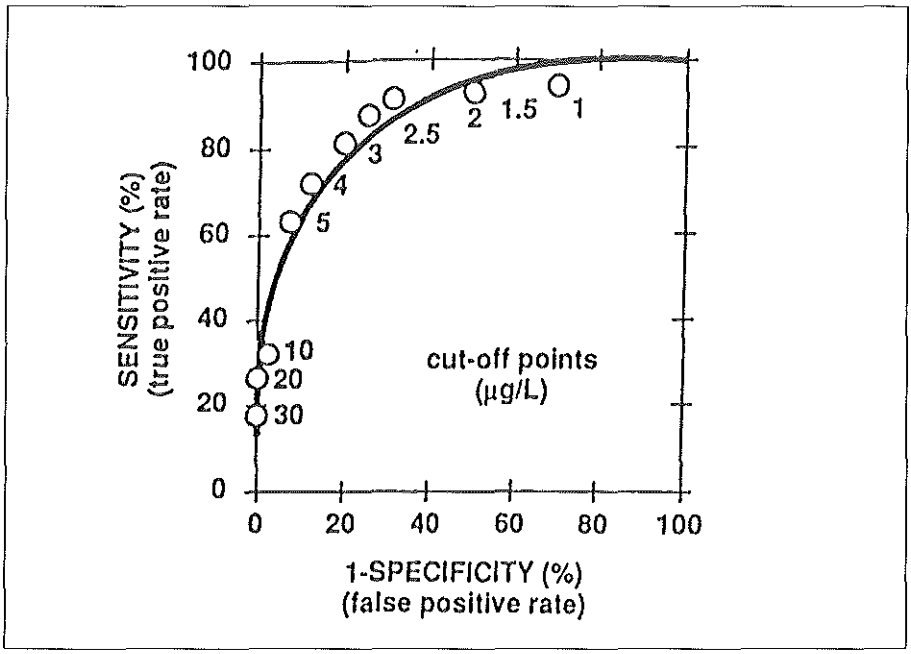

FIGURE 1

ROC curve calculated for serum PSA for detection of prostate carcinoma. Area under the ROC and its standard deviation are 87.8 +/- 3.3 %.

The Rotterdam feasibility studies of screening for prostate carcinoma. Obviously, the proper application of the available screening tests for prostate cancer to the general population still needs to be further determined. Within the European Randomized Study of Screening for Prostate Cancer (ERSPC), suitable population based data are in the process of being produced, which will eventually help to resolve this issue. A preliminary evaluation of pilot studies seems to indicate that neither rectal examination nor ultrasonography are useful or necessary if the PSA value is below 2.0 ng/ml. In these studies the detection rate amounted to 3.5 % in a population of 1,402 men aged 55 - 74 years. 4.1 biopsies were necessary to find 1 cancer. In the group of men with a PSA below 2.0 ng/ml only 4 % of all carcinomas were found, while one third of all the biopsies was carried out on the basis of false positive findings by rectal examination and/or transrectal ultrasonography. Based on these findings, it seems that rectal examination and transrectal ultrasonography may turn out not to be useful if the PSA value is below 2.0 ng/ml. If DRE and TRUS were eliminated in this very large group of almost 70 % of all participants, the biopsy rate per cancer found would fall to 2.7. In the same pilot studies the positive predictive value (PPV) of PSA alone versus rectal examination or transrectal ultrasonography as the only positive tests, is in the range of 2 fold higher (FIGURE 2). However, if the group of men who only have an elevated PSA above 4.0 ng/ml is compared to those men who in addition have an abnormal rectal examination or an abnormal ultrasonography, the positive predictive value for the combination is in the range of 2 to 3 times higher than for an elevated PSA alone. This indicates that

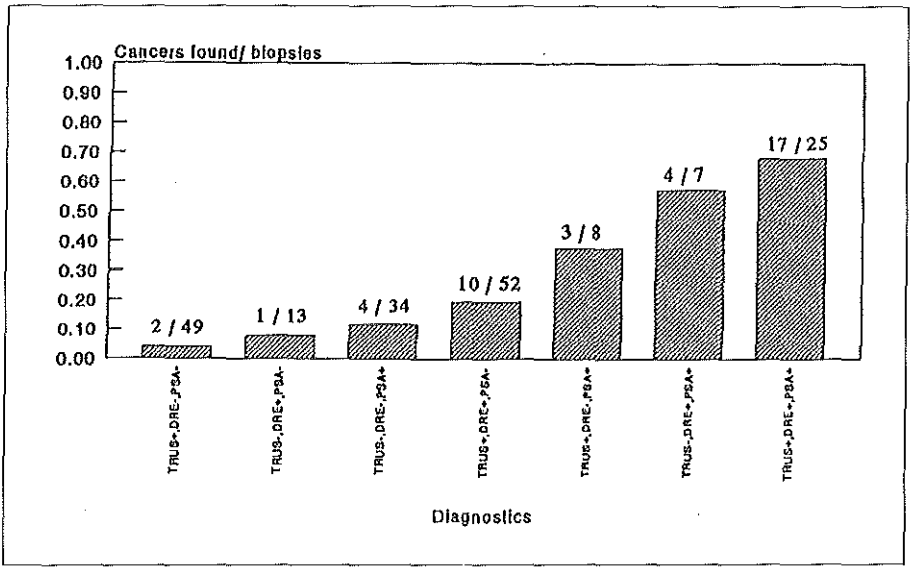

FIGURE 2
Positive predictive value of diagnostics in the Rotterdam feasibility studies for screening of prostate carcinoma; 1402 participants including 43 carcinomas.

rectal examination and ultrasonography will continue to play a role in screening for prostate cancer. The data referred to here are quite preliminary; the pilot studies are not sufficiently homogeneous with respect to the use of the screening tests. It can be expected that the ERSPC will produce more definite information on these issues within the year of 1995.

Volume adjusted PSA values
To increase the specificity of PCa detection serum PSA values have been adjusted for the ultrasonic prostate volume. The ratio between PSA and total volume (PSA density) was shown to increase the positive predictive value for PCa in selected outpatient populations if used instead of serum PSA alone [40]. In a study from the same institute, but in a different study population, a further improvement was made in predicting positive biopsies in the PSA range between 4.0 and 10.0 ng/ml by correction for the volume of the adenomatous transition zone only (PSAT) [41] (also: chapter 7). In the screening population of the Rotterdam feasibility study however, PSA values adjusted for ultrasonic volume of the total gland and adenomatous tissue hardly improved the detectability of PCa compared to serum PSA, nor did it differentiate between benign and malignant better than serum PSA in the men selected for sextant biospies by TRUS, DRE, and PSA [42] (also: chapter 6).

PSA velocity
Serial PSA measurements have been studied for early PCa diagnosis based on the concept that serum PSA in PCa is determined by tumour more than by BPH on a volume to volume base, and the growth of cancer is faster than that of BPH. In a retrospective community based study over 25 years it was seen that PSA increased significantly faster in PCa during the 5 years prior to diagnosis than BPH did [43]. In

this report the annual PSA increase (PSA velocity) of 0.75 ng/ml or more improved the specificity of PCa detection when measured over at least 4 years compared to a PSA cut-off value of 4.0 ng/ml. In a series of 121 men undergoing prostate biopsy, the short-term measurement over 450 days of PSA velocity could not stratify between benign and malignant disease [44]. In a community based population with annual PSA determinations the predictive value of the PSA velocity increased during a longer observation period, but was not better than the serum PSA for detection of PCa when taken over a maximum period of 3 years [45]. The variability of PSA determinations influences the calculation of PSA velocity too much to use annual increase based on a one year observation period as a discriminator between BPH and PCa (see also: chapter 8).
As a conclusion, volume adjusted PSA values do not increase the specificity of serum PSA in the detection of PCa. When PSA is corrected for volume, the age-adjusted PSA reference values loose their relevance because of their correlation to prostate volume. The annual increase of PSA might only become of clinical importance if an observation period of several years is implemented in a screening protocol.

PSA AS A MARKER FOR STAGING

Serum PSA shows considerable overlap between the different stages of cancer, as illustrated in FIGURE 3 of 162 patients with PCa with incidental carcinoma (T0), locally confined disease (T1,2), with locally extensive non metastatic disease (T3,4) and with metastatic disease (M+ or N+) [46]. Therefore, in order to differentiate between confined and non-confined carcinoma, PSA, combined with clinical stage, and biopsy grade have been evaluated in relation to pathological stage. In a study of 703 radical prostatectomy specimens Partin [22] constructed nomograms and probability plots combining these three preoperative parameters for predicting pathological stage including lymph node involvement.
The predicted lymph node status is not only of interest in preoperative evaluation, but also during operative procedures. Pelvic lymphadenectomy in patients clinically staged T1a to T3a resulted in negative histology in 25 % of these patients. They could be selected with an accuracy of 97 % by a combination of serum PSA, clinical stage, and prostate biopsy grade [47]. Omitting peroperative frozen section of lymph nodes on this base in patients undergoing radical prostatectomy might reduce operation time (also: chapter 10).
In all patients with a serum PSA less than 10 ng/ml [48,49], clinical stage A, or pathological grade 1 [50], preoperative bone scans were found to be negative [48]. All patients with a serum PSA of more than 20 ng/ml were found to have extraprostatic disease and/or lymphnode metastases at operation, even when their bone scan was negative [49]. Omitting this diagnostic procedure under these specific conditions is helpful in the reduction of costs. In the follow-up of patients with advanced prostatic cancer serum PSA preceded or paralleled the 6-monthly bone scans in 54 of 59 cases [51].
As tumour volume is the predominant factor influencing serum PSA, ultrasonic determination of tumour volume has been analysed to improve staging. Unfortunately ultrasonic tumour volume did not correlate well with histological tumour volume [52]. PSA density, calculated with ultrasonic total gland volume, was not a better parameter than serum PSA in predicting organ confined disease [53], although it proved to be a better predictor of positive biopsies [40]. The additional value of PSA density to preoperative evaluation in addition to staging is reported for differentiating clinically unimportant tumours (well differentiated tumours smaller than 0.2 ml) from larger tumours in T1c carcinomas [53] which are not palpable or

visible. Combining ultrasonically derived parameters like prostate volume and the volume of the hypoechogenic lesion, with biopsy grade and with PSA, an accuracy of 83 % for staging preoperative patients was reported [54]. Adding the information given by the number of core biopsies, and the volume (or length) of tumour found in the biopsies, an estimation of tumour volume [55, 56], and extracapsular extension [57] could be given.

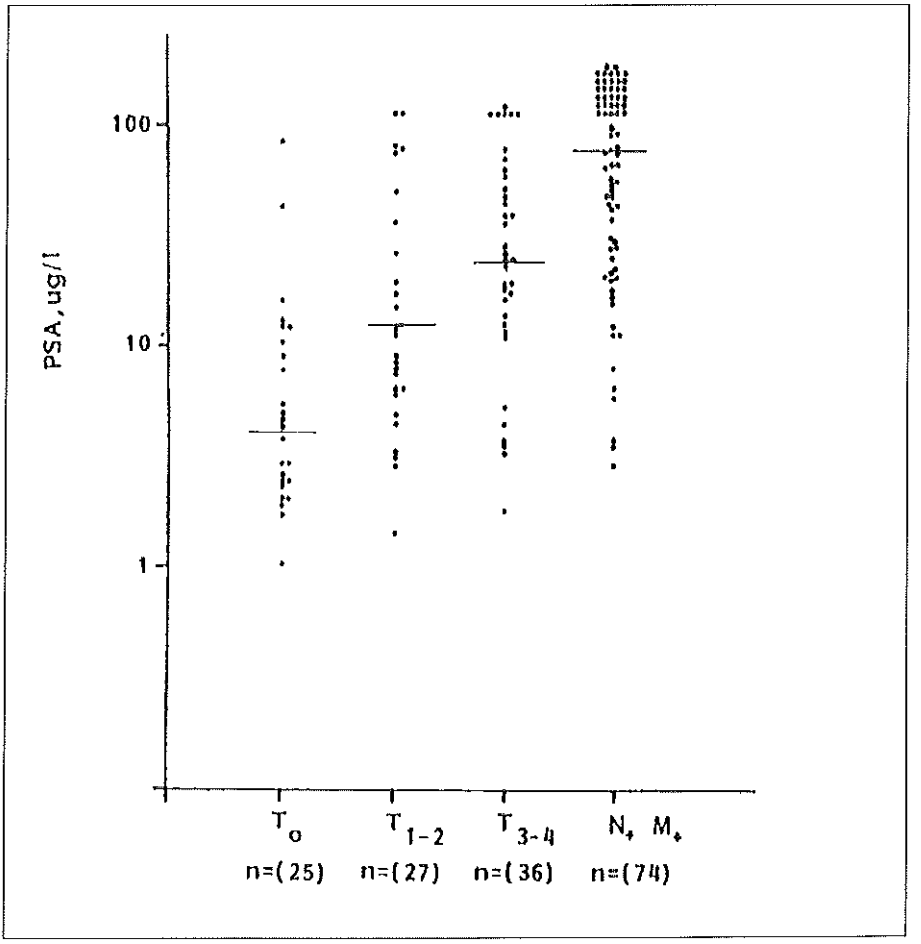

FIGURE 3
PSA levels in prostatic cancer patients according to clinical category. Lines represent median PSA values.

PSA AS A MARKER FOR PROGRESSION IN UNTREATED CARCINOMA

Local and distant progression of PCa manifests itself by changes in physical examination, or on abdominal CT or bone scan. Often clinical symptoms accompany progression. Increased levels of serum PSA, and other less specific markers like prostatic acid phosphatase (PAP) and alkaline phosphatase (AF), are correlated with higher stages. It is of interest to see whether or not PSA increase in time, follows

local cancer growth and metastases, whether it correlates with other diagnostic parameters, and if so, whether or not PSA increases predict clinical progression. Therefore serial PSA determinations were studied in men with untreated PCa.
In the retrospective study by Carter [43] serial PSA measurements provided information concerning PSA increase for different stages of PCa. The average annual increase (PSA velocity) of each group was used to calculate the time needed for doubling PSA concentration as a reflection of carcinoma growth. An exponential increase of PSA from 5 years before the diagnosis onwards was seen in patients with PCa in contrast to asymptomatic control subjects or BPH patients. PSA doubling times for benign groups were between 12 (BPH) and 17 years (controls) (standard deviation 5 years), which corresponds to cross sectional community based studies [34]. For confined and non-confined disease PSA doubling times of 2.4 and 1.8 years were calculated (without significant difference).
Serial PSA determinations of 29 patients with clinically confined PCa stage T1-2 N0 M0 were analysed in the authors' clinic and correlated to their clinical course. Local progression occured in 13 patients, but no difference in grade, stage, initial PSA level, or PSA increase was seen between non-progressive and progressive patients. The average PSA doubling time observed over a period of 39 months was 5 years (also: chapter 9).
In an even smaller study by Cadeddu [58] Gleason score and nuclear morphometry in 5 of 16 patients with confined PCa and a poor outcome (defined as metastatic disease or death of PCa) were statistically different from patients without evidence of metastatic disease.
Davidson reported on 54 untreated patients with lymphnode positive PCa in the delayed hormonal treatment arm of E.O.R.T.C.trial 30846 [59]. After a mean follow-up of 41 months the PSA doubling time in 13 men who developed bone metastases was 13 months, versus 42 months in 41 non-progressive men. Progressive patients showed a higher grade of disease. The increase of PSA after 6 to 12 months in percent of the initial PSA was predictive of progression: a PSA doubling time of 12 months was correlated with progression in 100 % of patients.

PSA AS A MARKER FOR RESPONSE UNDER TREATMENT

After radical prostatectomy

After radical prostatectomy PSA levels should be undetectable. Minimal levels of detection of serum PSA normally may range among different assays from < 0.3 ng/ml to < 0.1 ng/ml. Some assays have been developed as 'ultrasensitive' assays for detection ranges below 0.1 ng/ml, but they do not as yet have any clinical impact [60]. An increase of PSA is evidence of residual disease or recurrence. The half-life time of the serum PSA concentration during the first weeks after operation has been used to study effectiveness of the therapy [61]. In patients with residual disease, or recurrent disease later on, PSA half-life time was on average 3.0 days, which differed from patients without a recurrence (1.5 days). Differentiation between local and distant failure in patients with residual disease after radical prostatectomy was evaluated by serum PSA velocity and by urinary PSA levels. In 16 men with documented local recurrence the median PSA velocity of 0.43 ng/ml/month was statistically not different from the median PSA velocity of 2.0 ng/ml/month in 35 men with distant residual disease, unless combined with pathologic grade and stage [62]. PSA increase preceded clinical recurrence by a median of 16 months in 226 patients after radical perineal prostatectomy, and the incidence of recurrence correlated with the histological radicality of the removed specimen [63].

The urinary PSA level could not distinguish between local recurrence and distant failure in patients with an increasing PSA after radical prostatectomy [64]. Because of PSA secretion from paraurethral glands urinary PSA levels also are not adequate as a marker for radicality [65]. It might well be that in the future biochemical residual or recurrent disease will have therapeutic consequences like adjuvant radiotherapy or hormonal manipulation. However, at present treatment is usually applied on symptomatic patients, or those with proven local recurrences.

After radiotherapy

Evaluation of the therapeutic effects of external beam radiotherapy or 125-I radioactive seed implantation for confined PCa is more complicated compared to radical prostatectomy due to the lack of pathological staging of local and lymphnode status. PSA also is a limited tool, as the decrease of PSA to baseline levels (the nadir) takes on average 6 months [6]. Normal benign prostatic tissue within the radiated gland is relatively resistant to radiation, and therefore will continue to produce PSA.

Irradiation of the normal prostate will decrease PSA in 50 % of cases to undetectable levels [66]. In series evaluating effectiveness various PSA cut-off levels have been chosen to define a complete and durable response. The likelihood of a disease free status after 4 years was significantly lower when a PSA nadir of 1.0 ng/ml was used as an end point rather than when clinical evaluation by physical examination and a bone scan was used [67].

The PSA kinetics after radiotherapy provided little useful clinical information in a study of 154 patients [68]. The PSA half-life time as a result of therapy was not related with subsequent PSA doubling-time in recurrent disease. Doubling times appeared to be longer in low grade tumours. PSA recurrence preceeded clinical recurrence in the majority of patients by more than 40 months [69].

The level of serum PSA has been reported as the only factor predictive of local progression in patients after radiotherapy who underwent salvage radical prostatectomy [70].

The effectiveness of adjuvant radiotherapy directly after radical prostatectomy of pT3 N0 M0 PCa is under evaluation in the E.O.R.T.C.trial 30913. In small uncontrolled studies PSA decreased, often to an undetectable level, after immediate adjuvant radiotherapy [71], and this delayed symptomatic tumour recurrence [72]. Delayed radiotherapy for histologically proven local recurrences also turned out to be effective in decreasing PSA. These studies illustrate the value of PSA as a marker after adjuvant radiotherapy.

After endocrine treatment

The growth of the prostate and the production of PSA in epithelial cells is dependent on the intracellular conversion of testosteron into dihydrotestosteron (DHT). By blocking this mechanism 5-a-reductase inhibitors can reduce serum PSA levels during the treatment of BPH [73]. The reduction of prostate volume and PSA not necessarily parallels clinical improvement as assessed by symptom score [75]. There is no evidence yet that endocrine treatment for BPH obscures detectability of PCa because of the reduction of PSA levels [76].

Medical castration by LHRH agonists also has shown to reduce serum PSA levels by decreasing testosteron to castration levels [74]. In PCa surgical or hormonal castration will also decrease serum PSA to a nadir within 6 months [6]. In lymph node positive patients (pN1-3 M0) undetectable PSA levels have been associated with a 5 % local or distant disease progression, proven histologically or by bone scan,

after 5 years, versus nearly 100 % for those patients who never reached undetectable PSA levels after hormonal ablation [77]. Patients with bone metastases after castration showed a longer remission duration (median 42 months) when their PSA nadir reached a level below 4.0 ng/ml.
After androgen ablation a subset of tumour cells persist, which not only determines the fate of the patient with respect to his carcinoma, but also independently continues to produce PSA, or PSA like substrates, as shown by immunohistochemical staining. These remaining cells have been shown to be predominantly undifferentiated, and are therefore not able to perform the normal functions of a more mature cell. The unpredictable way of PSA production of these cells makes PSA less suitable as a prognostic marker. It is unexplained at this time why PSA, being an androgen dependent protein, rises in most patients with hormone independent, progressive tumours. PSA is presently being evaluated as a response parameter in phase II chemotherapy studies.

CONCLUSION

Serum PSA is an accepted parameter to select men for prostate biopsy to detect prostate carcinoma. In screening, a PSA level of 2.0 ng/ml may become a criterium for the application of DRE and/or TRUS. Serum PSA is an excellent marker for radicality after radical prostatectomy. Changes of PSA may be used for early detection of disease progression in selected groups, and for identification of progressive disease. Further development of PSA assays, and clinical evaluation may provide information needed for better differentiation between aggressive and non-aggressive cancers and their treatment.

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2

IMMUNOASSAYS FOR DETERMINATION OF PROSTATE SPECIFIC ANTIGEN

Prostate specific antigen (PSA) is a protease mainly produced in the epithelial cells of the prostate, and excreted for liquefaction of the semen. A minor fraction of PSA also can be detected in the blood as a result of leakage from its production site. There it circulates in a free form with a molecular weight of approximately 30 kDalton, and complexed mainly to α-1-antichymotrypsin (ACT) of 90 kDalton. PSA can be measured by immunoassays, which are based on a reaction between an antigenic site or epitope (Ag) on the molecule, and an antibody (Ab). To illustrate the difficulties of this identification, a short survey on the basic principles of immunoassays in relation to PSA is given.

2.1 BASIC PRINCIPLES OF IMMUNOASSAYS: QUANTIFICATION AND ERROR MODEL

An immunoassay is a measuring system which is preferably used for the investigation of proteins, hormones, and other materials with biological activity. The measurement is directed towards a biological function, or to the chemical composition of the material investigated. To express a quantity numerically, a measuring unit must be selected. At the same time this furnishes the measuring scale. During the measurement an analytical procedure converts the material property into a measurable signal. In simple assays an antigen (Ag) on the material investigated reacts with an antibody (Ab). This complex may generate a signal directly, for example by nephelometry, or indirectly by a subsequent detection reaction. The signal is then related to a quantity which represents the standard. By using standard specimens of various concentrations (calibrators), a calibration function is obtained. The measurements of the material are related to this function, and the results can be calculated (FIGURE 1).

The quantity of the material, or the concentration, refers to a specific chemical entity. With simple low molecular weight organic compounds it is generally possible to give an exact chemical definition of this material. In larger molecules, like serum proteins, several difficulties may occur. It may happen that distinction from similar molecules is not possible, or, on the other hand, that unimportant minor molecular modifications of the molecule prevent detection. Alterations of the physical state of a macromolecule may occur by aggregation, changes of the matrix in which the molecule is dissolved, changes of the tertiary or quaternary structure of the protein, and other factors. All these changes may cause alterations in the configuration and distribution of the antigens, or epitopes, and therefore the Ag-Ab reaction.

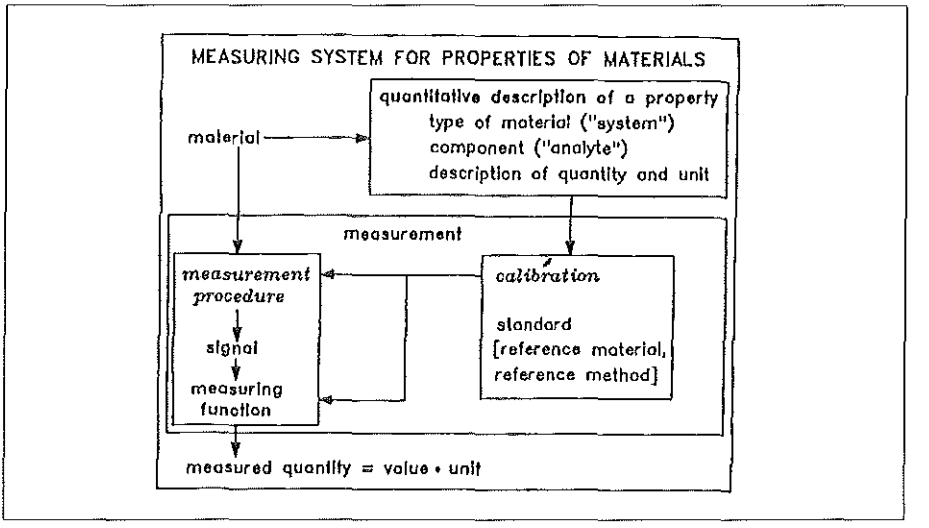

FIGURE 1
Measuring system for properties of materials [Büttner 1991]

When the chemical structure of the material determined is not known, or differs minimally from patient to patient due to genetic variability, quantification of the material by means of measurement of its mass is impossible. In that case a detailed operational definition of the standard and its units have to be defined, including the preparation procedure of the standard.
The analytical procedure converts the quantity of the defined material into analytical signals. In immunoassays the Ag-Ab reaction can be influenced by numerous factors other than those concerning the analyte (antigen). The antibody may be polyclonal, and its specificity for different epitopes (heterogeneity) may lead to differences in signal strength. When monoclonal the antibody may show a lower affinity to the analyte.
The Ag-Ab binding reaction itself is influenced by factors concerning the thermodynamics and kinetics. The serum matrix in which the reaction occurs, consists of multiple known and unknown substances, often varying between individuals or even within one individual at different times. The matrix (and also its absence) influences the state of the antibody and antigen, it may provoke cross-reactions with substances in the matrix, and effect the Ag-Ab reaction.
A central part of a measuring system is the calibration, this is the parallel measurement of standards along the sample sera. Classically a primary standard is selected, in which the pure analyte is dissolved in pure solvent, and its concentration is expressed in SI-units. Absence of the protective qualities of the matrix however may lead to considerable difficulties in immunoassays. Therefore matrixed standards have been produced to simulate the clinical situation. On the other hand the presence of a matrix makes it impossible to weigh or store the standard in the pure form, as lyophilization may denaturise the matrix. The matrix standard may even become infected, and changed by bacterial proteases. In such cases a reference material with reference method established values might better be used as a secondary standard.
The measuring system shows an error model according to FIGURE 2. The conventional true value in this figure can only be determined by calculation from

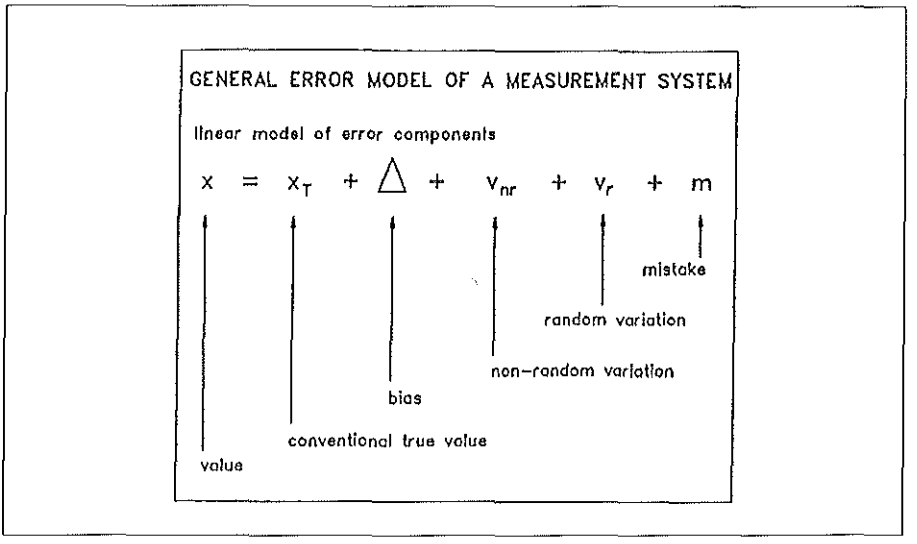

FIGURE 2
General error model of a measurement system [Büttner 1991]

the other variables. Some of the error components can be eliminated, some are unavoidable. Bias refers to the constant unavoidable part of the systemic error, which can be measured experimentally when appropriate standards are available. In immunoassays however, such standards or reference materials often are missing. The non-random variation refers to the variable part of the systemic error, for example the influence of temperature on the reaction, or the aging of the measuring instruments. These influences can be eliminated. The random variation or random error can be reduced, but never completely be eliminated, as it varies in an unpredictable manner. The deviation refers to the human error influencing the measuring system.
The result of various errors are seen in the performance characteristics of the immunoassay, as established by laboratory studies [Vadlamudi 1991]. These characteristics include reproducibility, recovery, linearity, stability, specificity, and sensitivity.
Reproducibility comprises the intra-assay variation (within run reproducibility), the inter-assay variation (between run reproducibility), the variation between reagent lots, and the reproducibility between laboratories. For determination of intra-assay variation usually three standard sera of low, medium, and high concentration are run several times, and the results are expressed as the mean, the standard deviation (S.D.), and their ratio the coefficient of variation (C.V.) expressed in %. A C.V. of 5-10 % is acceptable [Dalen 1993]. For the inter-assay variation the same standards are run over several days. The lot-to-lot variation compares the reagents of different lots of the same assay, also expressed in the C.V. Here, a C.V. of 5-8 % is acceptable: if this C.V. is exceeded, the manufacturer should normally reject the abberant lot. The reproducibility between laboratories in The Netherlands is organised on a voluntary base in a national quality control group for clinical laboratories, the L.W.B.A. (Landelijke Werkgroep Bindingsanalyse van de Stichting Kwaliteitscontrole Ziekenhuislaboratoria) (see further section 2.4).

Recovery shows the difference between the expected amount of a substrate and the amount actually measured by the assay when a purified form of the substrate is added to a purified amount of matrix. It therefore illustrates the effect of the matrix on the substrate. An acceptable difference lies within 10 %.
Linearity represents the correlation of the measurements of diluted specimens with a high substrate content with the regression line through these values. A false result often can be seen in clinical specimens with extremely high elevated concentrations of the substrate, also indicated as the hook effect.
Stability of assay reagents from different lots and of the various calibrators should be tested over a longer period of storage under different conditions. The same applies for storing the serum sample. Appropriate storing conditions assure stability of the assay reagents of at least one year, and of the serum of several years.
The analytical sensitivity shows the lowest measurable concentration in a specimen which can be distinguished from the 0 ng/ml calibrator (95 percent confidence interval).
The specificity of the assay relates to the quality of detecting the substrate without cross-reaction to other commonly interfering substances like endogenous metabolic products or drugs. Recovery after adding these substances illustrates the specificity.

2.2 DESIGN OF THE PSA IMMUNOASSAYS

The interpretation of results obtained by immunoassays is effected by many factors, especially by assay sensitivity and specificity. Basically there are two assay types: the (less sensitive) non-label inhibition method, and the sandwich method. The sandwich method can only be used for antigens large enough to bind two antibodies simultaneously. Most of the PSA assays make use of this sandwich design. The sensitivity of the sandwich method, and also reaction velocity (assay time) is proportional to the antibody concentration. Large amounts of labeled antibody may shorten assay time to several minutes, and increase sensitivity up to 100,000 fold. Specificity of the sandwich assay is improved by the use of two antibodies instead of one in the inhibition method.
In PSA assays the PSA molecule usually is first captured by a solid phase antigen. After that a second antibody, labeled by a radioactive or fluorescent or enzymatic marker, binds the PSA molecule on a different epitope. At least five epitopes have been described, and tenfolds of antibodies are known. PSA assays therefore differ mostly in their antibody selection and label. The situation has become more 'complex' by the discovery of serum PSA bound to serumproteins which may alter or obscure antigenic epitopes. The a-2-macroglobulin in serum completely obscures all known epitopes, rendering that portion of the serum PSA undetectable.

2.3 STANDARDIZATION OF PSA IMMUNOASSAYS

The difficulties in comparing one PSA assay to another have led to the call for standardization, as with other immunoassays. This can be discussed under two categories: analytical standardization, and clinical equivalence.
Clinical equivalence between PSA assays, measuring PSA in identical groups of patients, is expressed in the coefficient of correlation for a defined PSA range. Historically, assays have been compared to the Hybritech Tandem-R assay. For various reasons including marketing considerations, antibody selection, and choice of calibrators, it was not possible to choose a golden standard.
To measure a concentration of a substance in a test sample, the substance should be of a single molecular structure. In case of PSA the molecular weight and aminoacid sequence is known from analysis of purified seminal PSA. Although seminal PSA

shares at least some of its epitopes with serum PSA, it is physiologically and structurally different. The seminal PSA is predominantly unbound, and shows a proteolytic action in liquifying the semen. This in contrary to serum PSA, which is predominantlly complexed to ACT, and does not demonstrate proteolytic activity. This also means that recognition of the complexed state of PSA has added a further problem in standardization, as serum PSA appears to be a mixture instead of a single molecular entity. The use of purified PSA for preparation of a standard is therefore doomed to evoke problems, even when a correct and stable matrix would be found. Preparation of a standard of purified PSA complexed to ACT with purified PSA, in a physiological ratio (F/T ratio) between these two components, seemed necessary. At Stanford University Stamey et al. realised such a standard, with a F/T ratio of 10/100. This standard shares the commonly used epitopes of PSA assays with the serum PSA in vivo, and it was agreed on that it would be launched in the near future as an international standard.

With the 'complex' matter of free and bound serum PSA comparability of assays seemed to be further away than ever. The ratio between these two predominant PSA manifestations varies among relevant clinical conditions, and migth be used for differentiating between benign and malignant disease. This means that when an assay measures the total amount of serum PSA, the ratio between free and total PSA (F/T ratio) depends on the characteristics of the assay like the test kinetics of measuring both forms of PSA, and the F/T ratio of its calibrators. This item concerning the use of calibrators has been discussed in the literature [Graves 1993] and criticized, as the theoretical problems for calibrators seemed to overshadow the world-wide practical efforts to come to standardization [Stamey 1995]. In theory PSA assays measuring free and bound fractions of PSA equimolarly would give the most accurate representation of total serum PSA. In vivo comparison between polyclonal and monoclonal assays, and between equimolar and non-equimolar assays however has not shown a clinical advantage of one over the other yet. It was calculated that using calibrators of only PSA complexed to ACT would give a maximal difference from the molar value of 1-5 % only.

PSA-ACT might be more adequate than purified seminal PSA as a primary reference material. Secondary reference human sera will create significant logistical problems for international standardization in terms of production and storage, without solving the actual problem: the impossibility to create a standard for a mixture of PSA molecules of which the quantified relations may be of clinical importance. This may lead to the use of different tests for determination of the total serum PSA, of the ratio between free and complexed PSA forms, and even of the ratio between various PSA isomolecules when these turn out to have different physiologic or pathologic functions.

2.4 VARIABILITY OF VALUES FOUND FOR PROSTATE SPECIFIC ANTIGEN USING SIX METHODS OF ANALYSIS

To illustrate nowadays clinical situation a study was done at the University Hospital Rotterdam halfway 1993 to show the variability of PSA values between six PSA assays, regularly used in The Netherlands [Bangma 1994, see Appendix]. From a quality control study of the LWBA it was known that the variability of serum PSA of two test sera differed considerably between 90 voluntarily participating clinical laboratories in The Netherlands, using ca. 10 different PSA assays (FIGURE 3). The assays used in the Rotterdam study were the Pharmacia Delfia PSA (Kabi Pharmacia,

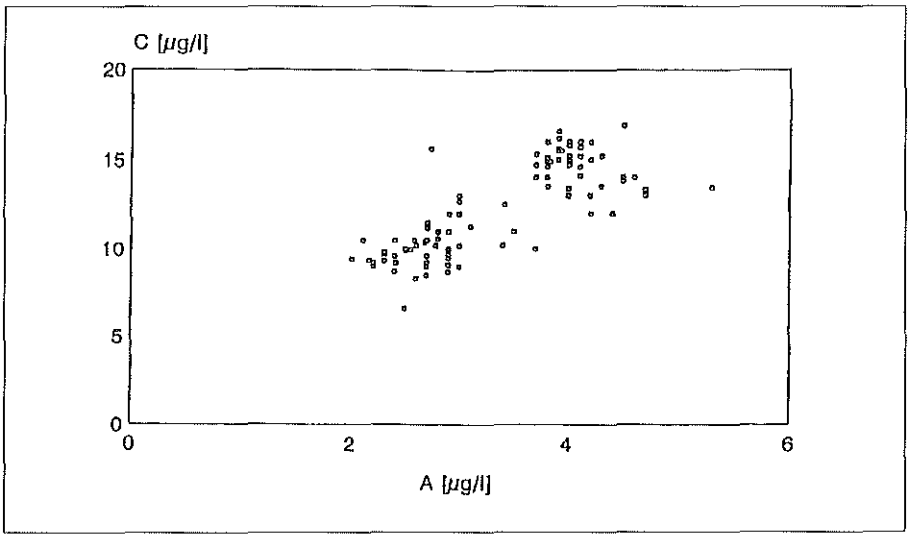

FIGURE 3
Results of serum PSA determinations of two testsera (A and C) in 90 laboratories

Woerden), the TOSOH AIA-1200 PSA (Eurogenetics, Moordrecht), the Abbott IMx PSA (Abbott, Amstelveen), the Corning ACS 180 PSA (Ciba-Corning Diagnostics, Houten), the Immulite PSA (Diagnostic Products Corporation, Apeldoorn), and the Hybritech Tandem-R PSA (Eli Lilly Hybritech, Nieuwegein).
These six different methods of PSA analysis were applied to sera of 19 participants of a the Rotterdam feasibility study for screening prostate carcinoma, 20 newly diagnosed patients with a prostate carcinoma and with a serum PSA between 0-20 ng/ml, and 15 patients 4 to 41 months after radical prostatectomy. All sera were determined once, according to the producers prescription. The Hybritech Tandem-R value was chosen as the standard to compare the methods of analysis for historical reasons [Myrtle].
FIGURE 4 shows the results of serum PSA of 19 outpatients with histologically proven prostate cancer. For each assay a regression line was constructed through the PSA values of those 19 sera. It was appreciated that these lines do not reflect the variation of the individual assays, as they were not given with the intention to

TABLE 1
Results of serum PSA determinations of 20 participants of a community based population, showing mean values and the range between brackets

	mean	minimum-maximum
Tandem-R	2.5	0.7 - 5.8
IMx	2.7	1.0 - 4.8
Delfia	3.2	1.2 - 6.4
ACS	4.6	1.1 - 8.6
AIA	2.8	0.9 - 5.6
Immulite	3.4	1.0 - 6.3

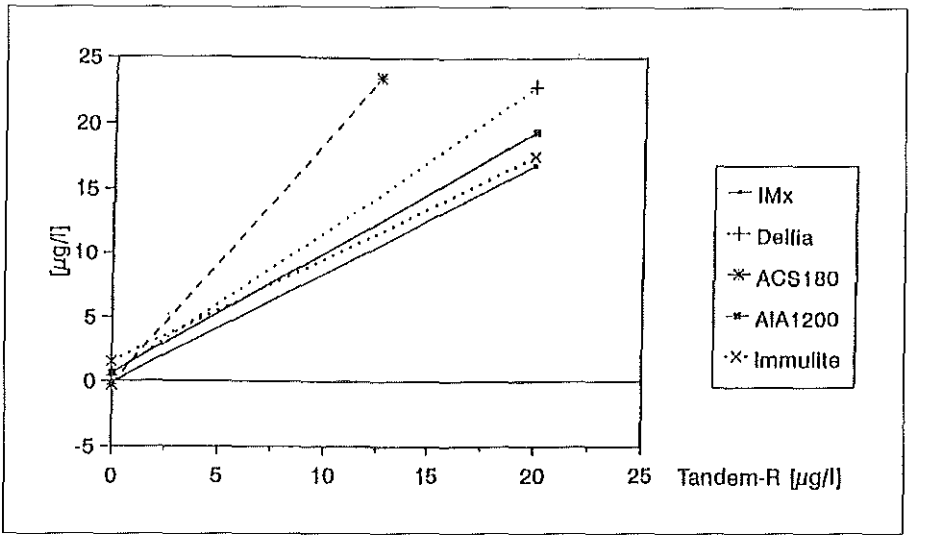

FIGURE 4
Results of serum PSA determinations with 6 assays (Delfia, AIA-1200, IMx, ACS 180, Immulite and Tandem R) of 19 outpatients with prostate carcinoma, with Hybritech Tandem-R as a standard.

compare the individual assays with each other. Around a standard of 4.0 ng/ml a range of 3.3 to 7.2 ng/ml was found; around 10.0 ng/ml there was a range of 8.7 to 18.5 ng/ml. The range of PSA values for 20 participants of the community based population was small (TABLE 1). After radical prostatectomy the different methods of analysis agreed completely with each other in 5 out of 15 patients with respect to serum PSA, whether detectable or undetectable (TABLE 2). When PSA was detectable, values varied between 0.1 and 0.5 ng/ml.
The variability of serum PSA between different PSA assays in these three clinically relevant groups of patients shows that confusion concerning PSA results is rised easily when interpretation of PSA values is done without basic knowledge of these assays.

TABLE 2
Results of detection of serum PSA with 6 PSA assays in 15 patients after radical prostatectomy.

detection							
negative	6	5	4	3	2	1	0
positive	0	1	2	3	4	5	6
incidence	4/15	5/15	2/15	1/15	1/15	1/15	1/15

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Part 2

Aspects of Prostate-volumetric Sonography

3

OBSERVATIONS ON THE RELIABILITY AND REPRODUCABILITY OF TRANSRECTAL ULTRASONIC VOLUME MEASUREMENTS OF THE PROSTATE: THE EFFECT OF EQUIPMENT AND OBSERVERS

A. Qais H.J. Niemer, Peter J.T. Davidson, Chris H. Bangma, Wim C.J. Hop, Fritz H. Schröder

ABSTRACT

Objectives. To determine the reliability and reproducability of transrectal ultrasonic volume measurements of the prostate.

Methods. In vitro and in vivo comparison of transrectally measured prostatic volumes were made between a 5 MHZ Aloka (chair mounted) and a 7 MHz Bruel and Kjær ultrasound scanner. Intra- and interobserver variability of both total prostate and inner zone volume was assessed using the 7 MHz Bruel and Kjær sector scanner. Volume measurements were calculated by step section planimetry and using the prolate spheroid formula.

Results. This study showed a considerable difference in volumetric measurements between the two machines in vivo. There were no significant differences between the planimetry and prolate spheroid techniques of volume measurements for the total prostate. For the inner zone, only the planimetry technique was more reproducable. Length showed the least interobserver reproducability; height and width were reliable measurement when repeated.

Conclusions. Although measurements of total prostate volume are generally reproducable, there can be inherent differences between machines. In caliper measurements prostatic length has the least interobserver reproducability in comparison to width and height. Planimetry is the only reproducable method in volumetry measurements of the inner zone. In consecutive measurements, total prostate and inner zone volume change can be regarded as significant when exceeding resp. 26% and 40%.

Submitted.

INTRODUCTION

Prostate volume measurements are used in preoperative decision making, when deciding between a transurethral or open prostatectomy for benign prostatic hypertrophy. They are required in diagnosis, when calculating prostatic specific antigen density [1] [2]. Further, serial prostate volume measurements have been utilised in the monitoring of response to therapy [3] [4] [5] [6]. There exist many different techniques of measuring prostatic volume. The traditional, and still most commonly employed, method of prostatic volume estimation is by digital rectal examination (DRE). DRE has been shown to be inaccurate in the estimation of prostatic volume [7] [8]. Ultrasound estimation has been shown to be superior [9]. The planimetry and prolate spheroid techniques of ultrasonic volume calculation have been found to correlate most closely with that of radical prostatectomy specimens [10].

At the authors' institution serial volume measurements are utilised in ongoing studies. As a part of a change from a Aloka USI-82 module with a 5MHz ASU8T chair type transrectal radial scanner in 1991, to a Bruel and Kjær (B&K) type 1846 module with a 7MHz type 8551 multiplanar transrectal sector scanner a comparison of volume measurements by the two machines was made. Furthermore, the reproducibility of both planimetry and prolate spheroid volume measurements by the 7MHz probe was assessed for the whole prostate and the inner zone.

MATERIAL AND METHODS

In these studies, prostate volume measurements were calculated by two different techniques. The first was the step section planimetry technique. Transverse prostate images at 5mm intervals were made. The geometrical average of two surface areas multiplied by 5mm gives the volume for each step, and the sum of these gives the total prostate volume. The second technique of volume measurement assumes the prostate to have the shape of a prolate spheroid, and by measuring the width (W) and height (H) as measured at the point of greatest transverse diameter , the volume was derived from the formula $V = 0.52 \times W \times W \times H$.

We performed eight separate studies. In our first study the two machines were compared *in-vitro*. Planimetric volume measurements of 10 catheter balloons in a water bath were performed by both machines. The balloons were filled with measured volumes of water, varying from 10 to 70 ml. This was followed by a second *in-vivo* study. One hundred consecutive patients referred for transrectal ultrasound of the prostate had planimetric volume measurements by first the 5MHz Aloka, and then the 7MHz B&K.

Our third study was a separate group of 10 patients coming for a radical prostatectomy having had similar planimetric measurements by the two machines preoperatively. The weight of the fresh radical prostatectomy specimen, after removal of the seminal vesicles at the base, was compared to these preoperative volume measurements. The weight of the prostate in grams was directly compared to the volume in cubic centimeters, as the specific gravity of prostatic tissue is 1.050 [11].

The inter- and intra-observer reproducability of volume and caliper measurements with the 7MHz B&K was then assessed. In study four, a further *in-vitro* study, using the same balloon model, two volume measurements were made of each balloon. Between measurements the stepping device was dismantled and reassembled. Then, in study five, in 42 consecutive patients, *in-vivo* measurements of length, height, width and planimetric total volume of the prostate were performed first by one observer (A), and then by a second (B). Between observations the ratcheted stepping

device was again dismanteled and reassembled, but the probe itself was not removed from the patient. Length was calculated as the distance from the apex to the bladder neck in the midline image of the sagittal plane. The interobserver difference was evaluated for length, height, width and volume measurements derived by both the planimetry and prolate spheroid techniques.
In study six, intraobserver differences were also assessed in the same manner, in 22 patients by observer A and then in a further 22 patients by observer B. The difference between the two consecutive measurements was assesed for length, height, width, and total prostate volume (by both techniques).
The (study seven) inter- and (study eight) intraobserver error was also calculated for caliper and volume measurements of the inner zone in respectively 47 and 52 consecutive patients. The inner zone is identified as the cranioventral more hypoechogenic heterogenous area of the prostate, and complies with the periurethral tissue, anterior fibromuscular stroma and the transition zone as discribed by McNeal [12]. In the stastistical analysis Pearson's correlation coefficients are given. Systematic differences between machines and observers were assessed using the paired t-test. In these analyses all volume measurements were transformed logarithmically to obtain approximate normal distributions.

RESULTS

Comparison between machines (study one to three)
In study one, the *in-vitro* balloon study, there was a good correlation (r = 1.00) between the 5MHz and 7MHz machines (FIGURE 1A). However on average, the B&K 7MHz measured a 5% greater volume than the Aloka 5MHz machine (p=0.02). Both machines measured a volume that correlated well with the known amount of water instilled in the balloon.
In study two, the *in-vivo* study, in plotting measured volumes Aloka 5MHz versus B&K 7MHz, the scatter of points became more spread out with increasing prostatic size. This indicates that measurement errors were related to the size of the prostate. The logarithmically transformed values were plotted and analysed (FIGURE 1B).

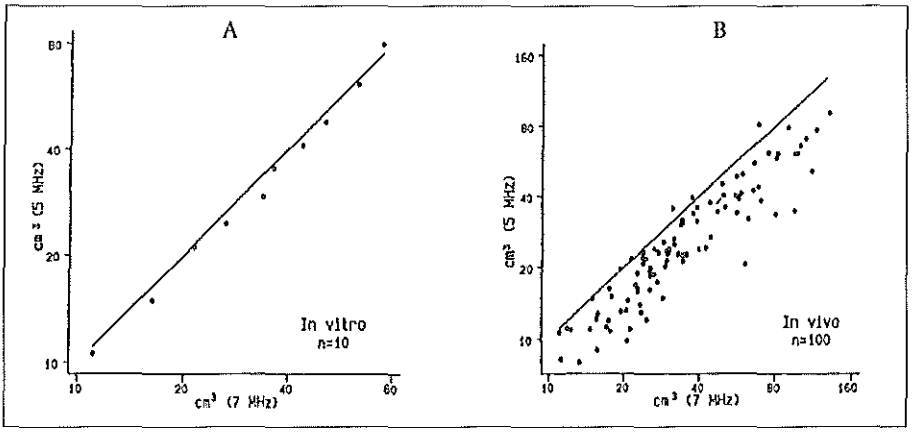

FIGURE 1
Comparison between planimetric volume measurements with the 5 MHz and 7 MHz probes, with line of identity drawn
A) in vitro balloon measurements
B) in vivo whole prostate volume measurements

Although the correlation between prostate volume measurements by the two machines was good (r =0.93), the 7MHz machine measured on average a 40% greater volume than that by the 5MHz machine (p<0.001).
In study three, of the 10 patients that underwent a radical prostatectomy the weight of the fresh specimen was always greater than the volume measured by the 7MHz

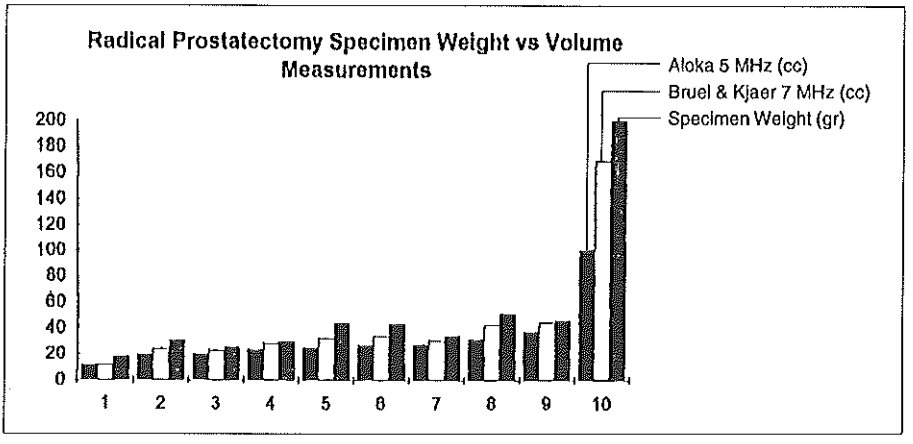

FIGURE 2
Comparison between TRUS volume measurements with the 5 MHz and 7 MHz probes and the radical prostatectomy specimen weight in 10 patients

machine, and the measurement by the 5MHz machine was in all cases less than that by the 7MHz (FIGURE 2).

Intraobserver in-vitro variation (study four)
The correlation was very strong between the two in-vitro measurements (r =1.00), with a 1:1 volume relationship and minimal scatter of values around the line of identity (FIGURE 3A).

Interobserver in-vivo variation of the total prostatic volume (study five):
Correlation between observers was good for both the planimetry and prolate spheroid methods of volume measurement (FIGURE 3C). The mean difference between observers was 2% when using the planimetry technique, and 3% by the prolate spheroid formula technique. Both differences were not significantly different from zero, indicating that there were no systematic differences in measurements between both observers. However, the standard deviation of the differences in the individual measurements was somewhat higher, with the mean varying from 13% (range -21%/+30%) between two observers for the planimetry method, and 16% (range -34%/+40%) for the prolate spheroid method (TABLE 1). There was also a good correlation between observers for width, height, and length. While there was no significant difference between observers in the measurement of width and height, there was a significant difference in the measurement of length (p<0.001). The poorer reproducibility of length is also reflected in a greater standard deviation of the difference.

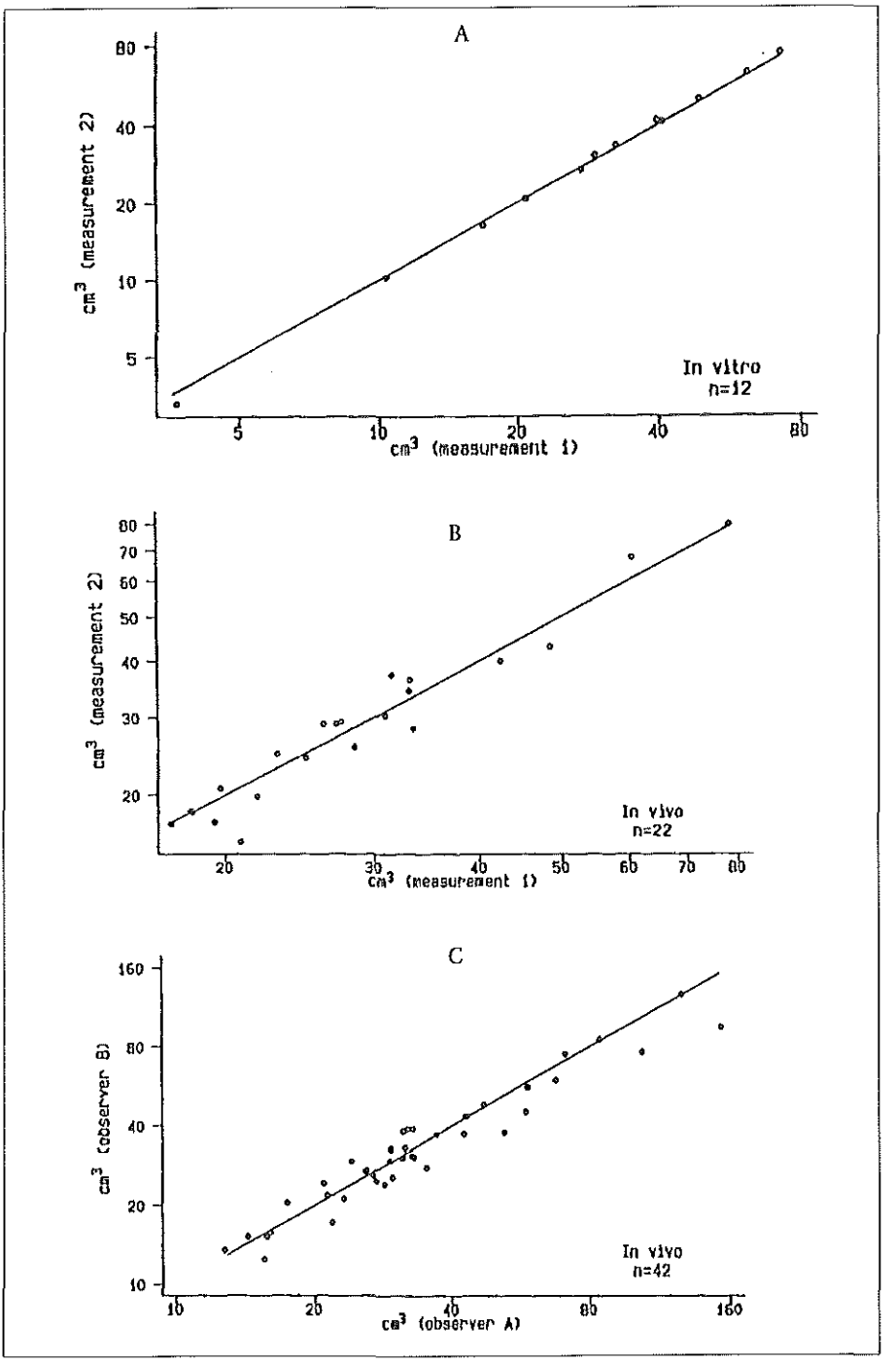

FIGURE 3

7 MHz TRUS total prostate planimetric volume measurements

a) Intraobserver variation in vitro

b) Intraobserver variation in vivo

c) Interobserver variation in vitro

TABLE 1
Variations between observers in 42 patients TRUS measurements of the whole prostate

	Correlation coefficient	*Mean Difference* *	*Stand. Deviation of the Diff.* *	*Range of Difference**
Planimetry	0.97	1.4 cm^3 (2%)	6.5 cm^3 (13 %)	- 6.7/+28.3 cm^3(-21/+30 %)
Prolate Spheroid	0.95	0.9 cm^3 (3%)	7.2 cm^3 (16 %)	-20.5/+22.3 cm^3(-34/+40 %)
Width	0.94	0.01 cm (0%)	0.29 cm (6 %)	- 0.5/+ 0.7 cm (-14/+19 %)
Height	0.92	0.04 cm (2%)	0.28 cm (9 %)	- 0.8/+ 0.6 cm (-20/+21 %)
Length	0.90	0.27 cm (3%)**	0.45 cm (16 %)	- 0.6/+ 1.4 cm (-34/+40 %)

* : difference as percentage of measurement by observer A

** : significantly ($P < 0.001$) greater than zero

Intraobserver in-vivo variation of the total prostatic volume (study six): The correlation between volume measurements for a single observer was good for the planimetry and prolate spheroid techniques (FIGURE 3B). The intraobserver coefficients of variation were 8% by the planimetry technique and 10% by the prolate spheroid technique for observer A, and 11% and 7% for observer B. The intraobserver coefficients of variation for width, height and length were 4%, 7% and 6% respectively. In all of these there was also a good correlation between two measurements by a single observer, as shown for one observer in TABLE 2. Similar results were obtained for the other observer.

TABLE 2
Intra-observer variation in 22 patients. Comparison of first and second TRUS measurements of the whole prostate

	Correlation coefficient	*Mean Difference**	*Stand. Deviation of the Diff.**	*Range of Difference**
Planimetry	0.97	0.0 cm^3(-1 %)	3.5 cm^3 (11 %)	-6.4/+8.7 cm^3 (-25/+19 %)
Prolate Spheroid	0.90	1.3 cm^3(8 %)	5.0 cm^3 (19 %)	-7.5/+14 cm^3 (-18/+64 %)
Width	0.90	0.0 cm (1 %)	0.3 cm (6 %)	-0.5/+0.5 cm (-9/+10 %)
Height	0.92	0.1 cm (5 %)	0.2 cm (10 %)	-0.2/+1.0 cm (-8/+43 %)
Length	0.85	0.0 cm (0 %)	0.4 cm (9 %)	-1.1/+0.6 cm (-26/+16 %)

* difference as percentage of first measurement

Interobserver in-vivo variation of the inner zone volume (study seven)
There was a high correlation (r =0.94) between planimetry measurements of the inner zone volume between the two observers (TABLE 3), with a mean difference of 7% which is not significantly different from zero. This did not apply for the prolate spheroid technique. The differences between both observers varied considerably from patient to patient. The range of differences varied from -57% to +44% for the planimetric method, and from -82% to +298% for the prolate spheroid technique. FIGURE 4B shows the comparison between planimetric volume measurements by observers A and B.

TABLE 3
Variations between observers in 47 patients TRUS measurements of the inner prostate zone

	Correlation coefficient	*Mean Difference**	*Stand. Deviation of the Diff.**	*Range of Difference**
Planimetry	0.94	1.1 cm^3(7 %)	3.2 cm^3 (20 %)	- 8.5/+9.5 cm^3(-57/+44 %)
Prolate Spheroid	0.82	4.2 cm^3(32 %)**	5.5 cm^3 (59 %)	-10.0/+18.1cm^3(-82/+298%)
Width	0.82	0.3 cm (8 %)**	0.6 cm (19 %)	- 1.9/+1.5 cm (-54/+55 %)
Height	0.87	0.1 cm (8 %)**	0.3 cm (18 %)	- 0.6/+0.9 cm (-30/+67 %)
Length	0.74	0.2 cm (-5 %)**	0.6 cm (18 %)	- 1.8/+1.5 cm (-36/+56 %)

* : difference as percentage of measurement by observer A
** : significantly (p <0.01) different from zero

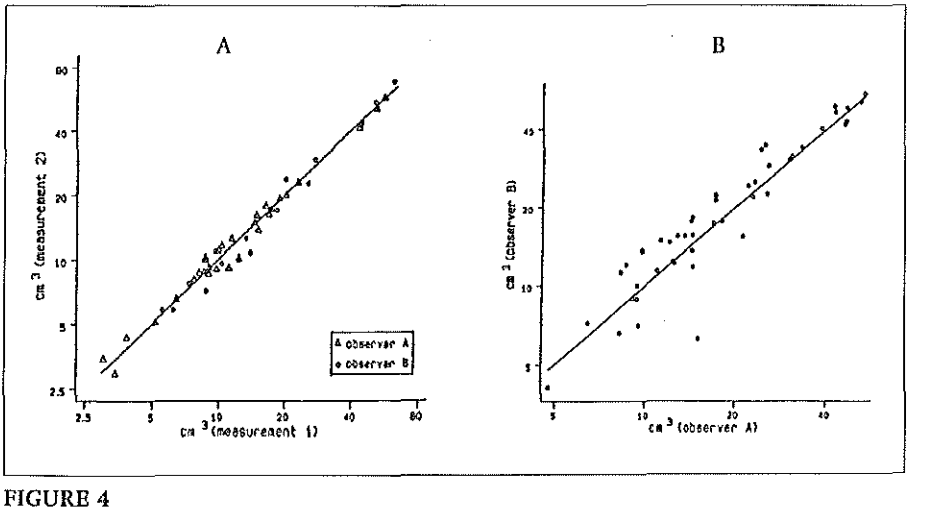

FIGURE 4
7 MHz TRUS internal zone planimetric volume measurements
a) Intraobserver variation in vivo
b) Interobserver variation in vivo

Intraobserver in-vivo variation of the inner zone volume (study eight)
TABLE 4 gives outcomes of the intraobserver study of 52 patients. For planimetry the intraobserver coefficient of variation was 7%, for the prolate spheroid technique however it was 20 %. For caliper measurements of length, width, and height the coefficients of variation were resp. 6%, 7%, and 8%.
FIGURE 4A shows the agreement between the first and second planimetric measurements. There were no statistically significant differences in variations of measurements between observers.

TABLE 4
Intra-observer variation in 52 patients. Comparison of first and second TRUS measurements of the inner prostate zone

	Correlation coefficient	*Mean Difference* *	*Stand. Deviation of the Diff.* *	*Range of Difference**
Planimetry	0.99	-0.1 cm^3 (0%)	1.5 cm^3 (10 %)	- 3.2/+3.5 cm^3(-22/+17 %)
Prolate Spheroid	0.96	0.2 cm^3 (9%)	3.2 cm^3 (27 %)	-11.6/+8.8 cm^3(-43/+68 %)
Width	0.95	0.0 cm (3%)	0.3 cm (13 %)	- 0.8/+0.6 cm (-22/+40 %)
Height	0.97	0.0 cm (1%)	0.2 cm (9 %)	- 0.4/+0.3 cm (-25/+15 %)
Length	0.90	0.1 cm (-1%)	0.4 cm (12 %)	- 1.0/+0.6 cm (-24/+20 %)

* ; difference as percentage of first measurement

DISCUSSION
Prostatic volume has been measured by both transabdominal and transrectal ultrasound [13]. These measurements have been correlated with the weight of prostatic tissue, as removed for benign disease [9], by radical prostatectomy [10] and at autopsy [14] [15].
Although in studies, such as that by Myschetzky et al [16], different machines with probes of varying frequencies have been used, we can find no direct comparison of volume measurements between machines in the literature. In the *in-vitro* comparison between the 5MHz Aloka and the 7MHz Bruel & Kjær (B&K) there was less than 5% difference in volume measurement between the two. Thus one may conclude, that under the close to ideal *in-vitro* conditions, both machines are measuring the same volume. When the *in-vivo* comparison was made, the B&K 7MHz planimetric volume measured an average of 40% more than the 5Mhz Aloka. This difference, which was not evident in the *in-vitro* study, raised the question of whether with the 7MHz too great a volume was being measured, or with the 5MHz too little. To try and clarify this, volume measurements were performed with both ultrasound machines prior to radical prostatectomy. In every case, the 7MHz B&K measured the same as, or less than the volume, as calculated by weight, of the radical prostatectomy specimen after removal of the seminal vesicles. On the other hand, the 5MHz Aloka volume was in every case less than that of the 7MHz B&K. It

appears therefore that in the *in-vivo* situation, the 5MHz Aloka measures less than the true volume. The explanation for this discrepancy might be that in many cases the complete prostate is not well visualised by the 5MHz ultrasound because of poorer image quality. Indirect evidence that the older ultrasound machines with a 3 and 5MHz frequency do not always visualise the peripheral part of the prostate comes from older studies showing a good correlation between the ultrasonically measured volume and the weight of prostatic tissue removed by either TURP or open prostatectomy. [17] [18]. Two studies [9] [19] finding a 1:1 correlation between the resected weight and the difference between the pre- and postoperative volume determinations still do not establish that the whole prostate is seen, as the residual tissue could still be unresected adenoma, with the unresected central and peripheral zone not being visualized before and after resection. The only certain method to establish whether or not the entire prostate is being measured is to correlate the volume with the weight of the radical prostatectomy specimen, but even here the volume measured underestimates the prostatic weight in 74-90% of the cases, depending on the method of volumetry [10] [16].

In their series of 150 patients, Terris and Stamey [10] demonstrated that the prolate spheroid formula, which does not utilise length, had a better correlation and smaller mean difference than all of the formulas using length. The finding in this study that length is the least reproducable of the three diameters may explain their observations, and supports the clinical impression that the length (cephalocaudal diameter) is the most difficult diameter to measure. This was also the finding of Braeckman et al [20] when trying to measure dimensions of the prostatic adenoma, and by Yip et al [21] in a study of autopsy prostates.

Measurements of width and height for the whole prostate were highly reproducible by both a single observer and two different observers in our study. Therefore, when using a shape based formula, the prolate spheroid formula which is independent of length is the most reproducible for total gland volume. As both the prolate spheroid and the planimetry methods of total volume measurement were equally reliable and reproducable, the prolate spheroid method may be preferable as it is less time consuming and requires less equipment.

For the inner zone, caliper measurements were reproducable for a single observer, however between two observers caliper measurements were significantly different suggesting a greater difficulty in the interpretation of the images. Consequently, the prolate spheroid technique is inadvisable. Planimetric measurements proved to be more reproducable between observers.

In the *in-vitro* model, ultrasound volume measurements were both accurate and reproducable. This is under ideal conditions with a stationary balloon in a water bath. In the patient situation, several factors may influence the measurements. An empty bladder can make the limits of the prostate difficult to define cranially. Patient movements, breathing and rotational movements of the prostate may further affect volume measurements. Therefore, it is not surprising that a greater variation between sequential measurements was found *in-vivo*. In this series, all patients were included, regardless of their preparation or ease of prostatic visualisation. To asses the reproducability of the measurements the equipment was completely dismanteled and reassembled between measurements, so as to simulate as closely as possible the normal working clinical situation. To assess the reproducability of serial volume measurements, the mean difference is important. This is an indicator as to whether or not a systematic difference exists. For total volume by both the prolate spheroid and planimetry techniques, the mean difference was less than 3%, not significantly different from zero. This indicates that

no systematic difference existed between observers. This does not, however, give any indication as to how great a variation in sequential volume measurements may exist in any one individual.
Styles et al [13] found that in 95% of the cases, a variation from -18 to +25mls in volume measurements was found between two planimetric measurements by the transrectal route. In our study the standard deviation of the difference was 13% for the planimetric method and 16% for the prolate spheroid method. This means that in any individual there can be a considerable variation between subsequent volume measurements. Therefore, in considering serial volume measurements in studies assessing the effect of an intervention, a change of greater than 26% should be seen before this is accepted as a real change. With these inherent variations, minor differences in volume should not be regarded as significant. For the inner gland, caliper based measurements are not acceptable. As in our study the standard deviation of the difference for planimetric measurements was 20%, changes in serial volume measurements greater than 40% have to be regarded as significant.

CONCLUSION

Although measurements of prostatic volume are generally reproducable, there can be inherent differences between machines.
In consecutive measurements, total prostate and inner zone volume change can be regarded as significant when exceeding resp. 26% and 40%.
In caliper measurements prostatic length has the least interobserver reproducability in comparison to width and height. Planimetry is the only reproducable method in volumetry measurements of the inner zone. These observations should be taken in consideration in ongoing screening studies and follow-up of prostatic volumetry.

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4

ERRORS IN TRANSRECTAL ULTRASONIC PLANIMETRY OF THE PROSTATE*

Computer simulation of volumetric errors applied to a screening population

Chris H. Bangma, Evert Jan Hengeveld, A.Qais H.J. Niemer, Fritz H. Schröder

ABSTRACT

Three systematic errors in routine ultrasonic planimetric volume measurements of the prostate were assessed. A computer model using ellipsoids was used to simulate the step section technique and different forms of rotational movements of the prostate during planimetry. The planimetric volume was up to 12 % smaller than the exact volume, depending on the degree of rotational movement, the shape, and the length of the ellipsoid.

In-vivo study of a screening population showed that it is worthwhile to compare caliper length with the number of planimetric steps, as the difference might be an indication of the difference between planimetric and caliper measured volume. In shorter prostates the planimetric volume was smaller than the prolate spheroid volume when compared to longer prostates, as was seen in the computer simulation.

INTRODUCTION

The volume of the prostate is a useful parameter in clinical decisions. Various techniques are available to determine prostate volume *in vivo*. Digital Rectal Examination (DRE), and Transrectal Ultrasonography (TRUS) are widespread methods of volumetry. Estimation of prostate volume by TRUS mainly is done two different ways: by step-size planimetry, or by mathematical formulas using one or more ultrasonic calipers.

Terris and Stamey [1] assessed the accuracy of ultrasonic volumetry comparing the volume of radical prostatectomy specimens with planimetric and caliper measured volumes *in vivo*. Planimetry underestimated the specimen volume in 86 %, while caliper calculated volumes by prolate spheroid formula overestimated this volume in 26 %, and elliptic formula in 90 %. This suggests a systemic error in planimetry. In our institution Niemer et al [2] tested reproducibility of ultrasonic volumetry, and found prolate spheroid and planimetric measurements of the whole gland to be highly reproducible by both single and different observers. Measurements of the inner zone of the prostate gland were best reproducible using planimetry.

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Variability in planimetry may be explained by various factors. Involuntary movements of the patient, and rotational movements of the prostate around the ultrasonic transducer during planimetry may disturb an optimal sequence of step-sections. The ultrasonographers may have variable interpretations of prostatic boundaries, which can be the result of ultrasonic disturbances, such as reverberation and deflection. The planimetric summation formula, like the caliper formulas, gives rise to geometrical simplification of the prostate, influencing the volumetry.
Longitudinal studies of ultrasonic volumetry are scarce due to its recent development and the ongoing changes of ultrasonic equipment. However, longitudinal studies of Prostate Specific Antigen (PSA) are of growing interest. The combination of PSA and volumetry, reflected in volume adjusted PSA-values, are likely to remain important. Therefore a continuing interest in volumetry of the prostate may be expected. Further improvement of volumetry, minimizing variability, is desirable.
We assessed three volumetric errors in planimetry, described as the salami effect, the capsizing effect, and the first step effect. The salami effect is the sectional effect of the planimetric method, which may leave small amounts at the extremes of the geometrical body unmeasured (FIGURE 1).

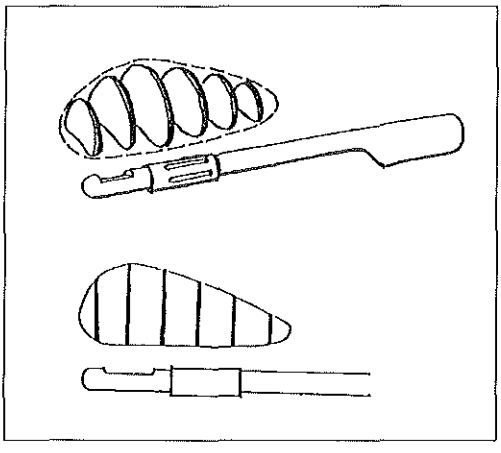

FIGURE 1
Schematic representation of planimetry of the prostate, with the ultrasonic probe parallel to the cephalo-caudal axis

Furthermore, when transverse sections are not perpendicular on the longitudinal axis of the geometrical body, the angulation gives rise to slices of different surface and thickness, and might even influence the number of step sections. The angle between the longitudinal axis of the prostate, and the longitudinal axis of the ultrasonic transducer was defined as α (FIGURE 2).

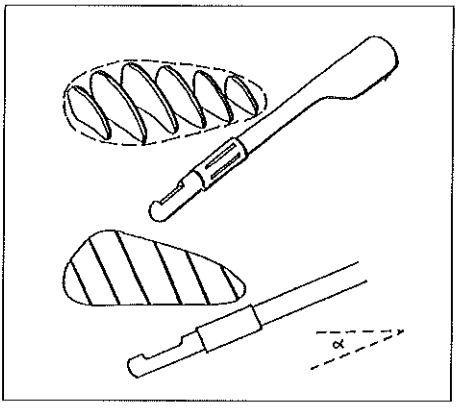

FIGURE 2
Schematic representation of the salami effect during planimetry with a fixed angle alpha

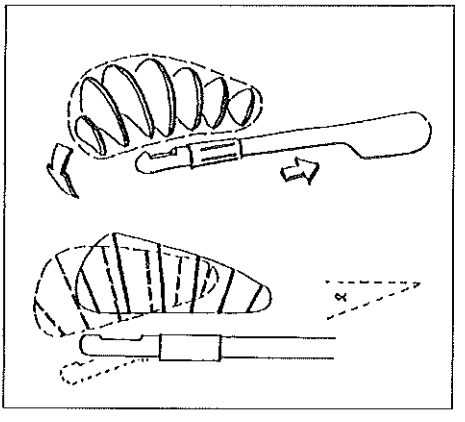

FIGURE 3
Schematic representation of the capsizing effect during step section planimetry with a continuously changing rotation angle alpha

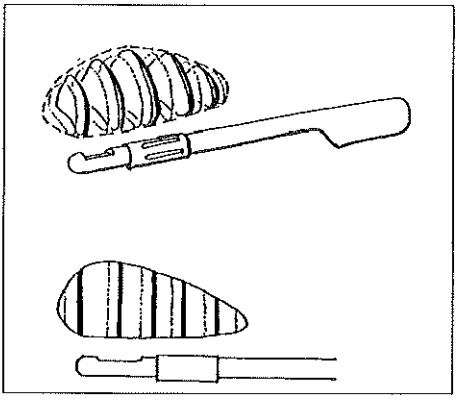

FIGURE 4
Schematic representation of the first step effect during step section planimetry

During a pilot study the capsizing effect was also observed, which occurs during planimetry when the cephalo-caudal or longitudinal axis of the prostate continuously changes (FIGURE 3), resulting in measurements of non-parallel transverse cross-sections. The first step effect is an observer dependent error, which was described as the reduction of number of step sections due to recognising the first step section too far into the prostate (FIGURE 4).
In a computer simulation the maximal volumetric differences between real (caliper measured) volume and planimetry of several ellipsoids were determined. Also the shape of the ellipsoids was analysed. The results were compared with volumetric data of 59 randomly choosen participants of a screening population for prostate cancer in Rotterdam, obtained by TRUS with a 7 MHz biplanar rectal probe on a Bruel and Kjaer 1846 ultrasound machine.

MATERIALS AND METHODS

Pilot study
In a pilot study 5 prostates between 26 and 91 ml (planimetric volume) were analysed. Step section planimetry with 5 mm steps was completed within 8 to 12 steps. Mean stepsize was calculated by the ratio of cephalocaudal length and number of steps, and varied between 3.5 to 5.0 mm. At each step the angle of rotation of the prostate in longitudinal direction around the ultrasound probe was determined from videophotographs, and was between 0 and 7 degrees for each step. The total capsizing rotation was between 12 and 32 degrees, independent of the prostatic volume or step size. It was not possible to determine whether the rotation was gradual or during a specific part of the procedure, for example at the end when the probe is almost completely retracted from the rectum.

Computer simulation
A computer model was created to simulate the salami effect, the capsizing effect, and the first step effect. The prostate was geometrically simplified into an ellipsoid. Ellipsoids were chosen with varying length, width, and height within physiological ranges as obtained in the Rotterdam feasibility study for screening of prostate cancer. Width or height was chosen never to exceed length. 74.244 different ellipsoids were analysed, arranged according to length, as length theoretically correlates best with the number of planimetric steps. 24 classes of 2 mm steps were created, containing 234 to 7634 ellipsoids of different shapes and sizes. During planimetry the salami effect and capsizing effect were analysed while the cephalocaudal axis of the ellipsoid was varied gradually over an angle α between 0 to 45 degrees with 5 degrees steps, compared with the axis of the planimetric probe. The difference between planimetric volume and exact calculated ellipsoid volume, defined as delta-volume, was classified in relative values (the percentage of the exact ellipsoid volume).
During the simulation described above, the first section was made exactly at the edge of the ellipsoid, simulating the perfect observer. When starting a planimetric measurement of a geometrical body *in vivo* the first section is made through one end of this body with an ultrasonic appearance just recognisable as part of this body. Usually the surface area of this first section is between 1 and 2 cm^2. By recognising the first section area too late, that is, too far into the ellipsoid, a reduction of the number of step sections may occur, deminishing the measured total volume. This was referred to as the first step effect. The effect may be dependent on the shape of the ellipsoid. In our computer simulation model we created a series of ellipsoids

with identical volume (31.4 ml, approximating the median volume in our screening population) but 7 different shapes. This was done by varying systematically one of the parameters length, width, or height by a chosen factor 1.5, and correcting one of the other parameters. While gradually capsizing these different ellipsoids over 30°, we were able to determine a critical surface area above which the first-step effect occured and which was the cause of an increased volumetric error. An additional error occurs if also the last step area is not recognised, when equal or less than the first step area.

Comparison to in vivo data

To detect missing or extra steps during planimetry, we noted in 59 randomly chosen participants of the Rotterdam feasibility study for prostate cancer the number of steps during measurement of total volume. Also caliper measured volumes were calculated. The product of number of steps times the standard step size was calculated to predict the length of the prostate. The absolute difference between predicted length and caliper measured length was supposed to be insignificant when smaller than 5 mm, as this would not induce extra or missing planimetric steps. All larger differences were divided in classes. The differences between planimetric volume and caliper measured volumes were also ordered in classes of percentage of the caliper measured volume. This volumetric difference was correlated to the number of steps and the caliper length. The prolate spheroid volume was chosen as reference.

RESULTS

With the salami effect delta-volume appeared to be larger in shorter ellipsoids, and generally increased with increasing angle α (FIGURE 5). Volumetry was optimal when planimetric slices exactly fitted with the ellipsoid length, as illustrated by the dips in the graphical representation. For the curve with sections perpendicular to the longitudinal axis (α = 0) the volumetric error was predominantly seen to be less than in case of angulation. The number of planimetric steps was always equal or one less than expected from the ellipsoid length.

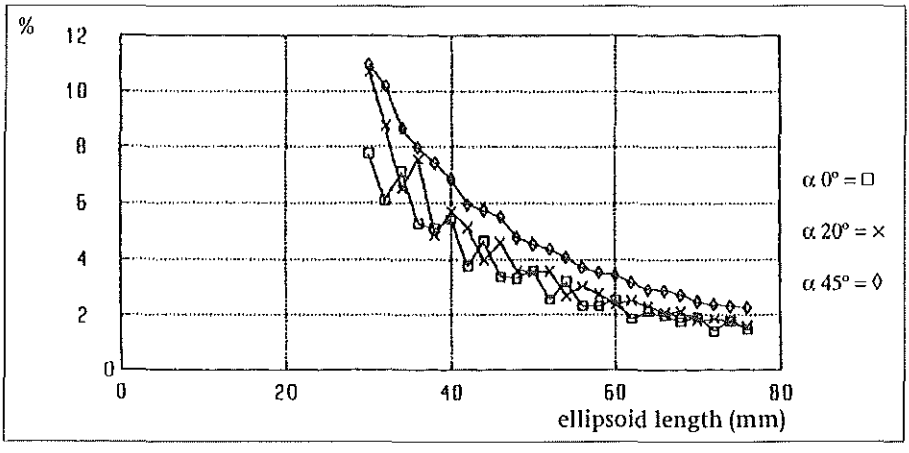

FIGURE 5

Volumetric difference between exact volume and planimetric volume of ellipsoids in percentage of the exact volume as a function of increasing ellipsoid length for the salami effect with angles α of 0, 20, and 45 degrees.

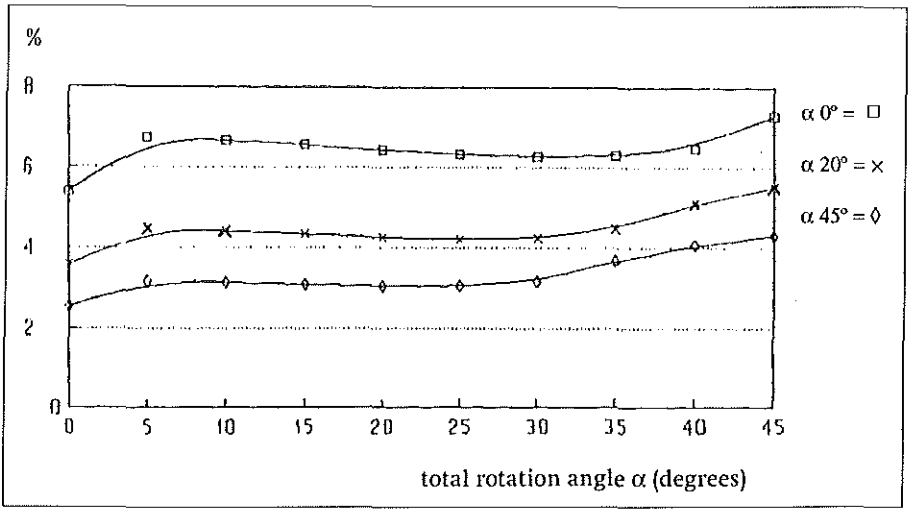

FIGURE 6

Volumetric difference between exact volume and planimetric volume of ellipsoids in percentage of the exact volume as a function of initial rotation angle α for ellipsoids during the capsizing effect in three different length classes of ellipsoids.

With the capsizing effect delta-volume was larger in shorter ellipsoids, and increasing rotation angle α (FIGURE 6), as was seen for the salami effect. The number of planimetric steps was always equal or one less than expected from the ellipsoid length. Volumetric difference between exact and planimetric volumes of various ellipsoids of the same length showed a progressive increase after a critical rotation angle which was smaller for longer ellipsoids (FIGURE 5). This means that the influence of capsizing of the ellipsoid during planimetry is relatively constant in shorter ellipsoids, although the difference in volume is relatively larger than in longer ellipsoids (FIGURE 6: upper curve). As the critical angle of rotation is smaller in longer ellipsoids, effects of capsizing will be seen more early, only the relative volumetric difference will be smaller than in shorter ellipsoids (FIGURE 6: lower curve). For each length class the mean minimal volumetric difference was 0.4 ml, and this was seen to occur in the ellipsoids of low volume. The salami effect was responsible for 0.3 ml of this volumetric difference.

The volumetric error due to the first step effect in the computer simulation was especially seen in ellipsoids which shape is low and broad (TABLE 1: numbers 2 and 6), resembling a rather usual configuration of a prostate. The total volume may be diminished up to 11 % in a ellipsoids of 31.4 ml.

Missing or extra steps during planimetry *in vivo* were calculated in 59 prostates (TABLE 2). Missing steps were noticed in 13 prostates (22 %). In 10 of these prostates the planimetric volume was less than the prolate spheroid volume, in 8 of them even more than 10 %. In 22 prostates, too many steps for planimetry had been taken (37 %). In 25 patients the prostates were in the range of equal length; in 8 out of 25 prostates the volumetric difference between prolate spheroid and planimetric volume exceeded 10 %. The number of missing steps correlated with the loss of planimetric volume compared to the prolate spheroid volume ($r = 0.52$).

In 60 % the planimetric volume was smaller than the prolate spheroid volume. In

TABLE 1

Volumetric difference between exact volume and planimetric volume in percentage of the exact volume of different ellipsoids of 31.4 ml, while planimetry started at a first section area of 1.0 or 2.0 cm². Critical first section area for occurance of first step effect noted in square centimeters.

ELLIPS	WIDTH cm	HEIGTH cm	LENGTH cm	PERCENTAGE OF VOLUMETRIC ERROR 1.0 cm²	2.0 cm²	CRITICAL AREA cm²
1	4	4	4	5	4	-
2	6	2.7	4	4	11	1.6
3	2.7	6	4	7	5	-
4	4	6	2.7	2	8	1.2
5	6	4	2.7	6	5	-
6	4	2.7	6	4	8	1.6
7	2.7	4	6	4	8	1.2

TABLE 2

Number of volumetric differences (prolate spheroid volume minus planimetric volume) in percentage of prolate spheroid volume (%) in 59 prostates, and differences between predicted and real length (mm) (step section size = 5 mm)

percentage volumetric difference	*missing steps* 2	1	*equal* 0	*extra steps* 1	2	3
< -10	-	3	3	6	1	2
-10 - 0	-	-	6	3	2	1
> 0 - 10	-	2	11	-	-	-
> 10	1	7	8	4	2	-
Total number	13		25	21		

shorter prostates (as in the computer simulation) the planimetric volume was smaller compared to the prolate spheroid volume (r = 0.29), but this was very weakly correlated to missing steps (r = 0.18). The number of planimetric steps was well correlated with caliper measured length (r = 0.65) and the planimetric volume (r = 0.80), as might be expected.

DISCUSSION

Step-section planimetry is a well-accepted method of volumetry of the prostate. It was described by Basset et al [3] as an accurate technique *in vitro*. In our institution it is the method of choice due to its reproducability of total and inner zone volume measurements [2] (chapter 3).

Terris and Stamey [1] compared the various ultrasonic volumetric methods *in vivo* with the weight of the radical prostatectomy specimens, unfortunately without performing ultrasonography of the post-operative specimen. Prolate spheroid measurements correlated best with the prostatic weight (r = 0.94), only slightly better than planimetry did (r= 0.93). The mean difference, as an index for accuracy, in 2 mm step section planimetry was as high as in prolate spheroid volumetry. Both Niemer et al. [2] and Terris and Stamey [1] mentioned the variability of caliper measurements of prostatic length. Collins et al [4] however described a reproducable technique of cephalo-caudal length measurements.

Though accuracy and reproducability of planimetry is as good as prolate spheroid caliper measurements, it is remarkable that planimetric volumetry results in smaller prostatic volumes compared to caliper measurements. We showed the effect of three potential errors, all of them giving rise to smaller planimetric volumes compared to optimal caliper measurements.

The salami effect was mentioned by Dahnert [5] as a methodological weakness. In the salami effect the angulation causes a mathematical error during the summation of step sections slightly larger compared to perpendicular slices. The combined error of salami effect with the step-section technique is in our study 12 % at maximum, and relativily larger in shorter ellipsoids.

The angulation which occurs during the salami effect might also occur during caliper measurements. Considering the same angle α, the decrease of caliper length will be related to the sinus of α. Ten degrees will diminish the calculated prolate spheroid volume by 1.5 %, 20 degrees by 6 %, and 30 degrees by 13 %. In calculating the volume of ellipsoids with 3 caliper measurements, the error is the product of three measurements, and only needs 5 % error in every caliper measurement (α = 20) 20° to produce a total decrease of 13 %. As a result we might say that in planimetry the salami effect is mainly dependent on the length of the ellipsoid (FIGURE 5), while in caliper measurements this angulation effect depends on the angle α. *In vivo* it is impossible to avoid the salami technique completely, as the longitudinal axis of the prostate is hard to define objectively, in contrary to an ellipsoid in a geometrical model. In caliper measurements the angle α can be avoided by measuring height perpendicular to length in the sagittal plane.

Capsizing occured in the pilot study in up to 30 degrees. In the computer simulation the capsizing effect caused only 2 to 7 % loss of volume. Longer ellipsoids were influenced by a larger extent than ellipsoids shorter than 45 mm. The capsizing effect is obviously of less importance than the salami effect. Missing a step section due to the first step effect is of considerable interest, as this may induce a volumetric loss up to 11 % in prostates of median volume. Starting the planimetric series of step sections we usually check the position of our first section by taking the ultrasonic longitudinal view, which indicates the position of the ultrasonic

transverse section (Bruel and Kjaer 1846 multiplanar probe).
The *in vivo* study showed that only in 9 out of 59 prostates the planimetric volume was smaller than the prolate spheroid volume, while also the number of planimetric steps was smaller than the expected number. Accepting the caliper measurement of length as a standard parameter in this study, the computer simulation predicting a smaller volume by a reduced number of steps was applicable only in 15 % of our *in vivo* population.
In contrast to missing a step section by the first step effect, an extra step may be induced, as illustrated in our *in vivo* study. Our computer model, with the point of rotation in the centre of the prostate, cannot account for these observations. They may be caused by other movements of the prostate resulting in series of fanlike overlapping slices. If during planimetry the amount of step sections exceeds the amount of steps as expected by the length of the prostate, the total planimetric volume might exceed the real volume, as too many planimetric slices are added into the planimetric summation formula.
Testing the relation between length and volumetric difference it was seen that in shorter prostates the planimetric volume was smaller than the prolate spheroid volume when compared to longer prostates. This was also seen in the computer model.

CONCLUSION

Planimetric measurements are influenced by non-parallel or oblique slices, described as the salami effect and the capsizing effect. These effects may diminish the total volume *in vitro* up to 12 %, especially in shorter ellipsoids. The shape of ellipsoids may cause small additional errors (the first step effect).
To minimize these errors *in vivo* it is worthwhile to compare caliper length with the number of planimetric steps. Also pressure of the ultrasonic probe against the prostate should be avoided, as this might promote capsizing and deformation of shape.
The intra-observer variation found in our institution of 12 %, and the standard deviation of the difference in inter-observer variation in planimetry (Niemer et al, 1994) might be partly explained by the described effects.
Increasing the number of steps by diminishing step-sizes theoretically improves accuracy and reproducability of planimetric volumetry of the prostate. This however lengthens the procedure considerably in daily practice. Simultaneous representation of the prostate in a sagittal and a transverse plane, as used in three-dimensional planigraphy, can be of additional value.

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5

TRANSRECTAL ULTRASONIC VOLUMETRY OF THE PROSTATE*

In vivo comparison of different methods

Chris H. Bangma, A.Qais H.J. Niemer, Diederick E. Grobbee, Fritz H. Schröder

ABSTRACT

In order to assess the accuracy of various volumetric methods for screening and follow-up of prostatic disease, total prostate volume and inner zone volume were measured by transrectal ultrasonography in a screening population of 716 men.
Semiplanimetric and caliper formula methods were compared with step section planimetry as the golden standard.
Planimetric volumetry of the prostate is regarded as the most reproducible method for individual follow up of total gland and inner zone volume. The prolate spheroid formula is the most reproducible of caliper formula methods for both volumes. In this study the elliptic volume was however more accurate than the prolate spheroid volume of the total gland, as the correlation coefficient between total elliptic volume and planimetry was higher compared to prolate spheroid volume (0.89 versus 0.83), and the standard deviation of the mean volumetric difference smaller. The mean total prolate spheroid volume resembled the mean total planimetric volume better than elliptic volume did, as the mean volumetric difference was smaller. For measurement of the inner zone volume the prolate spheroid volume was more accurate than the elliptic volume. The correlation coefficient between length and planimetric volume was similar to that of width and heigth, which accounted for more accuracy of the elliptic volume than of prolate spheroid volume in larger prostates.
The elliptic volume migth be used for incidental volumetric measurements of the total gland, and for comparison of different individuals, for example in preoperative evaluation or screening studies.

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* *Adapted from: Transrectal ultrasonic volumetry of the prostate, in vivo comparison of different methods, accepted for publication in The Prostate, 1995*

INTRODUCTION

The volume of the prostate is used in clinical decision making and follow up. It is also used to calculate prostate volume adjusted parameters like Prostate Specific Antigen Density (PSAD) during the diagnostic proces of prostate pathology. Volume measurements therefore should be reproducible and accurate. Accuracy represents the combination of precision, expressed in the correlation coefficient, and variation, expressed in standard error of a test (or the standard deviation of a sample) (NB in statistical terms, accuracy is often referred to as the ratio between the number of true positives plus the number of true negatives, and the total number of the population studied). Various techniques are available to determine prostatic volume in vivo. Digital rectal examination (DRE) gives an impression of the size of the posterior surface of the gland, but is unreliable in determining volume.
Transrectal ultrasonography (TRUS) has been introduced for evaluation of the prostate. Methods of measuring and calculating prostate volume by TRUS are mainly two-fold: step-wise planimetry, and mathemetical formulas using two or more ultrasonic caliper measurements.
In the Academic Hospital Rotterdam 5 mm step section planimetry with the Bruel and Kjaer 1846 ultrasonic equipment and its multiplanar 7.5 MHz transrectal probe is currently used as standard for volumetry due to its high reproducability [1]. Accuracy of the planimetric method was tested in a computer model in this hospital, and limitations of planimetry were described [2]. In order to test the accuracy of other volumetric methods for screening purposes and follow-up, these methods were compared to planimetry as a golden standard. The influence of various caliper measurements was analysed, especially the influence of prostatic length on volumetry and on volumetric differences between the various methods, as length is regarded in the literature as the most inconsistent parameter [1,3,4].

PATIENTS AND METHODS

As part of the Rotterdam feasability study for prostate cancer volumetry of the total gland and the inner zone in vivo were performed by five urological residents in 716 men between 55 and 74 years old. In 4 % of the participants a prostate carcinoma was diagnosed. In 6 % an operative procedure of the prostate had been performed previously (with a mean of 5 years before volumetry), and no men were on hormonal treatment. In ultrasonography the inner zone of the prostate, comprising the periprostatic tissue and the transition zone [5], is seen as a relative darker hypoechogenic heterogenous area. Volumetry was obtained using 5 mm step section planimetry, semiplanimetric ellipsoid formula (= 8 (maximum transverse area)^2 /3 x π x length), and caliper based elliptic (=0.524 x length x width x heigth) and prolate spheroid (= 0.524 x width x width x heigth) formulas. Width and heigth were measured at the maximal transverse diameter. Length was taken in cephalocaudal direction from the bladder neck (for both total gland and inner zone measurements) to the apex. The macroscopic ultrasonic contrast difference within the prostate accounted for the apex of the inner zone, while the contrast difference at the start of the relatively hypoechogenic urethral complex illustrated the apex of the total gland.
The data were analysed in a number of ways. First, using planimetry as a golden standard, Pearson correlation coefficients were calculated between planimetry and each of the other volumetric methods. Next, for total prostatic volume and inner zone volume, mean differences in volume between planimetry and each caliper based volumetric method were calculated with corresponding standard errors. Differences were tested using two tailed t-tests. Finally, to assess the modifying

effect of prostate length on the systematic differences in volumetric measurements, these differences were related to length in linear regression analysis. Similarly, using linear regression analysis, the best fitting weighing factors (coefficients) were calculated to relate calliper measurements to planimetric volume.

RESULTS

General characteristics and prostate volumes of the study population are given in TABLE 1. The mean total prostate volume using caliper measurements or semiplanimetry was smaller than based on mean planimetric volume. The correlation coefficient between planimetry and the prolate spheroid, elliptical, and ellipsoid was 0.83, 0.89, and 0.75 respectively, which were significantly different (TABLE 2, $p < 0.05$). Mean volumetric differences between planimetry and each other method were highly significant ($p < 0.001$). For the prolate spheroid volume this was 0.9 ml (S.D. 8.3 ml), for the elliptic volume this was 5.5 ml (6.7 ml), and for the ellipsoid volume this was 4.5 ml (10.3 ml). These standard deviations parallel the above mentioned correlation coefficients. Both are indices of volumetric precision. In 565 participants identical analyses were done for the inner zone volume (TABLE 3). The mean difference between planimetric and prolate spheroid volume was 2.2 ml (5.6 ml), for the elliptic volume this was 5.2 ml (5.1 ml), and for the ellipsoid volume this was 3.7 ml (7.4 ml) (all $p < 0.001$).

TABLE 1

General characteristics and prostatic volumes measured in various ways within the study population of 716 men

	mean	*(standard deviation)*	
age	64.3	(6.4)	(year)
weight	78.1	(10.8)	(kg)
total prostatic volume			
planimetric method	35.9	(14.9)	(ml)
prolate spheroid method	34.9	(14.0)	(ml)
elliptic method	30.3	(12.4)	(ml)
ellipsoid method	31.4	(15.0)	(ml)
inner zone prostatic volume			
planimetric method	18.7	(11.4)	(ml)
prolate spheroid method	16.4	(11.2)	(ml)
elliptic method	13.4	(9.0)	(ml)
ellipsoid method	15.0	(11.7)	(ml)

TABLE 2

Correlations, and mean volumetric difference (standard deviation) between planimetry and caliper based measurements of total prostatic volume (split up for all participants, and planimetric volume less or more than 40 ml)

method	*correlation coefficient*			*mean volume (S.D.)*	*total difference (in ml)*
	all	*< 40 ml*	*> 40 ml*		
prolate spheroid	0.83	0.62	0.70	0.9	(8.3)
elliptic	0.89	0.68	0.78	5.5	(6.7)
ellipsoid	0.75	0.45	0.67	4.5	(10.3)

TABLE 3

Correlations, and mean volumetric difference (standard deviation) between planimetry and caliper based measuerements of inner zone volume

method	*correlation coefficient*	*mean inner zone volume difference*	
		(S.D.)	*(in ml)*
prolate spheroid	0.71	2.2	(5.6)
elliptic	0.66	5.2	(5.1)
ellipsoid	0.46	3.7	(7.4)

Prostate length measurements were analysed with respect to the mean volumetric differences between planimetry and each other method (TABLE 4). For the prolate spheroid method each cm increase in length of the prostate resulted in an average of 3.9 ml increase in volumetric difference compared with planimetry, for the elliptical formula this was 0.9 ml, and for the ellipsoid formula this was 7.2 ml (all methods $p < 0.001$). When split up for planimetric size less or more than 40 ml, the higher accuracy of the elliptic formula method with increasing size was illustrated by a higher correlation coefficient between planimetry and elliptic formula for larger sizes compared to prolate spheroid volume (TABLE 2). Correlations to caliper measurements were calculated (TABLE 5). Regression analysis generated a formula predicting a caliper used volume fitting the planimetric volumes of this study population: predicted volume = - 64.3 + 7.4 (width) + 7.4 (length) + 12.1 (heigth).

TABLE 4
Increase in volumetric difference in ml per cm prostate length increase

method	*total gland volume*	*inner zone volume*
prolate spheroid	3.7	1.5
elliptic	0.9	2.5
ellipsoid	7.2	3.5

TABLE 5
Correlation coefficients between volumetry and caliper measurements for total prostatic volume and inner zone volume

caliper	*planimetry*	*prolate spheroid*	*elliptic*	*ellipsoid*
total volume				
length	.70	.53	.79	.14
width	.66	.88	.73	.36
heigth	.75	.82	.85	.31
inner zone volume				
length	.62	.71	.79	.29
width	.70	.86	.75	.42
heigth	.72	.69	.77	.46

DISCUSSION

For the determination of accuracy of ultrasonic volumes of the prostate, this study of a community based population shows that elliptic volumes of the total prostate are most closely correlated with planimetry, as illustrated by the highest correlation and the smallest standard variation in mean difference (TABLE 2 and 3). The absolute mean prolate spheroid volume resembles the mean planimetric volume better than elliptic volume does, as the mean difference in volume is smallest, but the variation in this volumetric difference is higher. Moreover, with increasing prostatic length the volumetric difference between total prolate spheroid and planimetric volume increases far more than between elliptic and planimetric volume, which

illustrates that in larger prostates the prolate spheroid volume is less accurate than in smaller glands. This is also illustrated by the higher correlation coefficient in glands more than 40 ml in elliptic volume measurement (TABLE 2). The similar correlation between prostatic length and planimetric volume, and the other caliper measurements (width and heigth) expresses the influence of length on volumetry. For volumetry of the inner zone of the prostate the prolate spheroid volume correlates better with planimetry than elliptic volume does. Even in modern ultrasonic equipment with a balanced Time Gain Compensation for adequate resolution of all prostate gland boundaries, the inaccuracy of inner zone measurements might be due to the uncertainty of inner zone boundary designation at the level of the apex.
The choice of planimetry as the golden standard is a limitation of this study. Accuracy of ultrasonic equipment is preferably tested to an in-vitro gold standard like a stable non biological object, such as a fluid filled balloon [6], or biological object, such as a cadaver prostate [7]. In those studies planimetry showed the best correlation with the actual volume. In vivo a volumetric method might be compared with the volume of fresh anatomic specimens, as obtained after radical prostatectomy with flush removal of the seminal vesicles [8]. We chose 5 mm step section planimetry as a standard because of its high reproducibility: in this hospital the in-vivo intra-observer variation was up to 11 % for planimetry for the total gland, and 10 % for the prolate spheroid method [1]. In other studies the intra-observer variation for planimetry of total prostatic volume only was 6 %, and for 'ellipsoid' (caliper measured) volumes 7 % [9]. The inter-observer variation between 2 of the 5 residents concerned in this study was for total planimetric volume 13 %, and for total prolate spheroid volume 16 %. The mean differences between observers were not significantly different from zero [1].
In a study of 150 patients planimetric and prolate spheroid volume correlated sligthly better with the volume of radical prostatectomy specimens compared to 2 mm step section planimetry ($r = 0.94$ versus $r = 0.93$, reliability interval not described), but the mean difference and the standard deviation of the difference, as an index for accuracy, was equal to planimetric volumetry [8]. Planimetry underestimated the volume of radical prostatectomy specimens in 86 % of cases. In contrast to our report, the mean prolate spheroid volume was sligthly larger than the planimetric volume, and underestimated the radical prostatectomy specimens in 74 %. The elliptic volume correlated less with the specimen volume because of difficulties measuring length, unlike our material.
In vivo comparison of volumetric methods showed that semi-planimetric methods corresponded badly with planimetry, as was reported by others [6].
In this study an empirical formula was generated using caliper measurements to fit total gland planimetric volume. The formula showed that all caliper measurements are weighed equally without the use of an exponent, conform a study of autopsy specimens [10]. This formula has not been made with the intention for clinical use, as statistically it is not significantly better than the prolate spheroid or ellipsoid formulas.

CONCLUSIONS

Planimetric volumetry of the prostate is regarded as the most reproducible method for individual follow up of total gland and inner zone volume. Using planimetry as the golden standard, the prolate spheroid formula (width x width x height x 0.52) has been reported to be the most reproducable of caliper formula methods for both volumes. From this study it appears however that the elliptic (width x height x

length x 0.52) volume of the total gland is more accurate than the prolate spheroid volume, especially in larger prostates. The elliptic volume therefore might be used for incidental volumetric measurements, and for comparison of the total prostate volume, for example in groups of patients undergoing therapeutic procedures or incidental screening. For follow-up a more reproducible method might be used. The semiplanimetric ellipsoid volume is inadvisable due to its considerable variation compared with caliper measurements.
Application of different volumetric methods during individual follow-up should be avoided.

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Part 3

Sonography of the Prostate for PSA Adjustment

6

VOLUME ADJUSTEMENT FOR INTERMEDIATE PROSTATE SPECIFIC ANTIGEN VALUES IN A SCREENING POPULATION*

Chris H. Bangma, Diederick E. Grobbee, Fritz H. Schröder

ABSTRACT

In a screening population of 812 men between 55 and 77 years old and prostate specific antigen (PSA) below 10.0 ng/ml (Hybritech) digital rectal examination (DRE) and transrectal ultrasonography of the prostate (TRUS) were performed. Seventeen prostate carcinomas were detected. Four methods of prostate volumetry were used to determine volume-adjusted PSA levels. These were prostate specific antigen density for the total gland volume (PSAD), for the inner zone volume (PSAT), and population-specific excess PSA values. There was a significant difference between the benign and the malignant population for age, PSA, PSAD, PSAT, and excess PSA values. The maximal discriminatory potential, analysed by the area under receiver operator curve, was 0.90, reached for prolate spheroid determined excess PSA. For PSA alone this was 0.86. Volume-adjusted PSA values have no additional benefit beyond unadjusted values in screening for prostate carcinoma in this study.

INTRODUCTION

Screening on prostate carcinoma (PCa) might provide a means of detecting confined prostate carcinoma in a treatable stage [1]. While digital rectal examination (DRE) and serum prostate specific antigen (PSA) determination have a limited positive predictive value (PPV) [2], especially in the intermediate range of PSA values between 4 and 10 ng/ml, their combined application was shown to be more effective. Transrectal Ultrasonography (TRUS) of the prostate in combination with PSA and DRE may increase PPV and negative predictive values (NPV) for detection of PCa. Volumetric parameters have been thought to limit the number of transrectal biopsies of the prostate without increasing the number of false negatives. In this study, ultrasonic volumetry is performed to determine volume adjusted PSA-values, for example prostate specific antigen density (PSAD), which indicate abnormal PSA-production in the body. Several formulae of PSA-density have been reported, including PSAD (= PSA / total gland volume [3]) and PSAT (= PSA / inner zone volume, [4]). In the Rotterdam feasibility study for screening prostate carcinoma new volume-adjusted PSA-values were evaluated for their use in selecting participants for further diagnostic examinations and/or prostate biopsy.

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PATIENTS AND METHODS

Out of a screening population of 1739 men between the ages of 55 and 77 years, 812 participants were randomly chosen for diagnostic examinations by digital rectal examination (DRE) and transrectal ultrasonography of the prostate (TRUS) if PSA was below 10.0 ng/ml (monoclonal Hybritech Tandem-R Stratus). DRE and TRUS were performed by four urological residents with a 1846 Bruel and Kjaer 7.0 MHz biplanar ultrasound probe. Four different methods of prostate volumetry of total gland and inner zone were used: 5 mm step-section planimetry, prolate spheroid volume (0.524 x transverse x transverse x anteroposterior diameter), elliptic volume (0.524 x cephalocaudal x transverse x anteroposterior diameter), and the semiplanimetric ellipsoid volume (8 x [area of largest ultrasonic transverse section]^2 / 3 pi x cephalocaudal diameter [5]). Men with suspicous DRE findings and hypoechoic TRUS lesions larger than 7 mm in diameter were biopsied under ultrasound guidance.
From the relation between serum PSA and inner zone volume, a set of formulae for predicted PSA values was obtained for correction of inner zone volume for each volumetric method, as illustrated in FIGURE 1 for planimetry. The slope of the

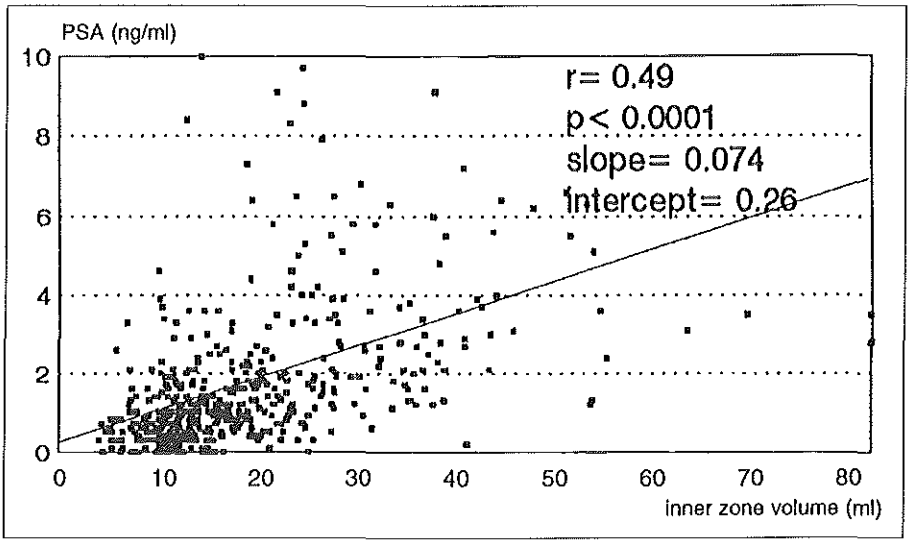

FIGURE 1
PSA as a function of planimetric inner zone volume. The linear regression line between inner zone volume and serum PSA indicates the formula for predicted PSA.
Predicted PSA = 0.26 + 0.074 x inner zone volume.

regression formula represents the mean PSA production per ml tissue of inner zone. Extrapolation of the inner zone volume to nil shows the intercept of the regression formula, which represents the mean PSA production of a peripheral zone of mean volume. Excess PSA values were calculated by subtracting the predicted PSA from the serum PSA.
Using logistic regression analysis the relative risk of presence of PCa was estimated for each diagnostic marker separately, and combined. Various contributions of PSA and measures of prostate volume were used to arrive at the model that predicted best the presence of PCa. Relative discriminatory potential of the different models was assessed by calculating the area under the ROC curve (Receiver Operator Characteristic [6]) of each model.

RESULTS

Of 812 participants the mean age was 64.5 years (S.D.: 5.3, range: 55-77), the mean body weight 78.3 kg (S.D.: 10.2, range: 51-116). Mean values of volumetric measurements were for total gland planimetric volume 35.5 ml (S.D.: 14.5, range: 7.7-117.0), and for inner zone planimetric volume 18.4 ml (S.D.: 11.1, range: 1.7-82.9). The mean serum prostate specific antigen was 1.7 ng/ml (S.D.: 1.7), and the mean PSAD 0.045 ng/ml/g (S.D.: 0.040). Bilateral biopsies were performed in 74 participants, and 17 prostate carcinomas were found. There was a significant difference between the benign and the malignant population for age, total gland height, serum PSA, PSAD, PSAT, and population derived excess PSA values. The discriminatory potential to detect prostate carcinoma of each parameter was determined by the area under ROC. For PSA alone the area was 0.86, for PSA in combination with age 0.88, PSA and prostatic height 0.86, and PSA with both age and height 0.86. The ROC areas of some of the significant volumetric parameters by four different methods are noted in TABLE 1. In the ROC-curve of serum PSA several PSA values are indicated (FIGURE 2). At a cut-off PSA level of 4.0 ng/ml sensitivity of PSA to detect PCa was 0.47 and its specificity was 0.91. For a cut-off level of 2.3 ng/ml this was 0.94 and 0.78 respectively.

TABLE 1

Area under ROC-curve for volume adjusted PSA

	PSA-density	*PSAT*	*Excess PSA*
planimetric	0.88	0.85	0.87
prolate spheroid	0.86	0.87	0.90
elliptical	0.86	0.83	0.89
ellipsoid	0.81	0.82	0.88

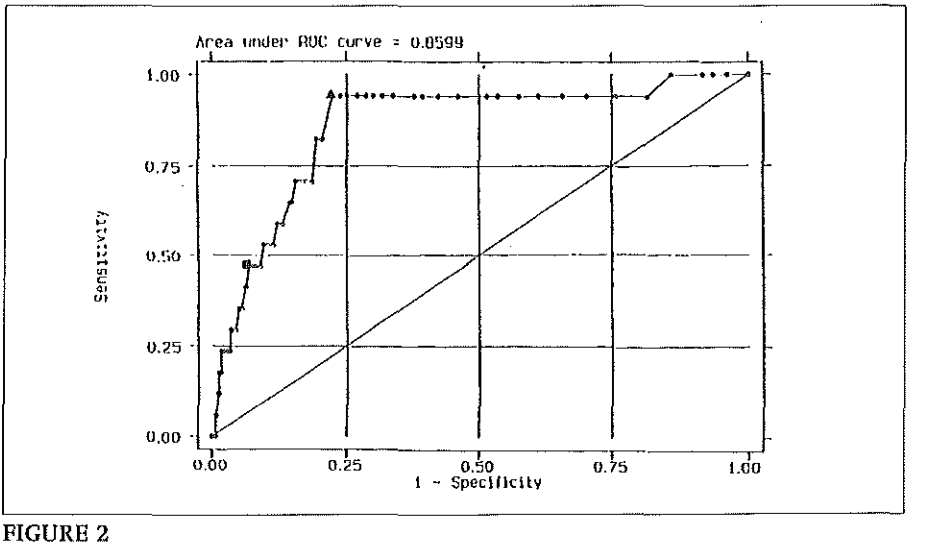

FIGURE 2

ROC-curve of serum PSA for detection of prostate carcinoma; serum PSA values of 2.3 (▲) and 4.0 (■) ng/ml are indicated.

DISCUSSION

Compared to serum PSA values, volumetric adjustments of those in the intermediate and low range do not improve the selection of men at risk for prostate carcinoma or for prostate biopsy in a screening population. When the volumetric corrections made by the PSAD, the PSAT, and the excess PSA, were compared, the best method of volumetric correction of serum PSA to maximise the diagnostic capacity for detecting prostate carcinoma appeared to be the excess PSA, increasing the area under ROC-curve from 0.86 to a maximum of 0.90 (prolate spheroid volumetry). For screening purposes, however, this slight improvement by volumetric measurements hardly offers an attractive procedure, as TRUS is expensive and time consuming, even when performed by skilled technicians instead of physicians. The correction of the serum PSA by the volume of the prostate or parts of it are based on the idea that the PSA production of normal or adenomatous prostatic tissue in the inner zone may conceal the abnormal PSA production in malignant tissue. The contribution of the PSA production of the peripheral zone, as illustrated by the intercept of the formula calculating excess PSA (FIGURE 1), is limited. The value of 0.26 ng/ml however is a median value, correlated to a peripheral zone of median volume (16.4 ml). The median PSA production per gram of peripheral zone tissue would be 0.016 ng/ml/g, which is far less than the PSA production in the inner zone of 0.074 ng/ml/g, illustrated by the slope of the formula. Therefore correction of the serum PSA for the inner zone volume only seems to be justified.

Volume adjusted PSA values are influenced by the method of prostate volumetry. Reproducability of inner zone and total gland volumetry is best in planimetry [7], and improvement is not expected [8]. The serum PSA concentration is influenced by various biochemical and biological factors, several of them still little understood. Values of PSA production in adenomatous tissue migth be dependent on the epithelial fraction [9], and leaking of PSA from epithelial cells to the serum is increased in malignancy [10], but these values show a wide variation. The mathematically derived value of PSA production in the inner zone of 0.074 ng/ml/g in our study corresponds well with the clinically determined value in the study of Mandell et al. [9].

In other studies, selection of men at risk for prostate carcinoma or for prostate biopsy in a screening population has been performed using PSA, DRE, and TRUS as discriminative parameters [11, 12]. The partial detection rates for PCa in the PSA range between 0 and 10 ng/ml in these studies were 3.7 and 1.5 %, respectively. By choosing a prescreen level of 2.0 ng/ml for performing DRE and TRUS, 11 % of cancers would have been missed in both studies, this is 15-17 % of cancers with a PSA between 0 and 10 ng/ml. By introduction of the prescreen level of 2.0 ng/ml as a cut off value for doing DRE and TRUS, Lee et al. calculated that the biopsy percentage would have been reduced by 50 %. The percentage of participants with a PSA smaller than 2.0 ng/ml was 69 % [11]. In our study the partial detection rate for PSA values till 10 ng/ml was 2.1 %. Only 1 of the 17 detected carcinomas had a PSA below 2.0 ng/ml, while 70 % of men had a PSA below 2.0 ng/ml. Using a prescreen level of 2.0 ng/ml, the number of biopsies would have been reduced from 74 (9.1 %) to 39 (4.3 %).

A correlation of serum PSA with age, which may be of importance to discriminate between participants with and without prostate carcinoma in screening populations, has been reported (Labrie [11] $r = 0.18$; Oesterling [13] $r = 0.43$). In the Rotterdam study the correlation of PSA with age was 0.25. Adjustment of the serum PSA for age resulted in a slight improvement of the diagnostic capacity as expressed by the area under ROC from 0.86 to 0.88. Although more easy than correction by prostatic volume, the improvement is considered too minimal to be worth persuing.

Diagnostic use of TRUS in screening for prostate carcinoma might increase the detection rate of prostate carcinoma [14]. In that study 42 % of detected tumours were in the PSA range below 4.0 ng/ml. In 41 % of patients, who underwent a radical prostatectomy for prostate cancer, the lesion was only detected by TRUS. Of these tumours 68 % appeared histologically organ-confined. In the study of Catalona [15] in 79 % of men with a prostate carcinoma and a PSA below 10.0 ng/ml the malignancy was histologically organ-confined, while in the group with a PSA of more than 10.0 ng/ml this number was 13 %.
According to Lee et al.[16], this implies that in order to detect non-palpable and curable (confined) carcinomas TRUS has to be performed particularly in the group with intermediate and low PSA values. Unfortunately this also is the largest group. In the Rotterdam study, all detected cancers in this group were organ-confined [17], and detected by TRUS in 100 % and DRE in 82 %. Limiting TRUS for screening participants with a PSA between 2.0 and 10.0 ng/ml would have reduced TRUS performance by 70 %, only missing one of 17 carcinomas.
In conclusion, volumetric adjustments of PSA-values up to 10.0 ng/ml have no additional benefit beyond unadjusted values for selection of males at risk for prostate carcinoma or for prostate biopsy in a screening population. A prescreen level of 2.0 ng/ml selects 30 % of the screening population in which 94 % of prostate cancers detectable by TRUS or DRE are found.

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7

POSTSCRIPTUM: IS PSAD USEFUL FOR SCREENING PROSTATE CARCINOMA?

The question whether volume adjustment of serum PSA is an adequate tool to select men in a community based screening population for prostate biopsy, or to limit the number of negative biopsies, was presented in chapter 6. The answer on this question is closely related to other problems, which deal with the evaluation of the various screening studies active at this time in Western Europe and Northern America [1-5]. For the correct interpretation of the value of volume-adjusted PSA, starting points and endpoints in these studies should be comparable, for example:

- are different screening populations comparable regarding age, race, method of sampling the community, etc.?
- which screening modalities are used among different studies: DRE, PSA, TRUS, or a combination of these, and what are their intrinsic qualities?
- how are these parameters used to select men for prostate biopsies, and what is the detection rate?
- which patients should be rescreened, at which frequency, and how should they be evaluated?
- what is the character of the carcinomas detected, and which are used as a study endpoint?

These and similar questions have to be solved in due time when the results of different community based screening studies have matured. Till that time continuous discussion concerning preliminary results will appear in the literature. The calculation of positive and negative predictive values (PPV and NPV), or of sensitivity and specificity, is widely used in papers concerning the capability of PSA, DRE, TRUS, and parameters derived from these, for detection of PCa. The positive predictive value of a test is the proportion of people with a positive test who actually have the disease for which they are tested. The negative predictive value is the proportion of people with a negative test who are free of the disease. The sensitivity is the proportion of people with the disease who have a positive test, and the specificity is the proportion of people free of disease who have a negative test (TABLE 1). Strictly spoken, these parameters can only be truly calculated when the real prevalence of prostate cancer in the population studied is known [7]. The detection rate in screening studies is between 2 and 5 % [1-5]. The detection rate (prevalence) is normally used for calculation of PPV, sensitivity, etc. In autopsy studies, in which the prevalence of PCa is around 32% [8], a large proportion of the population appears to have asymptomatic unpalpable focal carcinoma. Some of these focal carcinomas may be detected by screening for PCa, and their detection might be foremost dependent on the number of biopsies performed, and the volume of the prostate gland [9]. A large proportion of focal carcinomas obviously remains undetected. Therefore the true sensitivity and specificity of a screening test cannot be known. As long as it is realized that PPV, NPV, sensitivity, and specificity are dependent on the

TABLE 1

Calculation of positive predictive value (PPV), negative predictive value (NPV), sensitivity, and specificity of a diagnostic test, given the results of a golden standard.

A: mathematics

		GOLDEN STANDARD DISEASE +	-		
TEST RESULT	+	A	B	→ PPV	= A/ (A+B)
	-	C	D	→ NPV	= D/ (C+D)
				→ SPECIFICITY	= D/ (B+D)
				→ SENSITIVITY	= A/ (A+C)

B: community based screening population, biopsy results as golden standard.

		CANCER +	-		
		10	90 (biopsy)		
TEST RESULT (PSA>4)	+	6	27	→ PPV	= 18 % SENSITIVITY = 60 %
	-	4	63	→ NPV	= 94 % SPECIFICITY = 70 %

C: community based screening population, autopsy results as golden standard

		FOCAL CARCINOMA +	-		
		30	70 (autopsy)		
TEST RESULT (PSA>4)	+	6	21	→ PPV	= 22 % SENSITIVITY = 20 %
	-	24	49	→ NPV	= 70 % SPECIFICITY = 65 %

D: outpatient population

		CANCER +	-		
		40	60 (biopsy)		
TEST RESULT (PSA>4)	+	30	18	→ PPV	= 62 % SENSITIVITY = 75 %
	-	10	42	→ NPV	= 81 % SPECIFICITY = 70 %

population studied, these may be used for easy comparison of articles concerning comparable populations. TABLE 1 illustrates the variation of these calculated parameters in a community based, and an outpatient population.
In TABLE 1C the prevalence of the cancer has increased from 10 to 30 % due to respecting the autopsy prevalence of 30 % in a community based population as a golden standard, instead of the biopsy prevalence in the identical population. An identical number of carcinomas (n = 6) is picked up by the diagnostic test (for example: a serum PSA of 4.0 ng/ml or more), but more false negatives (n = 24) are identified. This decreases the sensitivity of the test considerably. Assuming that an identical proportion of the histological non-cancers is detected as positive by the test (this is in TABLE 1B: 27/90, and in TABLE 1C: 21/70), the PPV will not change dramatically, but the NPV does. As a result, using the cancer prevalence determined by biopsy results, the test looks better regarding sensitivity (this is: the ability to detect cancers in a population) and NPV (this is: excluding men for prostate biopsy because the test predicts that they have no cancer).
In an outpatient population (TABLE 1D) prostate carcinomas are detected by biopsy, which is used as the golden standard. As the median PSA value of this population is higher compared to a screening population, the test detects a relatively larger number of carcinomas. Assuming that an identical proportion of the histological non-cancers is detected as positive by the test, the test performance shows a better sensitivity, specificity, and PPV compared to the same test in a screening population.

Due to the overlap of serum PSA values between benign and malignant prostatic disease, studies started to concentrate on the 'grey' area of intermediate PSA levels between 4.0 and 10.0 ng/ml. In this group of men a parameter had to be found which was able to discriminate unpalpable PCa better than serum PSA did, as the ratio between positive and negative diagnostic prostate biopsies seemed to be unacceptable, regarding costs and complications. Most studies of outpatient populations agreed that PSAD can select men with an intermediate PSA result and normal DRE better for prostate biopsy than PSA only [6,10-15]. There are however similar studies which cannot support the selective application of PSAD for the use in the intermediate PSA group [16, 17]. In all of these studies symptomatic outpatients usually had been selected for biopsy when their PSA was more than 4.0 ng/ml, and/or they had an abnormal DRE. The PCa prevalence in these studies varied between 6.7 and 53 %. The percentage of men undergoing biopsy varied from 16 to 100 %. Furthermore these studies varied in their age ranges, biopsy techniques (palpable or TRUS lesions only, with or without 4 to 6 random biopsies), and statistical evaluation (parametric versus non-parametric tests).
Prostate size was discussed as a factor causing sampling errors in the detection of prostate cancer with prostate biopsies [9]. In the absence of palpable or visible lesions, sampling by at random sextant biopsies might be less sensitive in larger than in smaller prostates. Differences in mean prostate size in a study population might attribute to the difference in sensitivity of PSAD to detect prostate carcinoma in various studies mentioned.
The commonly found cut-off value for PSAD of 0.150 ng/ml/cc was reported by Benson and Cooner in their first article on enhancing the predictive value for PCa detection of intermediate PSA levels [10]. Lee and Littrup used a PSAD cut-off value of 0.2 ng/ml/cc in an outpatient population with a polyclonal PSA assay [18], and optimalized a cut-off value of 0.12 ng/ml/cc for the screening study of the ACS-NPCDP with a monoclonal PSA assay [19].

Stamey [20] and Cooner [21] have suggested not to perform a biopsy on those men with a normal DRE and serum PSA between 4 and 10 ng/ml, unless the PSAD is increased. Benson and Cooner evaluated the value of PSAD in asymptomatic men. Benson used a PSAD value of more than 0.150 ng/ml/cc (Hybritech PSA assay, and elliptic prostate volume) prospectively to select patients with a serum PSA value between 4.1 and 10.0 ng/ml for biopsy if their DRE was unremarkable. Those with a negative result were closely followed by DRE and PSA (not TRUS), and in a report of 68 patients he detected 3 carcinomas after 10 biopsies for a PSA increase of more than 1 ng/ml/year. No results were reported on patients with an initial PSAD over 0.150 ng/ml/cc [22]. Benson pleaded by arguments of other reports that PSAD could select men safely for observation [23], however these reports did not concern community based populations.
The application of PSAD to community based screening populations is limited to those patients who undergo diagnostic TRUS combined with volumetry. In several screening studies the use of TRUS is restricted to participants with a serum PSA of 4.0 ng/ml and more [4], or those participants already selected for biopsy [2]. Limitation of this time consuming screening modality is based on the assumption that the yield of TRUS to detect carcinomas in the group with a low serum PSA is limited. Clinically important carcinomas are supposed to be unpalpable and to have low serum PSA as a reflection of their small tumour volume.
PSA cut-off values as an indicator for TRUS guided biopsies have been determined from meta-analysis of outpatient populations, or from analysis of data from real community based populations. Aziz analysed the data of 4200 men, made hypothetical cohorts of men between 60 and 70 years old, and determined the optimal PSA cut-off by maximizing the number of cancers detected, and minimizing the number of negative biopsies: he arrived at a PSA cut-off value between 3.0 and 4.0 ng/ml [24]. Labrie extracted an optimal PSA cut-off by analysis of 1002 participants of a community based screening population, who underwent prostate biopsy when selected by DRE and/or TRUS [3]. He arrived at a PSA value of 3.0 ng/ml by the use of ROC curves.
Catalona objected to the use of PSAD, based on the data of his multicenter screening study of nearly 5000 men [25]. Selecting participants for TRUS volumetry and random quadrant biopsies when the serum PSA was more than 4.0 ng/ml, or the DRE was abnormal, he used the same method as Benson to determine PSAD. 33 unpalpable carcinomas were detected in the PSA range between 4.1 and 10.0 ng/ml. A PSAD cut-off value of 0.150 for prostate biopsies would have missed 48 % of these unpalpable carcinomas, a cut-off value of 0.100 ng/ml/cc 21 %. The number of biopsies would have been reduced from 161 = 100 % to resp. 25 % and 58 %. Of this group of 33 patients with PCa 18 underwent a radical prostatectomy and 17 carcinomas appeared to be organ confined. A PSAD cut-off value of 0.150 would have detected 9 organ confined carcinomas.
In the ACS-NPCDP study Littrup reported in 1994 detection of 87 carcinomas in a group of 2558 participants with a normal DRE after a 5 year follow-up [26]. 60 of those carcinomas would have been detected on indication of a PSA of 4.0 ng/ml or more at the cost of 154 biopsies, and also 60 would have been detected at the indication of a PSAD of 0.12 ng/ml/cc or more at the cost of 136 biopsies. This result is difficult to interpret, as biopsy indications in this multicentre study are not uniform, nor are the applied PSA assays. In the group mentioned, 195 men with a PSA of 4.0 ng/ml or more were not biopsied. It is also not stated whether the 60 detected carcinomas by PSA or by PSAD were of identical persons, or that the 26 not detected would have been picked up only by diagnostic ultrasonography. Speaking

in terms of detection of PCa only, in a small subgroup of 48 participants with a normal DRE and a normal TRUS and biopsied on indication of an elevated serum PSA, PSAD would have selected less patients for biopsy than serum PSA, while detecting an identical number of carcinomas (PPV 79% versus 68%).
In conclusion, there is no evidence that PSAD as an indicator for prostate biopsy is better than serum PSA regarding detection of prostate carcinoma in a community based screening population. When limited to the PSA window below 10 ng/ml, PSAD or other volume adjusted PSA values might have a slightly better performance regarding the ratio between number of biopsies and detected carcinomas. However, the cut-off value has not been uniformly established. The relation between sensitivity and specificity is reciprocal, and it seems clinically more important to detect organ-confined carcinomas at the cost of a higher sensitivity with subsequently more biopsies, than to jeopardize specificity at the cost of missing curable cancers.

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Part 4

Serial Analysis of PSA and Prostate Volume

8

SINGLE AND SERIAL PSA DETERMINATIONS IN DETECTION AND FOLLOW-UP OF PROSTATE CANCER*

Chris H. Bangma, Bert G. Blijenberg, Fritz H. Schröder

INTRODUCTION

Since its discovery for clinical application in 1979 Prostate Specific Antigen (PSA) has become a widely used parameter for prostate pathology. Though not tumour specific PSA has especially been used for diagnosis, monitoring, and follow-up of prostate cancer (PCa). For the detection of PCa single PSA measurements have been analysed in selected and population based men in relation to various other parameters. As these combinations have a limited potential in differentiating between PCa and benign prostatic conditions, the time based relation of serial PSA measurements might offer a new modality for detection of PCa. In time, this might give also new insigths in the natural history of benign prostatic hyperplasia (BPH) and the development of PCa. After radical prostatectomy undetectable levels of PSA may change to detectable, and this often is an indication for tumour recurrence. Likewise, during the follow-up of untreated PCa changes of PSA may indicate tumour progression preceding clinical progression for long periods of time. Differences between PSA values become relevant when these differences exceed the variation which is inherent to the laboratory determination. Various biological factors influence the PSA level. Knowledge of these factors is of importance for the interpretation of PSA measurements in clinical practice. Misunderstanding the laboratory and patient related variability may lead to a wrong appreciation of the discriminatory potential of PSA measurements. The application of new time based parameters, which show statistical significance in selected study populations, have to be analysed with consideration of this variability.

BIOCHEMICAL FACTS AND PHYSIOLOGICAL VARIABILITY

PSA is a 34,000 Dalton serine protease which can be detected in semen and male serum. The single chain aminoglycoside is mainly produced in the epithelial cells of the prostate, but can also be detected in other male and female Wolffian duct derivates like the seminal vesicles, the bladder, the urethra, and the glands of Skene in minimal amounts. In clinical practice however PSA can be regarded as 'prostate-specific'.

The PSA concentration in semen is more than 10^6 ng/ml. Its physiological function is liquefaction of the seminal coagulum. Up to 70 % of the secreted PSA purified from

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* *Adapted from: Single and serial PSA determinations in detection and follow-up of prostate cancer, Update Series, European Board of Urology, 1995; 1: vol 4 (1): 2-7*

semen has been estimated to have enzymatic activity. It remains uncertain whether the partial inactivity is a manifestation of the zymogen, or due to degradation by internal bond cleavages in the PSA structure, or due to inhibition by one of the serpins, the family of extracellular serine protease inhibitors which include Protein C Inhibitor in semen, and α-1-antichimotrypsin (ACT) in serum. The 'purified' seminal PSA forms the base of standards in several PSA assays.
A small fraction of PSA leakes to the blood, and can be detected in serum in a free form, and in several protein bound forms. The free form is most likely the inactive (internally clipped) form of PSA with the same molecular weigth as the free active seminal form. The main complex formation occurs with the abundantly available ACT in up to 95 % of the detected serum PSA [1]. In contrary to the unbound form, this complex of ca. 90.000 Dalton can normally not be cleared by the kidney. A serpin complex receptor in the liver has been identified which may be involved in degradation of the complex. The serum half-time value of PSA has been determined between 2.2 and 3.2 days. It is likely that clearance of the free form of PSA is much faster than the clearance of the in serum predominantly circulating complexed form. At -20 degrees Celsius the PSA molecule is stable for a long period, and can therefore be kept for research purposes. It is custom to freeze sera at -80 degrees Celsius.
The individual PSA concentration in serum shows a fluctuation during the day between 7.2 and 17.6 %, independent of prostate pathology, and for various PSA assays. As no diurnal rhythm has been found, it is well possible that this fluctuation is caused by other factors. At least in young men the PSA seems to have a maximum during the spring, most likely due to hormonal influences.
Any trauma to the prostate may increase the serum PSA. The maximum levels after digital rectal examination (DRE), cystoscopy, or prostate biopsy are measured about one hour after the procedure. DRE seems to have a statistical relevant influence only in patients with a pre-examination value of 20 ng/ml or more, but altered PSA values after DRE would have changed clinical decisions only in 3 % due to an increase above a cut-off level [2]. Ejaculation statistically raises PSA by minimal amounts. Even transurethral manipulation like rigid or flexible cystoscopy was found to influence serum PSA minimally, resulting in a 4 % change of clinical importance. Transrectal ultrasonography and prostate biopsy however immediately raise PSA.

PSA IN RELATION TO PROSTATIC PATHOLOGY

In comparison to a control population of asymptomatic men, increased values of PSA are seen in benign and malignant prostatic disease. Prostatic infarction and prostatitis show reversible PSA increases during their clinical course. PSA elevations seen with these various conditions are overlapping. In benign prostatic hyperplasia (BPH) the epithelial, glandular, and stromal volumetric fractions may increase independently of each other. This results in an elevated PSA with a weak correlation between PSA and total prostatic volume or the volume of the prostatic adenoma: in a screening population of 502 men in Rotterdam the correlation coefficient was 0.58 [3], in a selected series of 69 patients undergoing transurethral resection of the prostate (TURP) this correlation was 0.61 [4]. From such TURP studies the PSA leakage into the circulation per gram of resected adenomatous tissue during prostatectomy has been estimated between 1 and 3 ng/ml. Surprisingly this PSA leakage has been found not to correlate with the ratio between PSA productive glandular tissue and non-productive stromal tissue [5].
In prostatic carcinoma the PSA production reflected by the serum levels per gram of malignant tissue is estimated to be 3.5 ng/ml/gram. Malignant tissue actually produces less PSA than normal prostate epithelial cells, so it has to be concluded

that tissue barriers in malignancies of the prostate are such that they result in more leakage than in normal tissue. As tumour volume is correlated to clinical significance, it is likely that serum PSA is a reflection of the significance of the condition [6]. However, tumour differentiation also contributes to the variability of this PSA production value [7], and its reverse relation to tumour grade decreases the correlation to serum PSA.

PSA-ASSAY VARIABILITY

For detection of serum PSA several commercial assays exist. The reproducibility or assay variability is dependent on various factors like laboratory technique (automated, or semi-automated), reagent and calibrator stability and preparation, sample handling, and other factors. Intra-assay variability, expressed in the coefficient of variation (CV), which is the ratio of the standard variation and the mean of repeated measurements of each serum sample, has been measured for several assays by their manufacturers, and normally is around 5 % [8]. The determination of CV should be given for various ranges of PSA (the low, intermediate, and high range), as the percentual variation is usually larger at the lower end of the detection range of the assay. The variability over several days (the inter-assay variation), and the variability between various batches of the assay (the lot-to-lot variation) often is sligthly more than 5% [8]. In the laboratory of the authors institution the inter-assay coefficient of variation for the PSA assay (IMx) measured over 22 consecutive days was 4.2 to 6.6 % for three commercial sera (with a PSA of 1.5, 5.0, and 40 ng/ml). The inter-assay CV expresses adequately the variation of PSA determinations during the normal laboratory routine, and should be mentioned in every scientific article reporting on PSA values.

It has been observed that different assays may give different results in the same patient. Comparison has been performed for various assays resulting in value differences for the same sample from 2 to 100 fold [9]. Also 'high dose hook effects' of false low results were seen for very high (more than 500 ng/ml) PSA values. The cause of this phenomenon is not fully understood, but is associated with an overdose of antigen.

Discrepancies are likely due to the assay design and due to the complex formation of PSA with proteins in blood, which may obscure antigenic epitopes. The choice of calibrators further disturbs uniformity. In calibrators based on female sera endogenous antibody to prostate-specific antigen have incidentally been detected. For calibrators based on the purification of seminal PSA no standard production technique exists. Dilution of the standards for use in very low PSA ranges introduces additional variation. Like in serum, the complex formation of PSA may occur. These factors all contribute to the need for standardization and reference procedures.

MORE ABOUT PSA ASSAY DESIGN AND COMPLEX FORMATION

Most PSA assays make use of a sandwich principle: a PSA-antibody, mostly connected to a solid carrier, captures the PSA molecule on one (monoclonal) or a combination of epitopes (polyclonal); a second antibody for this complex, bound to a label, is needed for the detection. This detection reaction is based on an enzymatic proces, the measurement of radio-activity, or fluorescence. There are at least 70 mono- and polyclonal antibodies known to react with PSA, and certainly 5 epitopes have been identified on the PSA molecule. These epitopes may be (sterically) obscured by serum proteins, predominantly ACT [1] (FIGURE 1).

Some proteins, like α-2-macroglobulin, even may obscure all epitopes, which makes PSA detection impossible. Different assays detect the free and complex PSA forms in

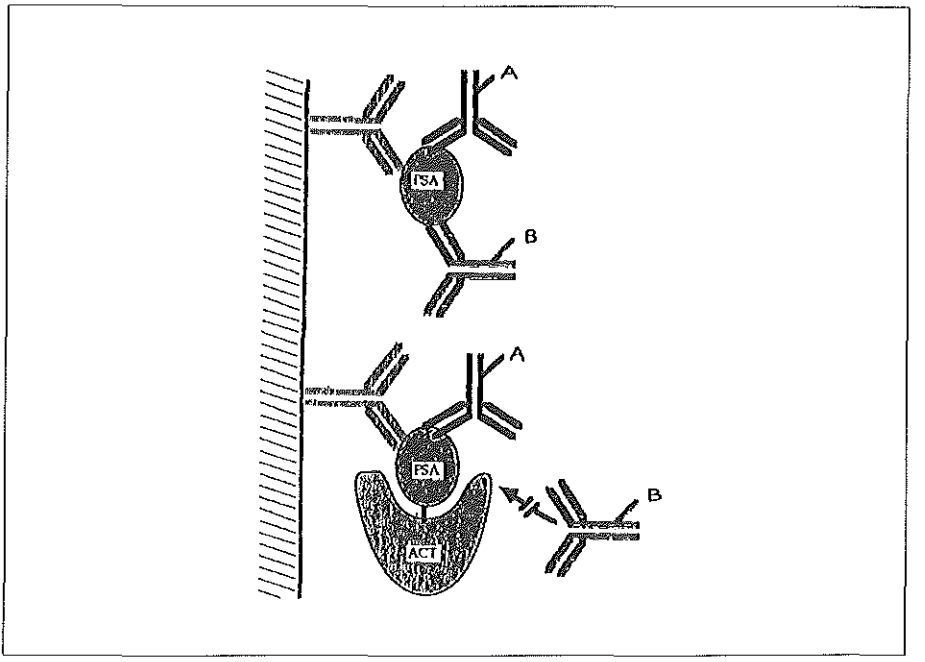

FIGURE 1

Schematic representation of free and complexed serum PSA (ACT). Antibody A will detect both free and complexed PSA, antibody B detects only free PSA.

different molar ratios [10]. Apart from the sterical shape of the PSA molecule, this may be due to the assay kinetics, in which the length of the incubation time influences the detection reaction. In assays with a short incubation time, the reaction between PSA and antibody may not come to an equilibrium, and induce skewed molar-responses regarding the different forms in which serum PSA is available. An assay is said to be equimolar when the ratio between the detected free and complex PSA forms is 1:1. The ratio between free and complex or total PSA (F/T ratio) is variable, and appears to be smaller in PCa compared to BPH. This might be due to the relative overproduction of ACT in prostate cancer cells compared to normal prostatic cells. Immunostaining in cell cultures showed that a high portion of cells expressed both ACT and PSA in low graded PCa, while only occasionally so in BPH. The F/T ratio therefore may be used for differentiation between benign and malignant prostatic disease [11]. In that study the F/T ratio improved differentiation between 57 patients with BPH and 32 PCa patients, increasing specificity from ca. 40 to 65 % at a sensitivity of 90 % by the application of the F/T ratio. In case the ratio between free and complexed PSA forms of a calibrator is not (completely) known, the equimolar assay is the best option for comparison of serum PSA with assay calibrators [12].

Standardization of calibrators and PSA assays is of utmost importance to be able to compare PSA values from different assays. This enables the clinician to make decisions especially in the low and intermediate PSA range of 2-10 ng/ml, in which the indication for transrectal ultrasonography and prostate biopsy still is under discussion.

SINGLE AND SERIAL PSA DETERMINATION

In this article PSA determinations are called 'single' when performed only once, or interpreted independently of former determinations. This in contrast to serial PSA determinations, in which repeated determinations are related to each other, especially by mathematical methods including a time factor. Serial measurements of PSA might be used to differentiate between benign and malignant disease, for follow-up after radical prostatectomy, and to predict progression in cancer already diagnosed.

Mathematical expressions of PSA change over time: PSA velocity and PSA doubling time

Cross-sectional studies of screening populations have shown that in participants of 50 years and older PSA shows a steady increase with increasing age. Bosch calculated the PSA concentration increases in 502 participants of a cross-sectional population based study, excluding PCa, to be 2 % per year [3]. Prostate volume also increased by 2 % annually, and had a stronger correlation with PSA than age (coefficient of correlation r = 0.58 resp. 0.25). Similar to other biological growth related markers PSA shows an exponential increase.

Basically there are two ways how to describe the increase of PSA mathematically. In a linear increase model (FIGURE 2A) there is a steady annual increase in PSA in absolute terms, resulting in an PSA VELOCITY expressed in ng/ml/yr, in formula PSAV = (PSA1 - PSA2) / t. In an exponential increase model (FIGURE 2B) there is a steady annual increase of PSA, which can be expressed in an annual percentage, or in a PSA DOUBLING TIME, by formula PSADT = log2 x t / (log PSA2 - log PSA1) [13]. The PSADT illustrates the time needed to double the value of PSA, calculated from two PSA values separated by the time t. PSADT corresponds better with PSA increase in a growing gland than PSAV does.

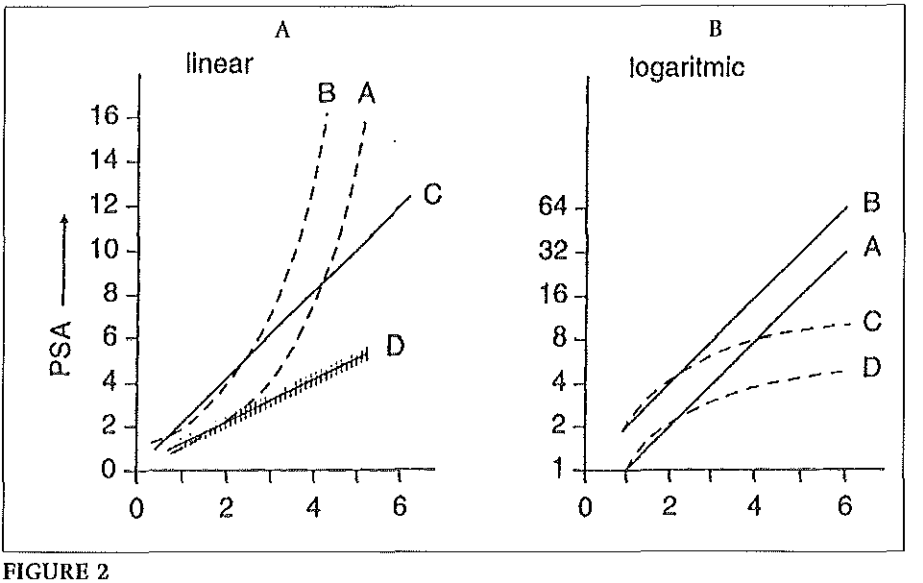

FIGURE 2

Representation of PSA versus time on a linear scale (A) and a logarithmic scale (B) for linear increase (C, D) and exponential increase (A, B) of systems with different initial values. The hatched band indicates the analytic variability.

During the early phase of growth the minimal increase, and the variability of the PSA measurements are responsible for the fact that linear and exponential increase may be indistinguishable from each other, as shown in FIGURE 2 by the overlapping bands. When illustrated on a linear scale identical increases in the absolute PSA concentration, or PSA-velocity, in prostates with various initial PSA values show parallel curves, while increases in prostates with identical doubling time, but different initial PSA values, are not easily identifyable. On a logarithmic scale for PSA however, identical exponential increases can be recognised as parallel lines. Changes in PSA production curves (for example due to the development of a carcinoma) in a prostate gland with a steady annual PSA increase, would therefore be best illustrated in a logarithmic model. Furthermore, PSADT is independent of the applied PSA assay. Comparison of PSA increase of men from different study populations using different assays can be done by calculation of PSADT.

PSA FOR DETECTING PROSTATE CARCINOMA

Single measurements

Single PSA determination are normally used with the intention to show the presence or absence of a prostate malignancy with an acceptable certainty, and to select patients for further investigation. This should always be accompanied by a digital rectal examination (DRE), and an adequate history related to the benign factors that may increase or decrease PSA.

The suitability of PSA for these purposes is expressed in the sensitivity and specificity of the test to detect PCa and to differentiate between benign and malignant disease. Calculation of the sensitivity and specificity varies with the incidence of PCa in the population studied [14]. The incidence is dependent on the method of detection of PCa, and the composition of that population. As the PCa incidence is different in autopsy studies, population based screening studies, and outpatient studies, sensitivity and specificity are difficult to compare among different studies. If the incidence is unknown it is not possible to calculate sensitivity and specificity.

In screening programs the aim is early detection of clinically important and treatable prostate carcinomas (PCa) by means of a sensitive, specific, reproducible, and most desirably non-invasive, fast, and inexpensive method. The sensitivity and specificity of single PSA determinations to detect PCa has been related to various cut-off values, showing that the sensitivity increases at lower values at the cost of the specificity. This leads to a large number (up to 25 % of the screened population) of diagnostic prostate biopsies for histologic confirmation of PCa, while most of them are negative due to the low specificity of PSA. Therefore PSA determinations have been combined with one or more of the following modalities especially to improve specificity: age, DRE, PSA index or density determination, or diagnostic transrectal ultrasonography (TRUS). The size of the prostate is the most important noncancer variable in determining serum PSA concentrations. The PSA-index or PSA density, this is the ratio between PSA and ultrasonic prostatic volume, has been shown to increase the positive predictive value for PCa detection in selected outpatient populations. Especially correction of PSA for the ultrasonic volume of the adenoma (PSAT) seemed to be better in predicting positive biopsies in the PSA range between 4.0 and 10.0 ng/ml.

In the Rotterdam feasibility studies for screening prostate carcinoma was observed that when the PSA value was more than 10 ng/ml approximately 50 % of all

screening participants were found to have a prostatic carcinoma confirmed by biopsy. Almost 70 % of participants had a serum PSA below 2 ng/ml, and only one carcinoma was detected in this group of 812 men. Especially in the PSA window between 2 and 10 ng/ml higher specificity was required to reduce the number of diagnostic biopsies with a negative result. However, within this window PSA indices related to total prostatic volume, or to the volume of the adenoma, or to both, were only minimally better in differentiating between benign and malignant and in selecting appropriate participants for prostate biopsy.

Serial measurements

The interpretation of serial PSA measurements is influenced by the analytical variation of the PSA assay, and the physiological variation over time excluding prostate pathology. The analytical intra- and interassay variation has been expressed in the coefficient of variation (CV), and normally does not exceed 10 %. The daily variation has been measured between 7.2 and 17.6 % [15].

Fluctuation of serum PSA over longer periods have been measured in normal men to determine the minimal change needed to exceed the physiologic variation of PSA. Combined with the analytical variation and knowledge concerning PSA increase in prostate pathology, this could lead to calculation of the minimal interval between PSA measurements needed for follow-up in different groups of patients. In patients with a short PSA doubling time, the PSA interval should be shorter compared to those with a long PSA doubling time. For example: in men with BPH and an annual PSA increase of approximately 3 % [3], this is a PSADT of approximately 35 years, the expected PSA change does not even overcome the analytical variation of the assay in two years time. In patients with progressive PCa, PSA doubling times of 13 months have been reported [see further, 13]: in 6 months a PSA increase may overcome a physiological variation of nearly 50 %.

Serial PSA determinations may relate to progression from undetectable to detectable stages of prostate carcinoma in community based samples. Changes of PSA however should exceed the physiologic variation to become significant. To study the physiologic variation in a group of 129 asymptomatic males two or more PSA measurements were done within the period of one year by Riehmann [16]. He found that the mean coefficient of variation of 58.0 % considerably exceeded the assay variation of 13.2 %. Decreasing PSA levels were at least as often seen, and of the same magnitude, as increasing levels, within a relatively narrow window of one year. In 10 of 39 men with 3 or more PSA determinations the slope of PSA increase over time exceeded + 20 %. The variation of slopes between two measurements was especially extreme when determinations were performed within a short period of time. The median follow-up time in men with at least three PSA determinations was 0.59 years.

The physiological variation of PSA in the 4.0 to 10.0 range observed by Stamey et al. [17] concerned observations in 91 volunteers, of whom two serum samples were taken within a 38 day period (mean: 22 days). The interassay CV was 3.9 %, with a 95 % confidence interval of 10.5 %. The CV of the two draws of an identical patient was 8.4 %, with a 95 % confidence interval of 23.5 %. Statistical analysis showed that a PSA change of 30 % or more (over the period of 22 days) could be interpreted as a real change.

Barry calculated the changes of serum PSA in 239 men with BPH by three-monthly determinations (Hybritech) for one year [18]. He showed that serum PSA had increased by at least 1.6 ng/ml in 20 % of men after 3 months. However, taking the average of two serum PSA determinations at baseline and 3 months, the increase

was 1.1 ng/ml in 20 % of this population, and 0.9 ng/ml after 9 months compared to the average of three previous samples. These studies illustrate that within the period of one year the PSA variation in absolute and in relative terms in normal men is considerable in a large portion of the populations studied. Multiple PSA determinations within the period of one year may increase the reliability of a single PSA value. It is not known whether the use of the average of two or more PSA determinations instead of the initial PSA value would influence clinical decisions in an important way.

To determine whether PSA changes could differentiate between benign and malignant disease in a community based population, several studies were analysed. In a longitudinal study of 376 men in a screening population with two PSA determinations over a period of 12 months Oesterling found an annual PSA increase between 5.1 and 11.4 % [19]. Only four carcinomas were detected (detection rate 1.1 %) with PSA and DRE; they showed an annual PSA increase of almost 60 %. Brawer described the annual PSA increase in 701 participants of a screening population with a follow-up of 12 months [20]. He did not show any difference in PSA velocity between benign and malignant disease. The absolute value of PSA however induced the detection of 1 % extra PCa in participants who previously had a PSA of less than 4.0 ng/ml, with an overall detection rate of 2.6 % in the first year. Participants with a PSA of less than 4.0 ng/ml had been excluded from DRE or TRUS. In a study of 121 participants of the same population undergoing biopsy because of a PSA value of more than 4.0 ng/ml neither the PSA velocity, nor a PSA increase of 20 % over one year, were able to stratify between 95 men with BPH and 26 men with PCa [21].

However, in a cohort of 54 men with a study period of 7 to 25 years with 2-yearly PSA determinations Carter showed in retrospect that a PSA velocity of more than 0.75 ng/ml/year (measured over at least 4 years) differentiated between 18 men with PCa and 36 men with benign prostates with a sensitivity of 78 % and a specificity of 90 % [22]. An absolute PSA value of 4.0 ng/ml or more also had a sensitivity of 78 % to detect PCa, but the specificity was 60 %. It was also noticed that during the study period of 7 to 25 years before the time of histologic confirmation patients with a prostate cancer in retrospect showed an exponential increase of PSA starting up to 5 years before diagnosis. Similar values of sensitivity and specificity were found in a screening study from which these 36 men were selected [23]. Also from this screening population, a preliminary analysis of serial biannual PSA determinations revealed that the PSA velocity of carcinoma patients was generally higher than for patients with normal biopsies or with normal PSA levels who did not have biopsies (1.32 vs. 0.28 vs. 0.02 ng/ml/year). The confidence intervals of the PSAV were too wide and overlapping to allow for a better discrimination between benign and malignant disease than by serum PSA. In a recent study of Carter [24] a computer simulation of PSA change over time was done in 56 PCa patients with a PSA sampling every two years, and in 223 men with BPH, in which PSA was drawn every three months. Using a PSA increase of 0.75 ng/ml/year or more as a predictor for PCa, a specificity of more than 90 % was obtained after 1.5 years (sensitivity of approximately 70 %). It was concluded that PSA measurements for the follow-up of asymptomatic patients with BPH should be taken with an interval of at least 1.5 years. In this report the annual PSA increase (PSA velocity) of 0.75 ng/ml improved the specificity of PCa detection compared to a PSA cut-off value of 4.0 ng/ml when measured over at least 4 years. PSA measurements with an interval of 2 years were obtained in the ACS-NPCDP screening study [25]. Mettlin analysed 1473 normal men and 84 men with PCa. The mean follow-up time was 2.9 years for the benign subgroup, and slightly shorter, 2.2

years, for the subgroup with PCa, as detection of carcinoma caused elimination for further follow-up. The PSA increase was computed by the difference between the first and the last PSA value over the time between those values. In 1354 men with an initial PSA below 4.0 ng/ml the average PSA increase was - 0.03 ng/ml/year, and for 35 men with cancer + 1.04 ng/ml/year. An increase of 0.75 ng/ml/year could detect less than half (46 %) of these men with cancer. From the identical screening study Littrup analysed 2120 men with more than one yearly PSA measurement [26]. Unfortunately the screening protocols and PSA-assays are different over the various institutes of this multicentre study. For use in a ROC curve 951 participants were selected, 63 of them with a prostate carcinoma, as men with a PSA decrease were excluded from evaluation. The group studied was dominated by participants with an initial PSA lower than 4.0 ng/ml. The annual PSA increase of 0.06 ng/ml was the best discriminator between benign and malignant in this subgroup with a serum PSA below 4.0 ng/ml. An annual PSA increase of 20 % showed a limited relative discriminatory potential.
These studies suggest that an absolute PSA increase (PSAV) of 0.75 ng/ml/year or more may have some potential in differentiating benign from malignant prostatic disease, but mainly so in selected groups of patients. In community based populations however the absolute PSA value seems to be more valuable as an indicator for prostate biopsy. The influence of the physiologic variation of PSA on the calculation of PSA velocity or percentual PSA increase becomes less when PSA determinations are separated by a period of 1.5 years or more. Decisions to biopsy for a PSA increase of 0.75 ng/ml/year or 20 % of the baseline in community based populations should be based on PSA observations spaced by an adequate interval of at least one year, but the optimal interval most likely is between 3 and 4 years, and still has to be determined in ongoing screening studies.

PSA IN STAGING

PSA has been related to stage especially for preoperative evaluation. The usefulness of PSA to differentiate between confined and non-confined carcinoma in an individual patient appeared to be limited [7]. Although there was a correlation between histologically determined tumour volume and serum PSA of 0.54, the volume of BPH showed a negative correlation with PSA. The unpredictable contribution of BPH in a prostate gland with a carcinoma together with the variable PSA production in relation to tumour grade explained that there was no linear relationship between serum PSA and stage. Final pathological stage was predicted better by a combination of serum PSA, Gleason score in the preoperative biopsy, and clinical stage, than by one of these variables as a single parameter. Nomograms and probability plots were constructed from a series of 703 radical prostatectomy specimens, also predicting nodal involvement.
Ultrasonically derived parameters were assessed for staging in combination with serum PSA in a series of 29 patients who underwent radical prostatectomy [27]. PSA, capsular characteristics and tumour volume, measured by one ultrasonographer, could determine or exclude extracapsular disease correctly in only 13 patients. Furthermore ultrasound underestimated histologic tumour volume by large amounts, and extracapsular disease, based on capsular irregularity or interruption, was picked up only in 6 out of 11 patients. In a retrospective study of 38 patients in the authors'clinic preoperative ultrasonic determination of the T-category was correct in 50 %, which was similar to clinical staging. This means that the subjective interpretation of ultrasonography does not help in staging prostate carcinoma. Ultrasonic prostate volume however appeared to be useful for the determination of

clinically important tumours by means of the calculation of volume adjusted PSA values. In a series of patients with unpalpable T1c PCa who underwent radical prostatectomy the combination of a PSAD of less than 0.15 ng/ml and a grade 1 or 2 in one diagnostic biopsy only (which gives an indication of tumour volume) was the best predictor for clinically unimportant T1c carcinomas, defined as confined well differentiated tumours of less than 0.2 ml [28].
In a series of more than 1000 radical prostatectomy patients the combination of PSA with clinical stage and biopsy grade could predict nodal metastasis with a false negative rate of less than 3 % [29]. This could prevent a pelvic lymphadenectomy in 25 % of patients clinically staged T1a to T3a. Pathological T-stage alone could be predicted by PSA in combination with various clinical parameters in different series between 70 and 90 %.
Metastasis was differentiated from BPH in 100 % by PSAD in a retrospective study, but the overlap with patients staged M0 was considerable [30]. A serum PSA of less than 10 ng/ml was found to correspond with a negative bone scan in all patients while staging PCa [7].

PSA FOR RESPONSE TO TREATMENT

Endocrine treatment

Repeated PSA determinations may be used to assess response to treatment. Not so much the rate of change in PSA has been evaluated, but predominantly the absolute decrease in ng/ml, the decrease in percentage of initial values or time to normalisation were analysed. The effect of endocrine therapy for BPH has been correlated to the reduction in prostate volume, and therefore PSA, but did not show a high correlation with a reduction in symptomscores. There is no evidence yet that endocrine treatment obscures detectability of PCa despite the reduction of PSA levels. In adenocarcinoma after surgical or hormonal castration plasma testosteron will go down. PSA is an androgen dependent enzyme which as a result will show a decrease, and can be used as a biochemical marker for progression later on.

Radiotherapy

Evaluation of the therapeutic effects of external beam radiotherapy or 125-I radioactive seed implantation for confined PCa is more complicated due to the lack of pathological staging of local and lymphnode status. PSA also is a limited tool, as the decrease of PSA to baseline levels (the nadir) takes on average 6 months. Normal benign prostatic tissue within the radiated gland is relatively resistant to radiation, and therefore will continue to produce PSA. Irradiation of the normal prostate will decrease PSA in 50 % of cases to undetectable levels [31].
In series evaluating effectiveness various PSA cut-off levels have been chosen to define a complete and durable response. The likelihood of a disease free status after 4 years was significantly lower when a PSA nadir of 1.0 ng/ml was used as an end point rather than when clinical evaluation by physical examination and a bone scan was used [32].
The PSA kinetics after radiotherapy provided little useful clinical information in a study of 154 patients [33]. The PSA half-life time as a result of therapy was not related with subsequent PSA doubling-time in recurrent disease. Doubling times appeared to be longer in low grade tumours. PSA recurrence preceeded clinical recurrence in the majority of patients by more than 40 months [34].
The level of serum PSA has been reported as the only factor predictive for local progression in patients after radiotherapy who underwent salvage radical prostatectomy [35].

The effectiveness of adjuvant radiotherapy directly after radical prostatectomy of pT3 N0 M0 PCa is under evaluation in the E.O.R.T.C.trial 30913. In small uncontrolled studies PSA decreased, often to an undetectable level, after immediate adjuvant radiotherapy [36], and delayed symptomatic tumour recurrence [37]. Delayed radiotherapy for histologically proven local recurrences also turned out to be effective in decreasing PSA. These studies illustrate the value of PSA as a marker after radiotherapy.

Radical prostatectomy
After radical prostatectomy PSA levels should be undetectable. An increase of PSA (above the detection level) is evidence of recurrence or residual disease. In the low range of PSA (less than 0.1 ng/ml) several 'ultrasensitive' assays have been developed to detect minimal residual PSA production. Repeated PSA determinations therefore have been advised in the follow-up of postoperative patients for early detection of recurrence. Isolated PSA recurrence often preceeds clinical recurrence by several months.
In patients after radical prostatectomy serial PSA determinations with the calculation of the half-life time of PSA have been used extensively to study effectiveness of the therapy. In 51 patients with a mean follow-up of at least 10 months, patients in which the postoperative PSA became undetectable, but who had a biochemical relapse later on, showed in the direct postoperative period of PSA disappearance a mean PSA half-life of 3.0 days. This was identical to the PSA half-life time of patients with residual disease, but different from the patients without PSA relapse (PSA half-life time 1.5 days) [38]. In 16 men with documented local recurrence the median PSA velocity of 0.43 ng/ml/month (4.8 ng/ml/year) was statistically not different from the median PSA velocity of 2.0 ng/ml/month (21.6 ng/ml/year) in 35 men with distant residual disease, unless combined with pathologic grade and stage [39]. Application of this method however has been limited, as there are no therapeutic consequences for these patients yet.

APPLICATION FOR DETECTION OF PROGRESSION

Detection of progression in untreated prostate carcinoma
The natural history of clinically confined prostate cancer is still incompletely understood. Available literature is biased by selection of patients left untreated. Median times to progression for focal disease (T1A) may be in the range of 13 years, and in up to 10 years for T2 disease.
Also the pattern of progression, either locally or to metastatic disease, of T1 and T2 tumours is not known. Available information seems to indicate that metastatic progression, independent of local progression, occurs in about 20 % of the patients with T1A (A1) tumours; in patients with T2 tumours 20-40 % developed metastases after 10 year [40]. This meta-analysis however reflects the selection bias of the included series of patients.
Uncertainty exists with respect to the definition of local progression in T1 and T2 disease. Usually the WHO rule indicating an increase in size of 25 % or more of the product of the two largest diameters as progression is applied. The EORTC also applies an increase in one T-category. A volume increase of more than 40 % measured by transrectal ultrasonography was described as an indicator of progression.
Concerning the correlation of PSA increase and progression in T1 and T2 tumours in time virtually no information is available in the literature. With regard of the

uncertainties of identifying local progression it may well be possible that an increase of PSA is a more accurate indicator of progressive disease in this group of patients.
At the authors instititution serial PSA determinations of a selected group of 29 patients with untreated confined adenocarcinoma of the prostate were correlated to their clinical course. Their mean age was 74 years. There were 15 T1A patients, and in only one patient histology was undifferentiated. Follow-up was at least twice yearly over a mean period of 39 months (FIGURE 3). Local progression occurred in 13 patients after a mean period of 31 months without relevant volumetric increase of the prostate on transrectal ultrasound. Metastatic progression was not observed. There was no difference in grade or stage with respect to time of progression

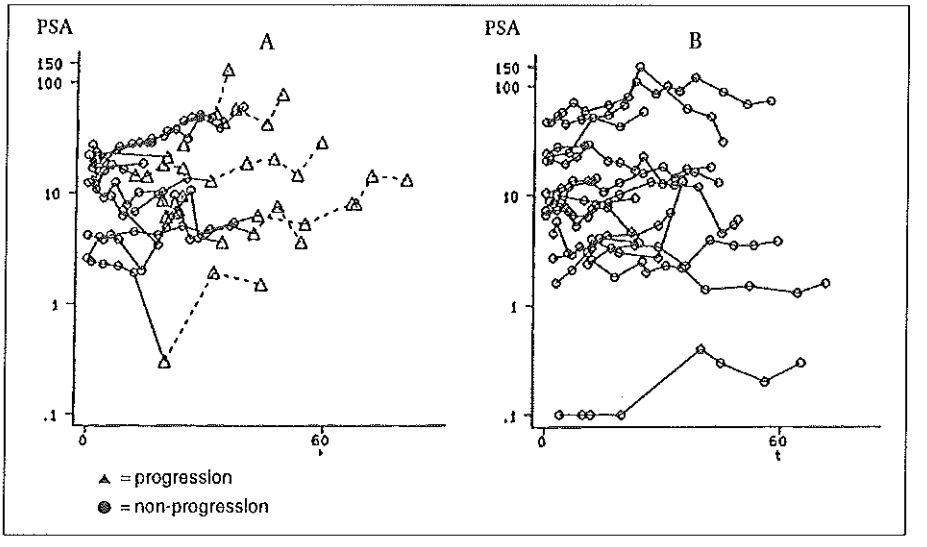

FIGURE 3
Scattergrams of PSA (ng/ml) over time (days) in logarithmic plotting for clinically progressive (A) and non-progressive patients (B).

between progressive and non-progressive patients. Percentual PSA increase, annual PSA increase during the first, second, and third year of observation, or the initial PSA value could not differentiate between progressive and non-progressive disease. The mean annual increase of PSA was 14 % (mean PSADT of 5 years), and of prostate volume 15 %, with a weak correlation between these two. FIGURE 3 shows the variation of PSA determinations. It is evident that the fluctuations of PSA within the curve of each individual patient, even on a logarithmic scale, influences the suitability as a predictor for progression. After more time has passed and objective metastatic progression occured, a difference between both groups might be observed more clearly.
In a retrospective study of 43 patients with untreated PCa Schmid found a significantly lower PSADT in patients with higher grade and stage [41]. In 20 of 28 patients with clinically organ confined carcinoma PSADT was more than 4 years,

while in metastatic disease the PSADT was in 9 of 15 patients less than 4 years. Although 14 % of all patients had a stable PSA, it was concluded that PCa has a constant exponential (log-linear) growth rate.
In 40 patients with confined PCa Wemyss-Holden was not able to find any predictive parameter for progression [42]. Progressive patients tended to have higher serum PSA levels, but PSA increase, grade or stage could not predict progression.
Contrary to localised PCa, Davidson found in 54 untreated patients with lymphnode positive prostatic cancer in the delayed hormonal treatment arm of EORTC trial 30846 at the authors institution a statistical difference in mean PSADT for 13 progressive (PSADT = 13 months) versus 41 non-progressive (PSADT = 42 months) patients [13]. Progressive patients showed higher grade disease and tended to have a higher stage at diagnosis. The increase of PSA in percent after 6 to 12 months after staging was predictive of progression (FIGURE 4). A PSADT of 12 months correlated with progression in 100 % of cases.

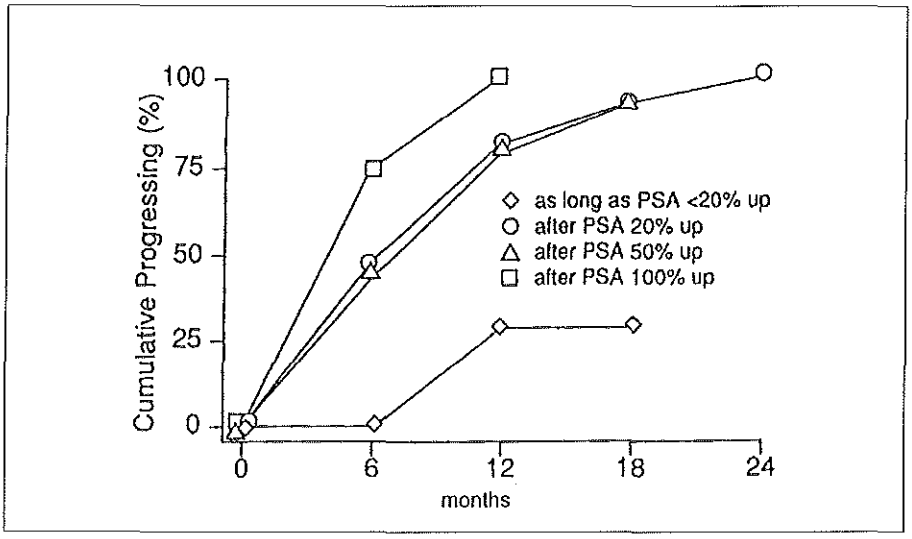

FIGURE 4
Cumulative progression (%) of untreated node positive patients with prostate carcinoma related to time (months) for various percentages of PSA increase after definitive staging.

CONCLUSION

Single measurements of PSA show an assay variability up to 10 %. Therefore individual changes of PSA have to be 20 % or more to be considered relevant. Serious manipulations of the prostate and prostatitis may cause excessive increases of PSA. Single PSA determinations are an adequate tool for prostate cancer detection if PSA levels are elevated. This can be improved by DRE, diagnostic TRUS, or prostate volumetry. Even if the reproducibility of ultrasonic prostate volumetry (and therefore PSA-index) migth be improved further, the inability to determine adequately the presence of PCa and tumour volume preoperatively is a major limitation of TRUS. Prostate screening programs may predict and exclude clinically relevant carcinomas in an early stage. PSA was shown to predict nodal metastases

and locally extensive disease in certain situations with acceptable accuracy if combined with other parameters. After potentially curative forms of treatment PSA is an excellent tool to predict residual or recurrent disease, but a biochemical PSA recurrence has no implication for therapy yet.
For carcinoma detection longitudinal studies with serial PSA determinations have not been completed. Serial measurements with calculations of PSA velocity are used in addition to single PSA determinations, but so far no additional value in predicting future progression has been shown.
Serial measurements of PSA in untreated prostate carcinoma show an exponential increase, and are therefore best evaluated by PSA doubling time (PSADT), which is less dependent on the chosen PSA assay, and the level of PSA at the beginning of observation, and corrects better for prostate volume than the absolute increase of PSA (PSA velocity) does. PSADT in benign disease is estimated on 35 years, in selected patients with confined prostate carcinoma 5 years, in non-progressive lymphenode positive disease 42 months, and in progressive lymphenode positive disease 13 months. A PSADT of 12 months is correlated with a 100 % progression rate in lymphnode positive disease, but in other patients PSADT has not shown to be a predictor for progression. During the follow-up of prostate carcinoma PSA determinations are probably advisable to be done not less than every 6 months, in combination with a physical examination.
Near future developments will comprise the application of the PSA Free/Total-ratio in population based screening programs, preoperative staging, and in follow-up of PCa. Like PSA-index and PSADT, the F/T-ratio was shown to differentiate between benign and malignant prostatic disease in some instances. As the mechanism for the formation of complexed PSA is still unknown, it is impossible to predict whether the F/T-ratio is correlated better with tumour volume or tumour grade than total PSA is. As the F/T-ratio is lower in patients with PCa compared to BPH, the change of F/T-ratio might correspond with tumour induction or progression.
Though serial PSA determinations with calculation of PSADT or PSA velocity have been promising, their impact as yet is not as large as the serum total PSA determination has been so far.

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9

SERIAL PROSTATE SPECIFIC ANTIGEN MEASUREMENTS AND PROGRESSION IN UNTREATED CONFINED (T0-3 Nx M0 G1-3) CARCINOMA OF THE PROSTATE*

Chris H. Bangma, Wim C. J.Hop, Fritz H. Schröder

ABSTRACT

Objectives. The contribution of serial Prostate Specific Antigen (PSA) determinations was studied to obtain better understanding of the natural history of clinically confined prostate carcinoma.

Methods. Serial PSA determinations of 29 patients with untreated confined prostate carcinoma were correlated to their clinical course over a mean period of 39 months.

Results. Local progression occurred in 13 patients after a mean period of 31 months. Metastatic progression was not observed. Grade and stage, nor PSA changes or initial PSA showed significant difference with respect to time of progression between progressive and non-progressive patients.

Conclusions. PSA does not parallel clinical progression in patients selected for watchful waiting.

INTRODUCTION

This study was carried out to contribute to a better understanding of the natural history of clinically confined prostate carcinoma, and to study the contribution of serial Prostate Specific Antigen (PSA) determinations. For this reason PSA determinations were correlated with the clinical course in 29 patients in whom a policy of watchful waiting was applied. In several studies a rising PSA predicts later clinical progression [1, 2, 3, 4]. The duration of the time interval between both events depends on tumour stage at diagnosis or treatment. Concerning the correlation of PSA increase and progression in T1 and T2 tumours in time very little information is available in the literature. With regard to the uncertainties of identifying local progression it may well be possible that an increase of PSA is a more accurate indicator of progressive disease in this group of patients.

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PATIENTS AND METHODS

29 Patients with histologically confirmed adenocarcinoma of the prostate were evaluated retrospectively from the time of diagnosis till the time of first treatment, if any, for cancer related progression. The mean age was 74 years (range 58 - 85), and the mean follow-up was 39 months (11 - 73). Stage was assessed clinically by digital rectal examination (DRE), according to the 1992 TNM-classification [5]. In 15 patients staged T1A, previously not suspected carcinoma was found in transurethral resection specimens. Grade was according to the Anderson-classification [6]. Metastatic disease was excluded by a normal chest x-ray and a normal bone scan. The decision not to treat at the time of diagnosis was made by the urologist in discussion with the patient and his family with respect to age, general health, clinical stage, and patient preference. All patients were felt to have the likelihood of surviving at least one year.

Follow-up

Patients attended follow-up clinics normally twice yearly (mean number of annual visits 2.7, range 1.4 - 4.3). Physical examination including DRE, serum PSA, and Alkaline Phosphatase were assessed at each visit from the time of diagnosis onwards. When no PSA value was available at the time of diagnosis (baseline-value), but one or more months afterwards, this value was extrapolated by linear regression from the logarithmically transformed PSA values versus time during the first two years from diagnosis for individual patients. This method was evaluated by applying the procedure to those patients in whom a baseline value of PSA was available (n = 10). In this group the correlation between the extrapolated value thus obtained, and the actual value was very good (r = 0.98), indicating its validity. The values thus obtained in case no baseline value was available were used in all further calculations when a baseline value was needed (except for one patient in which PSA follow-up started two years after diagnosis). Bone scan and chest X-ray were repeated regularly, and when clinically indicated. In 14 patients serial transrectal ultrasonic volumetry of the prostate was performed.

Staging and Progression

Of 29 patients who were classified according to the 1992 TNM system 15 had T1A, 13 T2, and 1 had T3 tumour. There were 11 Grade 1, 15 G2 and 1 G3 cancers (2 patients grading unknown). The mean age was 74 years (range 58 - 85). The distribution of age and grade with the T-categories is summarised in TABLE 1.

TABLE 1
Distribution of age and grade with T-categories

T-category	*Number*	*Mean age (year)*	*Number*		
			G1	G2	G3
T1a	15	74	7	7	1
T2	11	77	3	6	-
T3	3	72	1	2	-

Local and metastatic progression were evaluated. Subjective progression, like obstructive micturition or pain, was taken into consideration for treatment decisions. Local subjective progression occured in most patients at the same time as local objective progression, or later.
Local objective progression was defined as an increase in T-category, when prostate size increased on DRE by 25 % or more of the products of the two largest perpendicular parameters, or by ultrasound measured volume more than 40 %.
Objective metastatic progression was diagnosed by the appearance of new lesions on bone scan. Bone scans were carried out for initial staging, and afterwards on indication only. For the purpose of defining CLINICAL progression a rise in serum markers alone was not considered relevant. In case of progression the patients remained in this study up to the time of first therapy, which may have been initiated because of subjective or objective local or distant progression.
During the course of this study only one patient died of a non disease related cause (another malignancy).
The day-to-day coefficient of variation of the PSA assay (Hybritech) related to three "stable" standards during 50 days varied between 6.9 and 18.8 %.
Statistical analysis included Kaplan-Meier curves for analysis of time to progression in patients according to stage and grade. The changes in PSA and prostate volume were expressed by the slopes of their log-linear curves versus time. In one patient one of the serial PSA values was extremely low without evident reason. This outlier was not accounted for in the calculations of PSA slopes. The Mann-Whitney test was used to evaluate differences between various groups of patients regarding these slopes. The level of statistical significance was set at $p = 0.05$ (two-tailed). The relation between progression rates and the level of PSA or its change from the baseline value was evaluated using Cox-regression allowing for time-dependent variables [7]. The relation between PSA levels and simultaneous prostate volume measurements, allowing for inter- and intrapatient differences, was assessed using regression analysis [8].

RESULTS

During the follow-up period 13 of 29 patients had progression, all locally, after a mean follow-up period of 31 months. Metastatic progression was not seen. Six of these patients with progression started therapy, five of them for subjective symptoms at the time of progression or within 3 months afterwards, and one patient for objective progression only. There were two patients with local objective and subjective progression who did not start therapy. 16 patients did not progress, their mean time of follow-up was 40 months.
FIGURE 1A-C shows the Kaplan-Meier curves for time to progression for all patients, and separated for stage T1 versus T2-3, and for grade G1 versus G2-3. There was no statistical difference in time to progression between different grades, or different T-categories. The mean number of PSA recordings per patient was 8.7.
FIGURE 2A-B shows the scattergrams of PSA over time in linear plotting for clinically progressive and non-progressive patients. Slopes of PSA regression curves comparing progressive and non-progressive disease were not significantly different.
FIGURE 3 shows a plot of the PSA Doubling Time for the individual patients, separated for progressive and non-progressive patients. Also a percentual PSA increase from baseline of 20 % along time was tested as an indicator of progression (FIGURE 4). At no point on the individual curves was it possible to predict clinical progression. The same applied to the cut-off level of a 50 % increase of PSA along time.

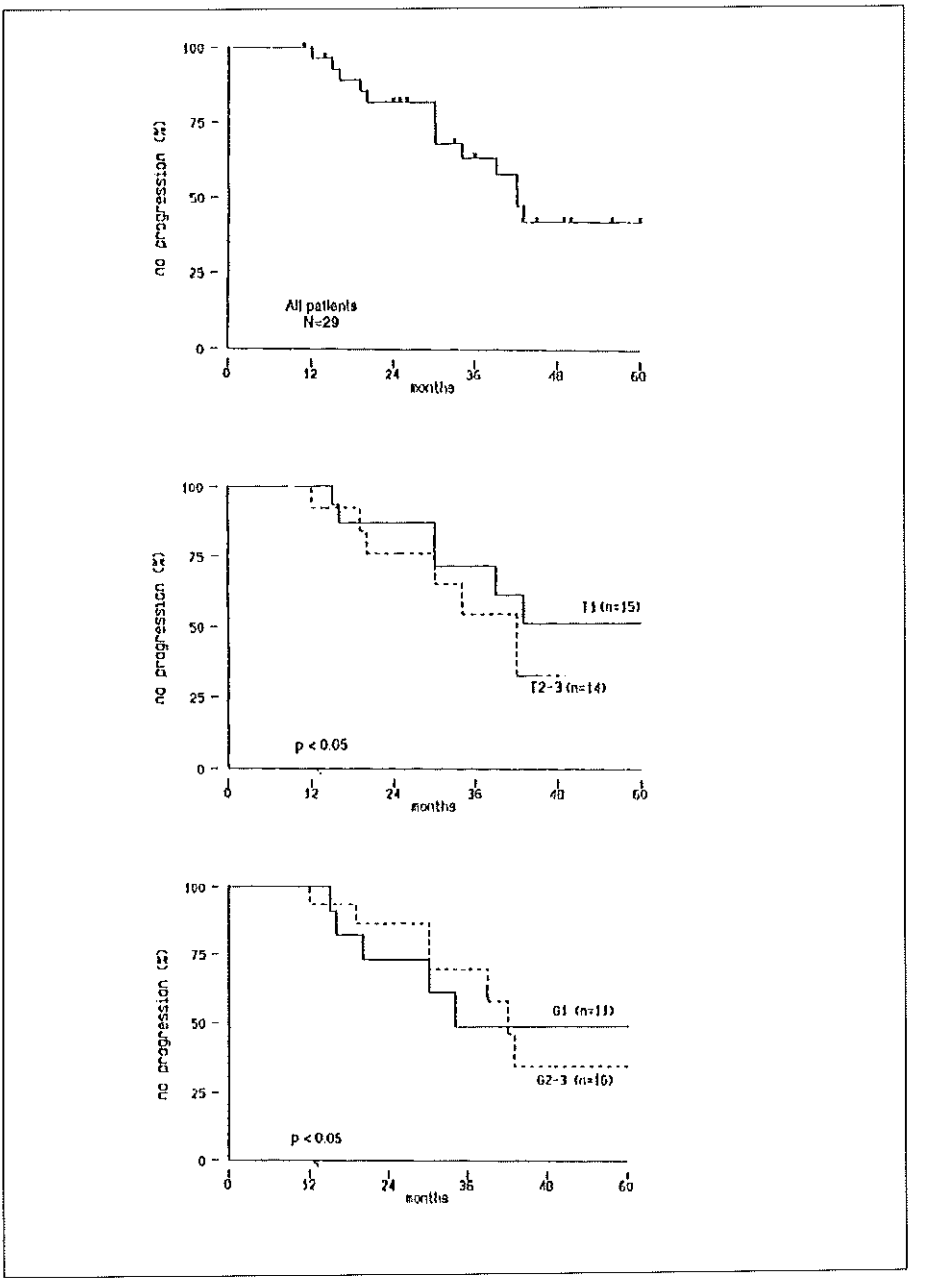

FIGURE 1A-C

Kaplan-Meier curves for time to progression for all patients (A), separated for stage T1 versus T2-3 (B), and for grade G1 versus G2-3 (C). Tickmarks along curves denote ends of follow-up of some patients.

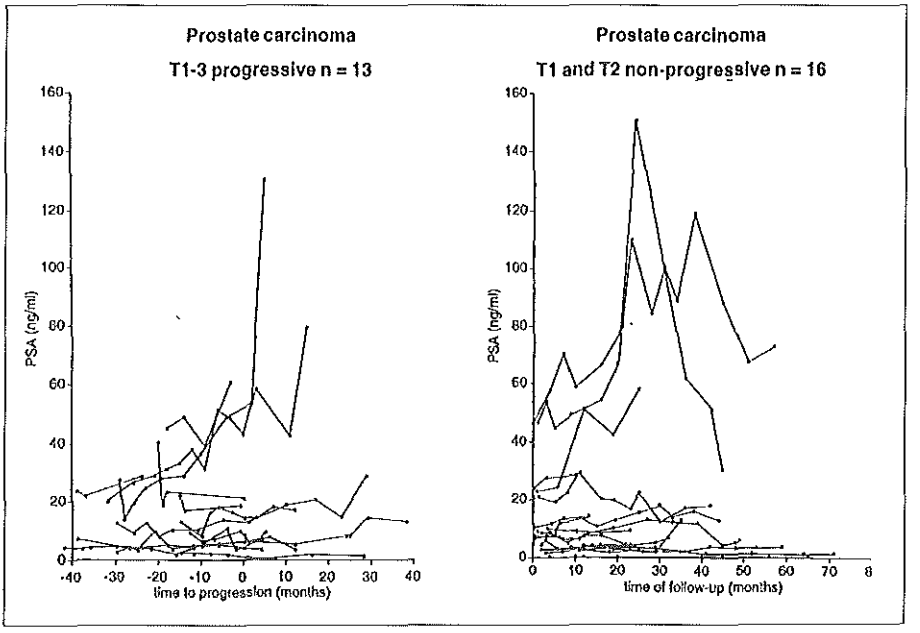

FIGURE 2A-B
Scattergrams of PSA over time for clinically progressive (A) and non-progressive patients (B).

Longitudinal measurements of ultrasonic prostatic volume were done in 14 patients. The mean annual increase of prostatic volume of these patients was 15 %. The mean increase in volume was not significantly different between progressive versus non-progressive patients. A volume increase of more than 40 % that would qualify a patient for clinical progression on the basis of transrectal ultrasonography was not observed. There was a weak, though significant, correlation between volume and PSA (FIGURE 5). Changes of both parameters within patients, however, were not significantly related to each other.

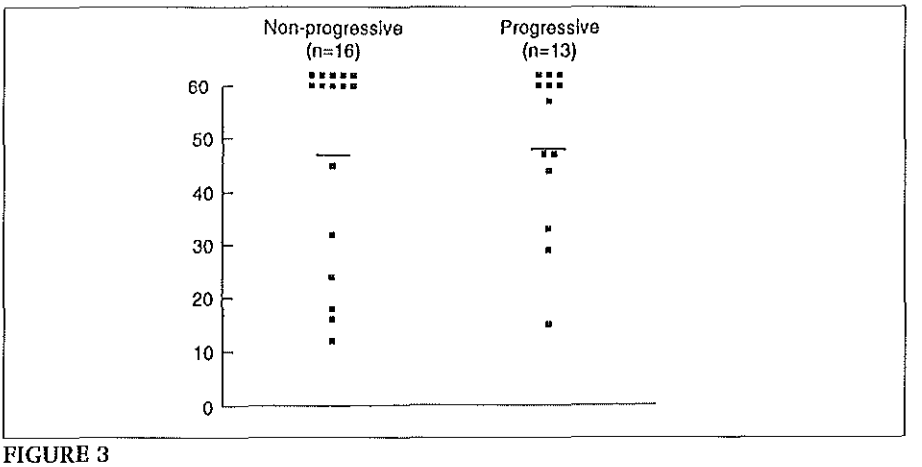

FIGURE 3
Plot of PSA doubling times for progressive and non-progressive patients.

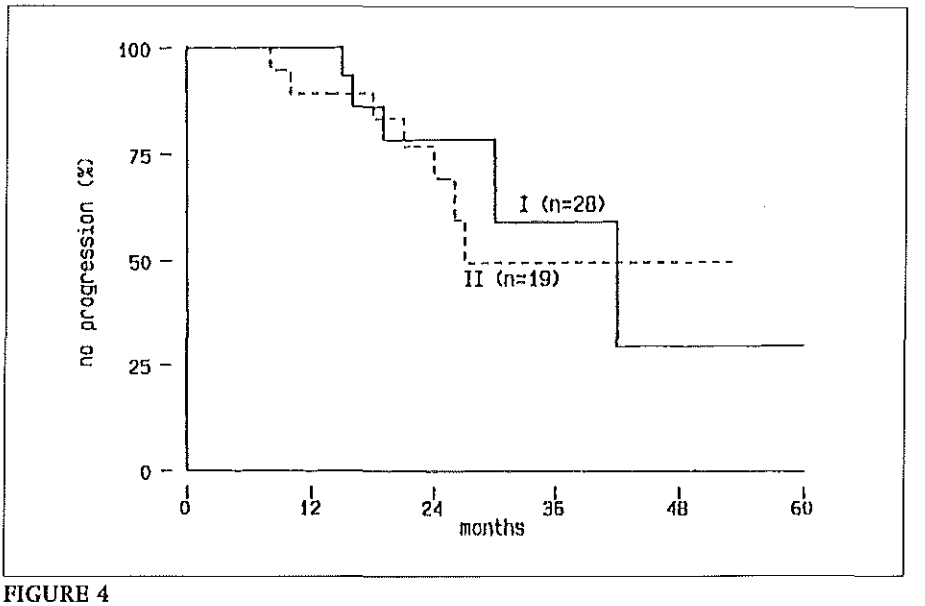

FIGURE 4

Kaplan-Meier curves for time to progression for patients from the time of a PSA increase of 20 % or more without prior clinical progression (II, n = 19), versus patients during the period as long as the PSA increase was less than 20 % (I, n = 28), p > 0,05.

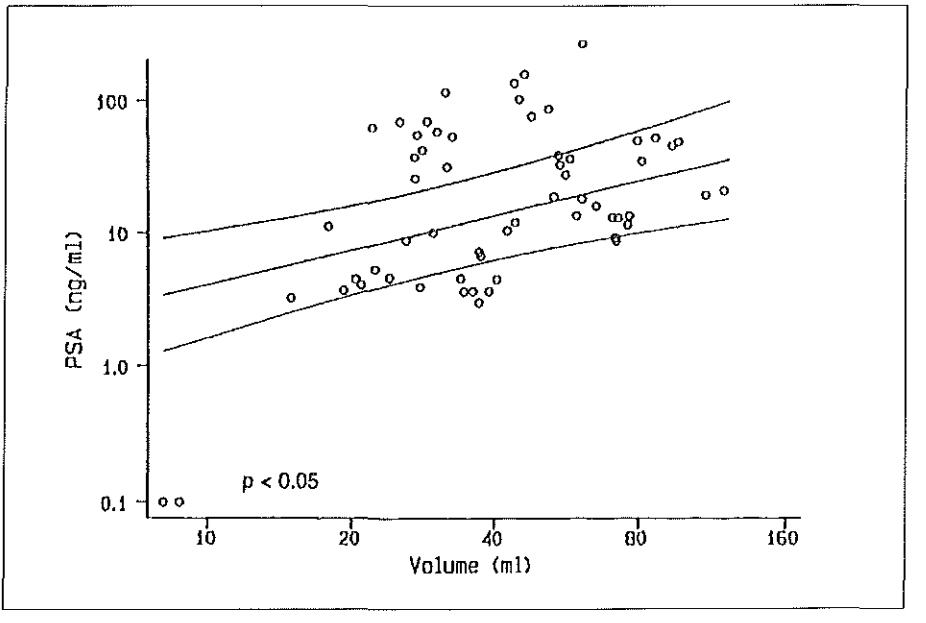

FIGURE 5

Mean PSA according to volume. Curved lines denote the 95 % confidence interval for the mean PSA. Disregarding the outlying 2 observations of a single patient (left hand lower corner), the relation was still significant (p < 0.05)

DISCUSSION

In this study an attempt is made to identify the rate and time of progression of 29 patients with locally confined prostate cancer in whom a policy of watchful waiting was applied. In no instance was systemic progression observed during the average observation time of 31 months. Local progression was identifiable as an increase of T-category or as a palpable increase of prostate size in conjunction with subjective progression of the tumour [9]. A volume increase in TRUS of 40 % or more was not observed [10]. PSA increase was independently studied and compared with clinical progression.

The information presented in this paper is very likely to be biased by patient selection, and is certainly not representative for those men who would normally be recommended to undergo radical prostatectomy. The material may however be compatible with patient populations presented in other surveillance studies such as summarised in the overview by Chodak et al. [11]. The selection bias is reflected in the high average age, the large proportion of grade 1 and T1A tumours, and the absence of high grade lesions. The median time to progression for T1 and T2-3 patients was for both groups around 42 months (FIGURE 1), which is shorter than reported by Lowe [12] and Schröder (1993) for T1A tumours (about 13 years), and by Whitmore [13] for T2 tumours (about 10 years).

Several methods of analysing PSA increase have been applied. PSA velocity, which represents the absolute annual PSA increase, was used in a study reported by Carter [14] in a selected group of 52 men to discriminate between prostate carcinoma and benign disease. A discriminatory cut-off value of 0.75 ng/ml/year was not confirmed by Brawer [15] in community based population of 701 men. In that study the participants were submitted to further evaluation by digital rectal examination when the PSA increased more than 20 % over the period of one year. In 5 % of these men eventually a prostate carcinoma was found. Oesterling [16] noticed an age independent annual increase in PSA in several agegroups in a community based cohort of 376 men. The mean annual increase of PSA was between 5.1 and 11.4 %, but extremely variable partly due to the fact that the follow-up was one year, and only two PSA measurements were taken. In 1.1 % a carcinoma developped, with a mean annual PSA increase of 59 %.

In the present study increases of PSA along time could not discriminate between non-progressive, often incidentally found, localised prostate carcinoma and progressive disease. Also the annual PSA increase during the first, second, or third year of the evaluation was no marker for progression. This means that although there was a wide variety in PSA velocity and PSA doubling times (FIGURE 3), these parameters were not useful in this patient selection over this study period.

The present study confirms the PSA doubling time for organ confined prostate carcinoma found in other studies. In 43 untreated patients with prostate carcinoma Schmid [17] observed that in 20 of 28 patients with organ confined carcinoma PSA doubling time was more than 4 years. The PSA doubling time in case of higher grades or stages were faster than others. Although 14 % of all patients had a stable PSA, it was concluded that prostate carcinoma has an exponential growth rate. In our study 17 of 29 patients had PSA Doubling Time of more than 4 years. In the BLSA study of Cadeddu [18] the PSA Doubling Time between 11 patients developing confined versus 5 patients progressing towards metastatic disease was not statistically different. Carter [14] mentioned that the linear increase of PSA between BPH and prostatic carcinoma patients (up to the time of diagnosis) was statistically different, but from the PSA curves it is obvious that this PSA velocity has no prognostic value.

A low level of PSA has been associated with long-term survival in a study of Belville [19] of 26 patients with prostate carcinoma stage A1. The patients had a similar age distribution to our study population (median 72 years). All had well differentiated histology. Ninety-six percent of PSA levels was below 3.0 ng/ml. Evaluation after a median follow-up of 12.5 years showed that in all patients the prostate carcinoma remained localised, although in 6 patients (23 %) progression to a palpable stage occured. In the present series in 5 of 14 patients with all PSA values below 10 ng/ml progression occured. Only physician dependent initiation of therapy was found more frequent in patients with higher PSA values.
Transrectal ultrasonic volumetry was not helpful as a parameter for progression.
In this study population and probably in similarly selected groups of patients watchful waiting is considered to be justified. The absence of metastatic progression is at variance with other findings in the literature [20, 21]. On the basis of this study it appears to be reasonable to follow patients with PSA determination, rectal examination, and the intermittent evaluation of related symptoms and signs, to obtain more information concerning PSA behavior in patients with untreated confined prostate cancer. It has to be realised that clinical progression may occur without changes in serum PSA.

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Part 5

Application of PSA in Staging Prostate Carcinoma

10

ELIMINATING THE NEED FOR PEROPERATIVE FROZEN SECTION OF PELVIC LYMPHNODES DURING RADICAL PROSTATECTOMY*

Chris H. Bangma, Wim C.J. Hop, Fritz H. Schröder

In 214 patients undergoing bilateral pelvic lymphadenectomy with the intention of retropubic radical prostatectomy for clinically confined prostate carcinoma, preoperative serum PSA, clinical stage, and Anderson biopsy grade were analysed to investigate their ability to predict peroperative frozen section results of pelvic lymphnodes. Serum PSA was the best predictor for frozen section results positive for lymphnode metastasis, followed by biopsy grade. Clinical T-category in combination with PSA predicted lymphnode status worse than biopsy grade, and did not have additional value when combined with biopsy grade and PSA.
Preoperative serum PSA in combination with Anderson biopsy grade could predict with 95 % certainty a negative frozen section result of the bilateral pelvic lymphadenectomy in at least 17 % of patients undergoing radical prostatectomy for clinically confined prostate cancer. In this series, frozen section had a false negative rate of 4.7 % compared to histological staging. Therefore, combining the group of patients with an estimated 95 % chance on negative frozen section results with the group of false negatives, 11 in 214 patients (5.1 %) would have undergone radical prostatectomy while having micrometastases. Selecting men for omission of peroperative frozen section may cause a relevant time reduction of the operative procedure, and helps in a more adequate planning of operation time.

INTRODUCTION

Bilateral pelvic lymphnode excision is used for patients with clinically localized prostate cancer to determine lymphnode status. Most lymphadenectomies are performed as the first step of a procedure culminating radical prostatectomy. In these cases analysis of peroperative frozen sections of the lymphnodes is needed to continue the operation; waiting for these results may cause delay and insufficient use of operation time and facilities.
The preoperative radiologic determination of lymphnode status is unreliable. Predicting lymphnode status on an individual basis by Gleason grading of the preoperative prostate biopsy alone also has been shown to be insufficiently reliable [1]. Preoperative serum prostate specific antigen (PSA) levels correlate with pathological stage, but a considerable overlap exists between different stages [2]. A combination of clinical stage, Gleason biopsy grade, and serum PSA has led to the production of nomograms to predict pathological stage, including lymphnode status [3].
In the present study Anderson grade [4] of the preoperative prostate biopsy and serum PSA level was used to predict negative lymphnode status, thereby eliminating the need for peroperative frozen section analysis in some patients.

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PATIENTS AND METHODS

From 1988 to 1994, bilateral pelvic lymphadenectomy was performed in 214 patients with clinically localized prostate carcinoma, with the intention of carrying out retropubic radical prostatectomy. In all patients serum PSA was determined preoperatively using a radio immuno assay (Hybritech Tandem R, Abbott IMx). Histological confirmation and grading were performed on carried out transrectal prostate biopsies. Staging was by digital rectal examination, bone scan, and computer tomography of the abdomen when indicated, according to the TNM-classification of 1982, later converted to the 1992 version [5]. The results of the frozen section analysis were used to determine the need to proceed with radical prostatectomy. The histological lymphnode status of paraffine-embedded sections was compared with to the results from the frozen sections to assess the accuracy of the latter. Continuous variables were compared between groups using the Kruskal-Wallis or Mann-Whitney U-test. Percentages were compared with the chi-squared test. Logistic regression was used to assess the relationship between negative frozen section results, Anderson grading, clinical staging, and PSA level, the latter after logarithmic transformation. The limit of significance was defined as $p = 0.05$ (two sided).

RESULTS

The number of patients with category T1, T2, T3, and T4 carcinoma were respectively, 23, 11, 74, and four, and for grades 1, 2, and 3 were, respectively, 42, 106, and 45. The biopsy grade was missing for 21 patients. The median PSA of the whole group was 14.2 ng/ml (range 0.1 to 361).

FIGURE 1 shows the serum PSA level according to biopsy grade and T-category. For all grades the median PSA values were significantly different from each other, as were the T-categories, except for T1 versus T2. In 57 patients (30 %) the frozen sections were positive for lymphnode metastasis, and the operative procedure was

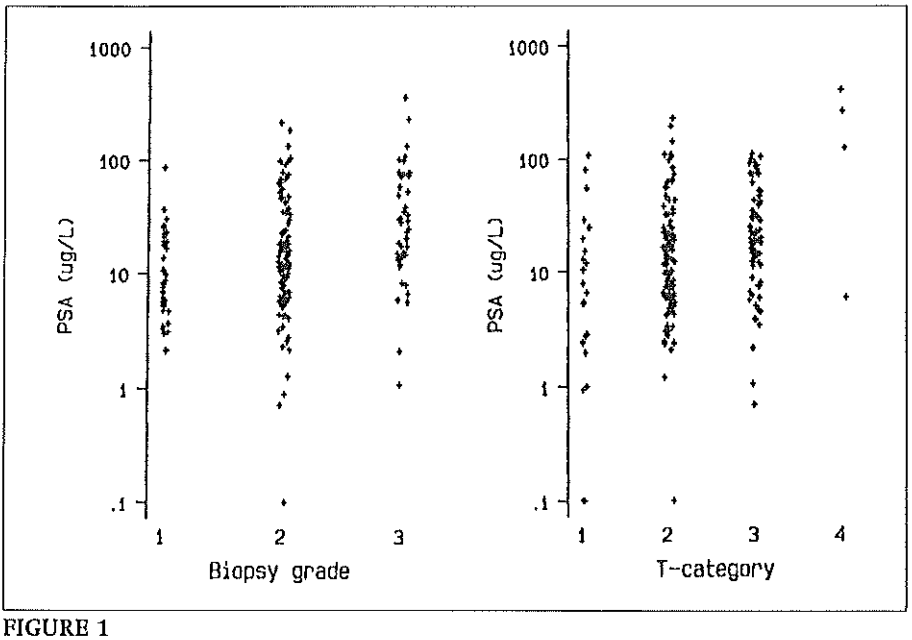

FIGURE 1

Serum PSA distribution along biopsy grade and clinical T-category in 214 men.

terminated after lymph-adenectomy. Histology confirmed metastasis in all 57 cases. There was a significant difference between patients with positive and negative frozen section results when related to both the biopsy grade and the T-category. The percentage of patients with a positive frozen section was 5, 23 and 51 % for G1, G2, and G3 respectively. This percentage for T1, T2, T3, and T4 was 17 %, 20 %, 36 %, and 75 % respectively. The median PSA value of 11.7 ng/ml in the group of patients with negative frozen sections was significantly smaller than the median of 31.3 ng/ml in those with positive frozen sections ($p < 0.001$). TABLE 1 shows the results of the multivariate analysis incorporating PSA level and biopsy grade. Serum PSA level was the best predictor of a positive frozen section, followed by the Anderson biopsy grade. Clinical T-category did not have significant additional value when combined with biopsy grade and PSA level. FIGURE 2 shows the probability of a positive frozen section related to the logarithm of serum PSA level for various biopsy grades. The distribution of patients among 5%-classes of predicted probability of a positive frozen-section is shown in FIGURE 3. Thirty-two patients (17%) had a predicted probability of a positive frozen-section of $\leq$ 5%; a positive frozen-section occurred in only one patient (3%) of this group.
Of 161 patients in whom the predicted probability was $\geq$ 5 %, positive frozen sections were found in 48 patients (30 %). TABLE 2 shows the PSA threshold value for each grade with increasing predicted probability of a positive frozen section. In TABLE 3 the predicted probability of positive frozen section is shown for a range of PSA values.

TABLE 1
Multivariate analysis of the probability of a positive frozen section according to PSA level and grade. (A ratio of odds = 1 indicates no increased risk).

Factor	*Odds-ratio*	*95 % confidence interval*	*p-value*
PSA	1.7 *	1.3 - 2.3	< 0.001
Grade 1	1 #	-	-
Grade 2	4.0 $	0.9 - 18.5	0.08 *
Grade 3	10.9 $	2.2 - 53.6	0.004 *

* effect of doubling of PSA
# reference category
$ significantly different from each other (P = 0.02)
* comparison with the reference category (grade 1)

TABLE 2
Treshold levels of PSA (ng/ml) according to the calculated probability of a positive frozen section for each grade

Probability	*5%*	*10%*	*15%*	*20%*	*30%*	*50%*
Grade 1	12.6	32.0	57.2	88.5	173.8	>400
Grade 2	2.2	5.7	10.2	15.7	30.9	89.4
Grade 3	0.6	1.6	2.9	4.5	8.8	25.3

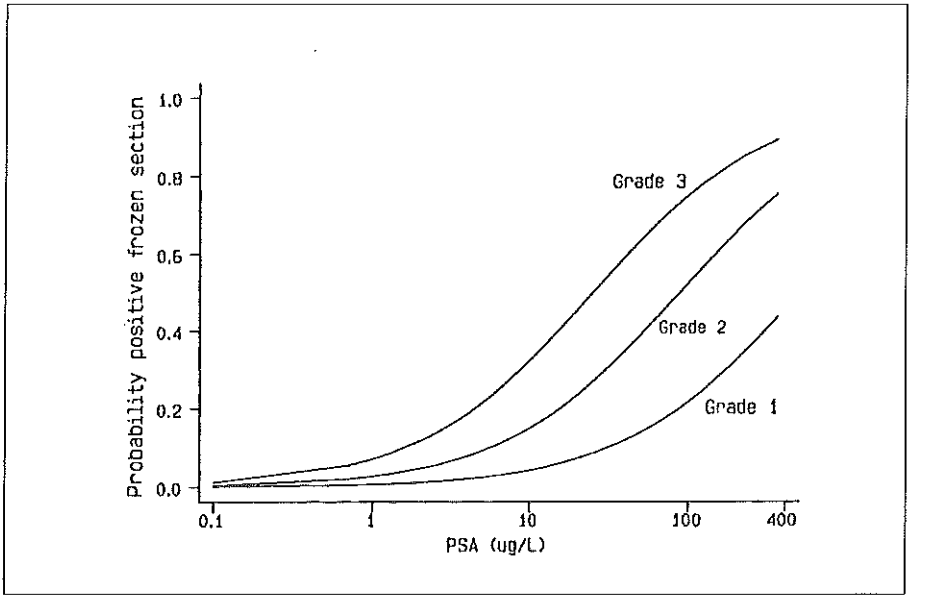

FIGURE 2
The probability of a positive frozen section in relation to serum PSA level for various biopsy grades.

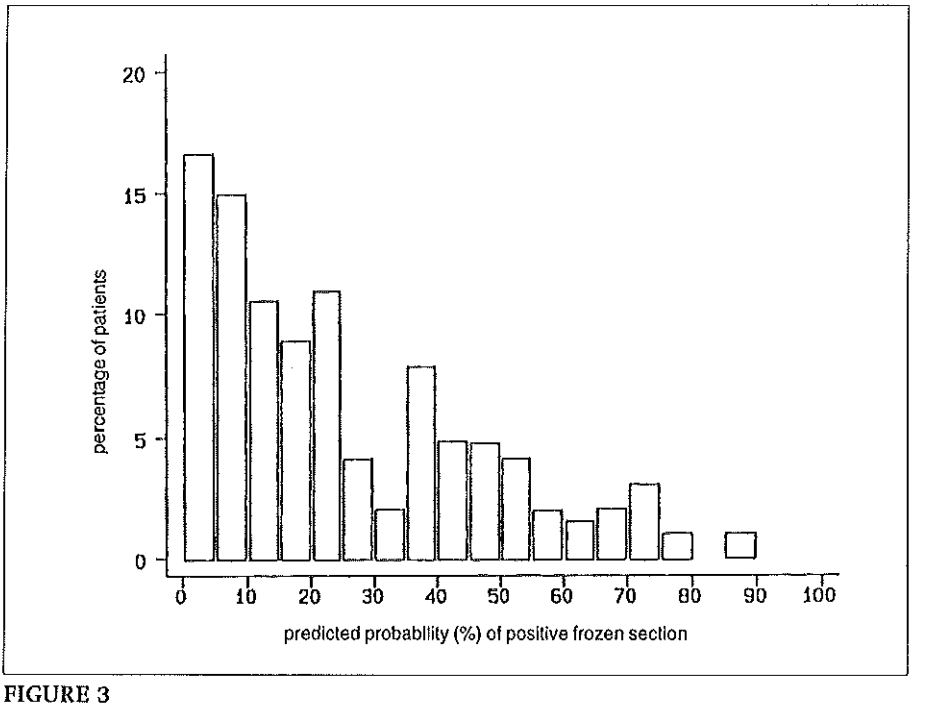

FIGURE 3
The proportion of patients in relation to the predicted probability of a positive frozen section (in classes of 5 %) in 193 men.

TABLE 3
Probability (%) of positive frozen section for chosen PSA values for each grade.

PSA (ng/ml)	*5*	*10*	*20*	*50*	*100*
Grade 1	2	4	7	14	22
Grade 2	9	15	23	39	52
Grade 3	22	32	45	63	75

DISCUSSION

Preoperative serum PSA combinated with the Anderson biopsy grade can give a clinically relevant probability of negative pelvic lymphnode status. In 17 % of patients with clinically confined prostate cancer, a negative peroperative frozen section of pelvic lymphnodes could be identified with a 95 % likelihood. Thus, in one of six patients the analysis of frozen sections during pelvic lymphadenectomy and subsequent radical prostatectomy could have been omitted, accepting a 5 % false negative result. In the authors' clinic the logistics are such that omitting one frozen section makes it possible to perform two radical prostatectomies within the time planned and available for one day of operation.

Recent advances in the diagnosis of prostate cancer have led to the selection of more favourable cases and it is likely that the group of patients in whom an accurate prediction is possible will be larger. During the last 12 months of the study period, from June 1993 till June 1994, 56 patients underwent a radical prostatectomy, and 6 patients (11 %) a lymphnode dissection only. In this group the median serum PSA level was 10.2 ng/ml, which was lower than the median serum PSA level in the complete study. Also the percentage of patients undergoing lymphnode dissection only was lower than that for the whole group, showing that the distribution of patients undergoing operation tended to move towards those with lower PSA ranges, and more confined disease stages. The percentage of patients wich could be spared frozen section analysis or lymphnode dissection might therefore become greater than 17 %. A false negative frozen section occurred in 10 of 214 patients (4.7 %), none of whom were within the group of 32 patients which would have been selected for the omission of frozen section analysis based on a 95 % certainty of negative lymph nodes. In this group, one patient (5%) had positive lymphnodes; thus, if the suggested criterion had been applied, in total 11 in 214 patients (5.1 %) would have undergone radical prostatectomy for micrometastases, which seems accepatable. Fortunately, no false positive frozen sections were seen.

Contrary to other published reports, the T-category had no additional value in predicting lymphnode status in the present study. However, in the studies of Kleer et al.[6] and of Partin et al.[3] the populations of patients were selected, as they all underwent radical prostatectomy. Bluestein et al. analysed 1632 patients who underwent bilateral pelvic lymphadenectomy, of whom 1586 patients had a retropubic radical prostatectomy regardless of lymphnode status [7]. Positive lymphnodes were found in 12% on definitive staging. Accepting a 97 % certainty of predicting negative lymphnodes, they reported that 61 % of patients clinically staged T1a to T2b and 29 % of patients staged T1a to T2c could have been spared pelvic lymphadenectomy; these patients comprised a subgroup of 406 men (25 %). Sands et

al. evaluated 569 patients undergoing staging lymphadenectomy, and, using gradeand stage to predict nodal status in patients with localized prostate carcinoma, concluded that the gains in predictive accuracy from PSA beyond that obtained from stage and grade were small; 194 patients (34 %) were found to have $\leq$ 5 % chance of having nodal metastases [8]. In the present study, 17 % of patients could be spared frozen section analysis during radical prostatectomy, which is similar to the 25 % found by Bluestein et al. when corrected for the number of positive lymphnodes (12 % vs. 30 %). It is not clear from Bluestein's article how much the grade and stage added to the serum PSA level in obtaining the maximum predictive potential. Therefore it is not possible to explane why stage did not have additional value in the present study.
The use of different grading systems makes comparison of these studies difficult. Kleer makes use of the Mayo system, Partin et al. and Bluestein et al. of the Gleason score. Sands et al. used both Anderson and Gleason grading, but found no practical difference between the systems in their analysis. In Europe, the Anderson grading is often used and, though not interchangeable, these systems seem all well applicable. In the author's institute, the main argument for omitting frozen section analysis of pelvic lymphnodes is to save time and money. In all cases, pelvic lymphadenectomy is performed for proper staging and consequent therapy and follow-up, often within international clinical trials. Therefore, the reduction of morbidity associated with lymphadenectomy is not considered under these specific circumstances. Radical prostatectomy is allways performed retropubically, which allows easy access to pelvic lymphnodes. In patients selected for radiotherapy, and in those with a high risk of nodal metastases, transabdominal laparoscopic bilateral pelvic lymphadenectomy is performed.

CONCLUSION

Preoperative serum PSA level combinated with Anderson biopsy grade can predict a negative result of frozen section analysis of bilateral pelvic lymphadenectomy in 17 % of patients undergoing radical prostatectomy for clinically confined prostate cancer with 95 % certainty. This may allow a significant reduction in the durational operation, and a more efficient planning. It may also eliminate the need for a pelvic lymphnode dissection in these patients. The population eligible for this selection may be expected to enlarge with the improving selection of patients and the more frequent diagnosis of confined lesions.

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Part 6

the value of free to total Psa ratio in Screening Adenocarcinoma of the prostate

11

FREE AND TOTAL PSA IN A SCREENING POPULATION*

Chris H. Bangma, Ries Kranse, Bert G. Blijenberg, Fritz H. Schröder

ABSTRACT

Objectives. The ratio between free and total PSA in serum (F/T ratio) improves the differentiation between prostate carcinoma and benign conditions in selected series of patients. The second generation DELFIA PSA assays were used to determine the F/T ratio in a screening population.

Methods. In 1726 men between 55 and 76 years old 67 prostate carcinomas were detected by DRE, TRUS, and total serum PSA (Abbott IMx, Hybritech Tandem E). The DELFIA ProStatus-TM PSA EQM and ProStatus-TM PSA Free/Total assays were applied in retrospect to determine total and free serum PSA.

Results. The specificity of the total serum PSA determination for the detection of prostate carcinoma was improved by the combination of free and total serum PSA. Predictors for a positive biopsy result were estimated as a function of total PSA, free PSA, and the combination of both. The relation of this approach to the F/T ratio is discussed. The F/T ratio may improve the specificity in the total PSA range of 7 ng/ml and more. There was an excellent correlation between the DELFIA ProStatus and the Abbott IMx and the Hybritech Tandem E assays ($r > 0.97$).

Conclusions. The F/T ratio may be valuable for the detection of prostate carcinoma in a screening population. Below a serum PSA value of 7 ng/ml the F/T ratio does not differentiate better between benign and malignant than the total serum PSA does.

INTRODUCTION

Prostate specific antigen (PSA) can be found in serum in a free form and bound to various serum proteins, predominantly α-1-antichymotrypsine (ACT) [1, 2]. Detection of PSA depends on the identification of antigenic epitopes on the PSA molecule by labeled antibodies. Some of these epitopes may be (sterically) obscured by binding proteins, predominantly ACT [3]. This introduces the possibility of discriminating between free (F) and complexed PSA, and of calculating the ratio between free and

* *Submitted.*

total PSA (F/T ratio). In selected groups the ratio between free and complexed PSA improves the differentiation between prostate carcinoma (PCa) and benign conditions [4-6]. It is not known whether the F/T ratio can improve the specificity of PSA to detect PCa in a community based population. In this study sera of participants of the Rotterdam feasibility study or screening for prostate carcinoma [7] were used to analyse in retrospect the value of the determination of total and complexed PSA measured by the DELFIA ProStatus-TM PSA Free/Total assay. This assay was compared to the DELFIA ProStatus-TM PSA EQM assay, and the Abbott IMx PSA assay, both measuring total serum PSA.

PATIENTS AND METHODS

Sampling

Serum samples of 1726 men, which had been stored at - 70° C, were used to determine total and free PSA by two DELFIA second generation PSA assays. The DELFIA ProStatus-TM PSA Free/Total assay provides simultaneous dual label measurement of free and total PSA by using time-resolved fluorimetry of europium (free PSA) and samarium chelates (total PSA) (FIGURE 1). The ProStatus-TM PSA EQM provides a highly sensitive single label (europium) assay of total PSA. Both assays measure total PSA with the same reagent combination resulting in equimolar detection of PSA in free and complexed form. The detection limits for the dual label assay are < 0.01 ng/ml, and < 0.1 ng/ml for free and total PSA respectively, and < 0.01 ng/ml for the single label total PSA assay.

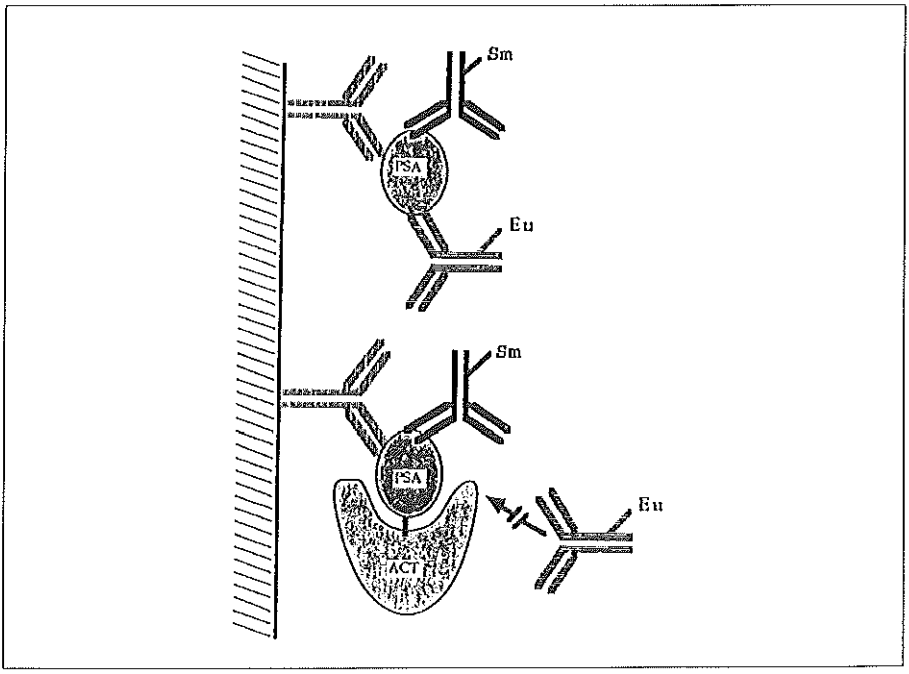


FIGURE 1
Schematic representation of the DELFIA ProStatus-TM PSA EQM and ProStatus-TM PSA Free/Total assay principle.

Methods

Two samples of men originating from the Rotterdam community were combined for this study: 903 participants of the European Randomised Study of Screening for Prostate Carcinoma (ERSPC), and 823 men of the Rotterdam feasibility study of screening for prostate carcinoma. All men aged 55 to 77 years had been randomised for screening by total serum PSA determination (Hybritech Tandem E for the ERSPC participants, Abbott IMx for the feasibility study), digital rectal examination (DRE) and transrectal ultrasonography of the prostate (TRUS). Blood sampling was done prior to further testing, and DRE was performed before TRUS by one of five urological residents. An 1846 Bruel and Kjaer 7.0 MHz biplanar ultrasound probe was used for diagnostic ultrasonography. Indications for ultrasound guided sextant prostatic biopsies differed slightly among four different pilot studies. Biopsies were taken in case of a suspect DRE and/ or TRUS, and/or an elevated PSA over 4.0 ng/ml. All men with a negative biopsy, and those in whom no biopsy was taken, were considered free of prostate cancer within this evaluation.

Statistics

The correlations between total serum PSA measured by the Abbott IMx, the Hybritech Tandem E, and both DELFIA assays were calculated for the total PSA range from 0.4 to 184 ng/ml (IMx) by the method described by Passing and Bablok [8]. Continuous parameters were compared by means of the Mann Whitney U-test, and a two-sided 0.05 level of significance was used. Predictors for a positive biopsy result were estimated as a function of total PSA, free PSA, and a combination of both by means of logistic regression analysis. These predictors were used to construct ROC curves, which illustrate their relative intrinsic discriminatory potential [9]. For the predictor with the 'best' properties (this is the largest area under the ROC curve) the

TABLE 1

Age, prostatic volumes, and PSA values (DELFIA ProStatus-TM PSA EQM, DELFIA ProStatus-TM PSA Free/Total, Abbott IMx, Hybritech Tandem E) of 1659 benign prostates and 67 participants with prostate cancer

	Benign			*Prostate Cancer*		
(units)	*number*	*median*	*range*	*number*	*median*	*range*
AGE (years)	1659	64	54-77	67	66.8	55.6-76.2
total prostatic VOLUME (ml)	1565	31	8-204	64	33	16-105
TOTAL PSA						
Abbott IMx	792	1.2	0-34	31	6.3	0.4-94
Tandem E	867	1.3	0-30	36	7.1	1.0-156
DELFIA						
ProStatus-TM EQ	1659	1.3	0-35	67	7.2	0.4-165
ProStatus-TM F/T	1659	1.2	0-30	67	7.6	0.5-150
FREE PSA ProStatus-TM F/T	1659	0.33	0.01-8.4	67	0.89	0.15-15.6

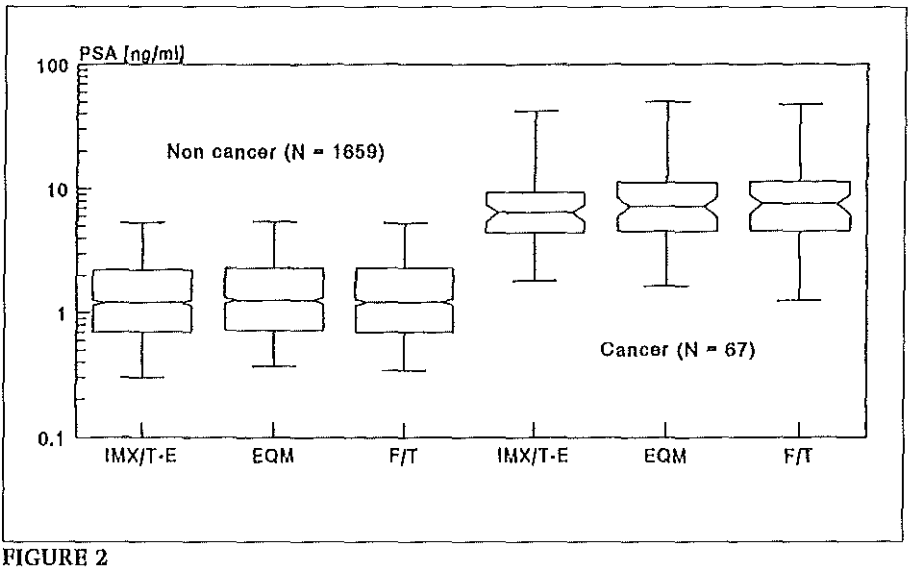


FIGURE 2

Total PSA; comparison of IMx (IMX) and Tandem E (T-E), DELFIA ProStatus-TM PSA EQM (EQM), and DELFIA ProStatus-TM PSA Free/Total (F/T) assays in 1659 benign and 67 prostate carcinoma patients, Box Whisker plots.

best predictive value was determined (this is the value which would have resulted in an equal sensitivity and specificity with respect to the prediction of a positive biopsy result).

RESULTS

General characteristics of all men are shown in TABLE 1. In 1726 men 67 cancers were histologically diagnosed by sextant prostate biopsies in 308 men. There was no significant difference between the median prostatic volume of men with and without prostate carcinoma. The values of total serum PSA measurements of both the ProStatus, IMx, and Tandem E PSA assays in relation to cancer detection are illustrated in FIGURE 2 by Box Whisker plots. The box includes the results of 50 % of the participants, with the median value plotted inside. The notch represents the 95 % confidence interval of the median, and the midle vertical line the 90 % interval of all values. The figure is broken up for benign conditions and PCa, and for the PSA assays used.

In FIGURE 3 A-C the results of the ProStatus-TM PSA Free/Total assay are illustrated similarly: the free PSA, the ACT-complexed PSA, and calculated F/T ratio are given for benign and malignant conditions.

Between both the ProStatus assays a coefficient of correlation of 0.96 was obtained for non-malignant cases (FIGURE 4 A), and of 0.99 for men with cancer (FIGURE 4 B).

Comparison between the IMx, or the Tandem E assay, and ProStatus-TM PSA Free/ Total assay measurements of total PSA resulted in a coefficient of correlation (r) of 0.97 in men without detected malignancy (FIGURE 5 A-B), and for cancer patients in a coefficient of correlation of 0.99 or more (FIGURE 5 C-D).

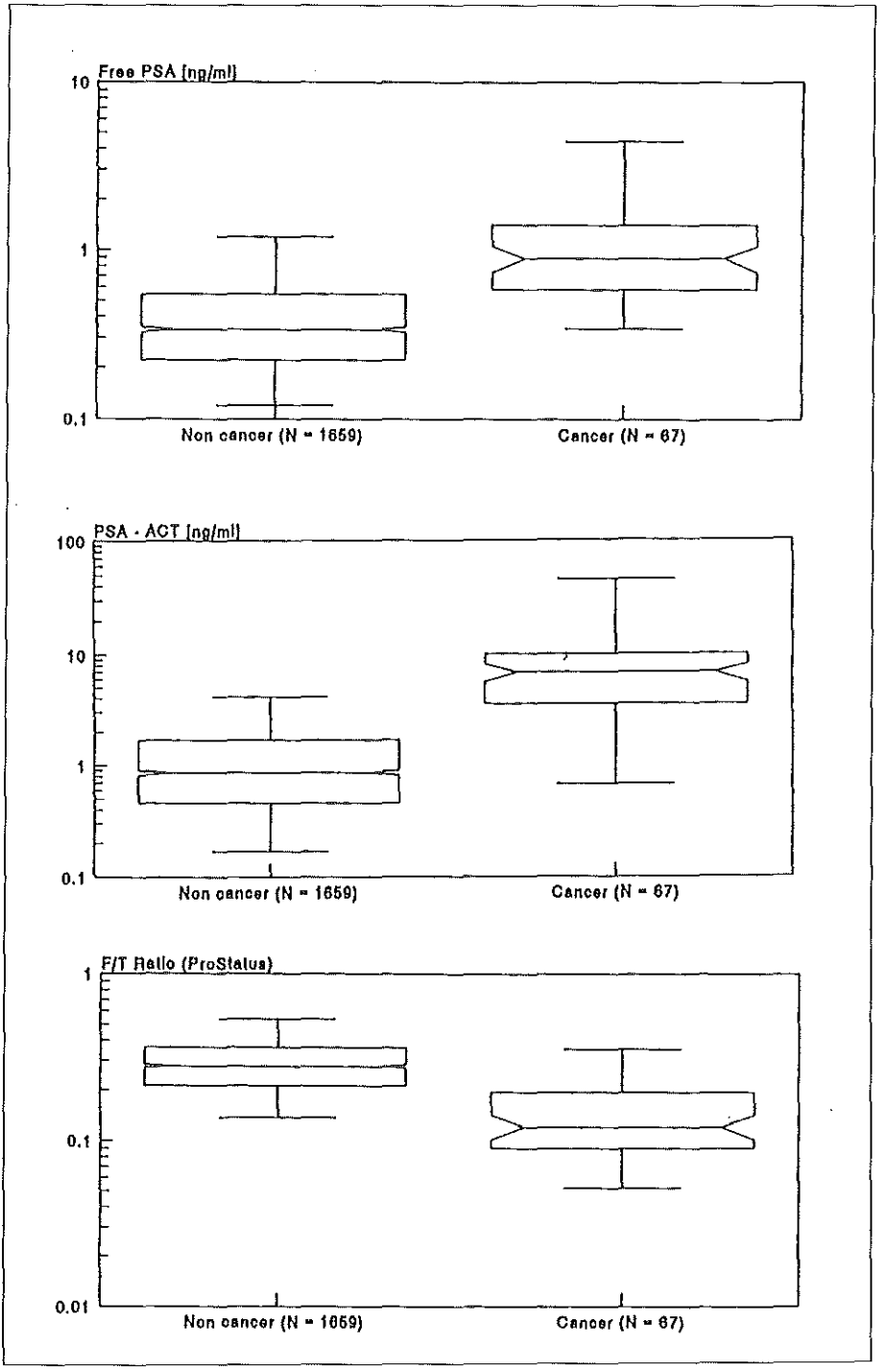


FIGURE 3

Free PSA, PSA-ACT, and PSA F/T ratio in 1659 benign and 67 prostate carcinoma patients, Box Whisker plots.

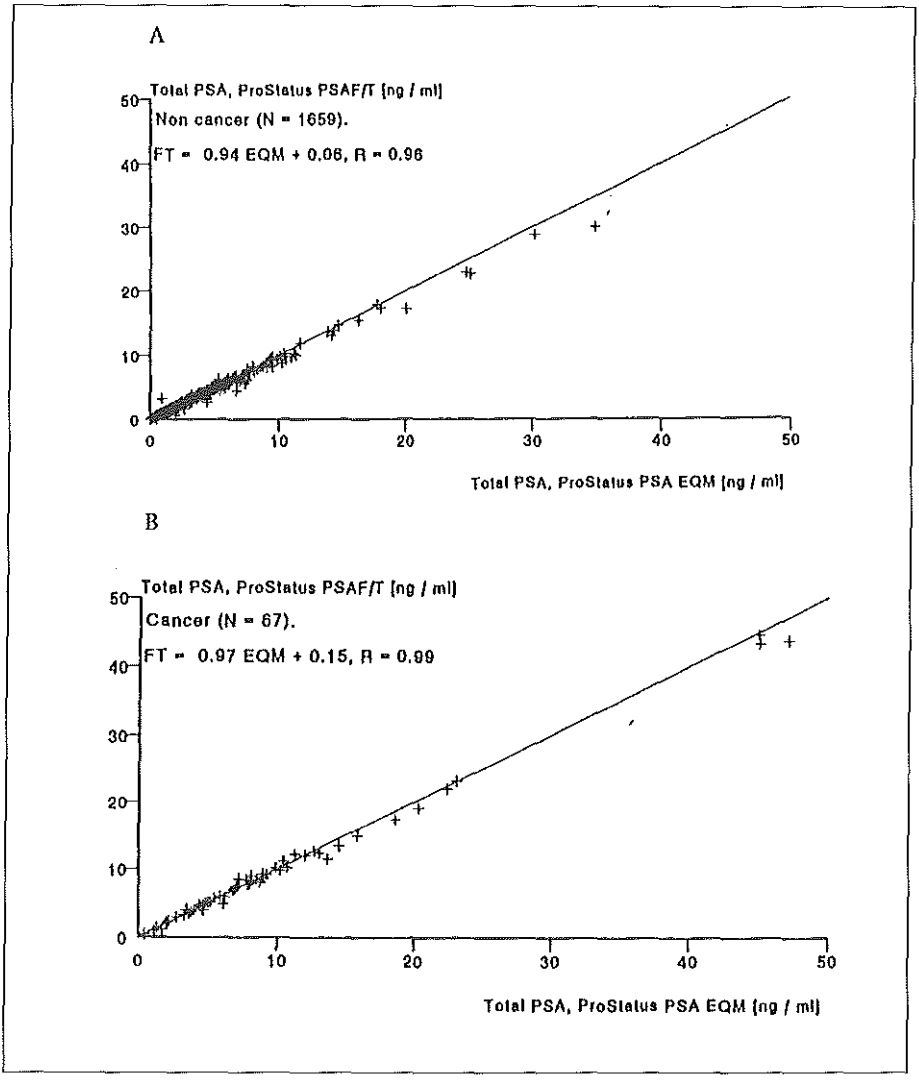


FIGURE 4

Correlation between DELFIA ProStatus-TM PSA EQM (EQM) and ProStatus-TM PSA Free/Total (FT) for 1659 benign participants (A), and 67 men with prostate carcinoma (B). The line of equivalence is indicated.

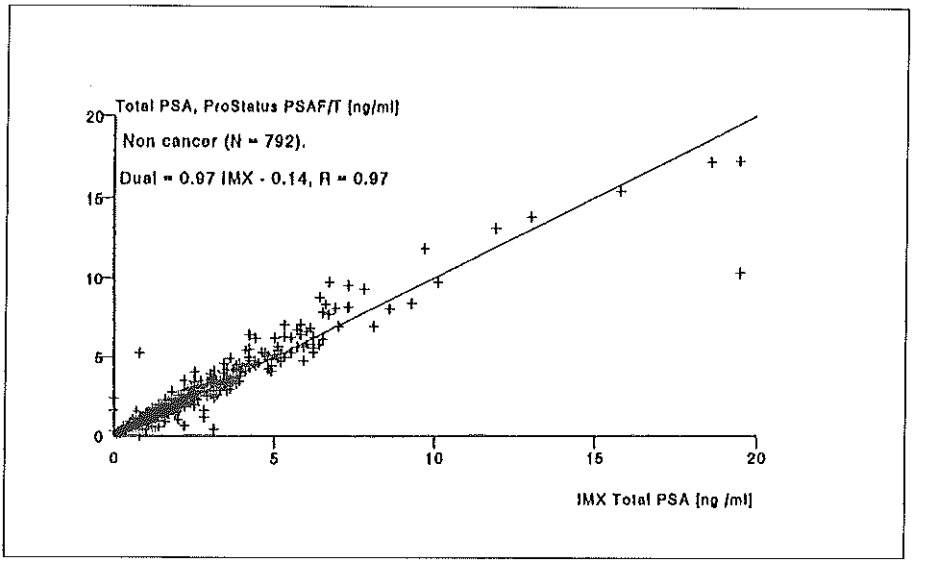


FIGURE 5A

Correlation between Abbott IMx (IMX) and DELFIA ProStatus-TM PSA Free/Total (Dual) assay for 792 benign participants. Illustrated is the PSA range between 0 - 20 ng/ml. The line of equivalence is indicated.

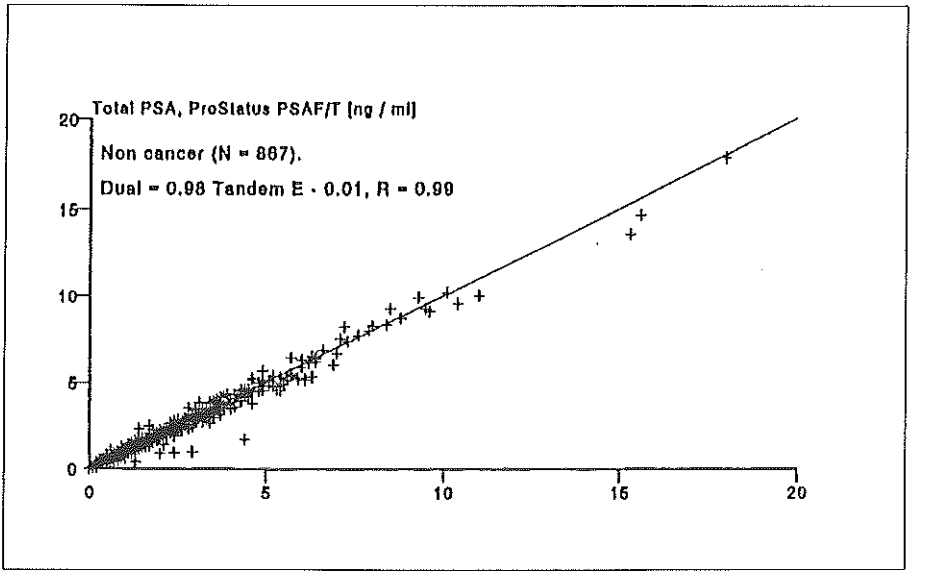


FIGURE 5B

Correlation between Hybritech Tandem E and DELFIA ProStatus-TM PSA Free/Total (Dual) assay for 867 benign participants. Illustrated is the PSA range between 0 - 20 ng/ml. The line of equivalence is indicated.

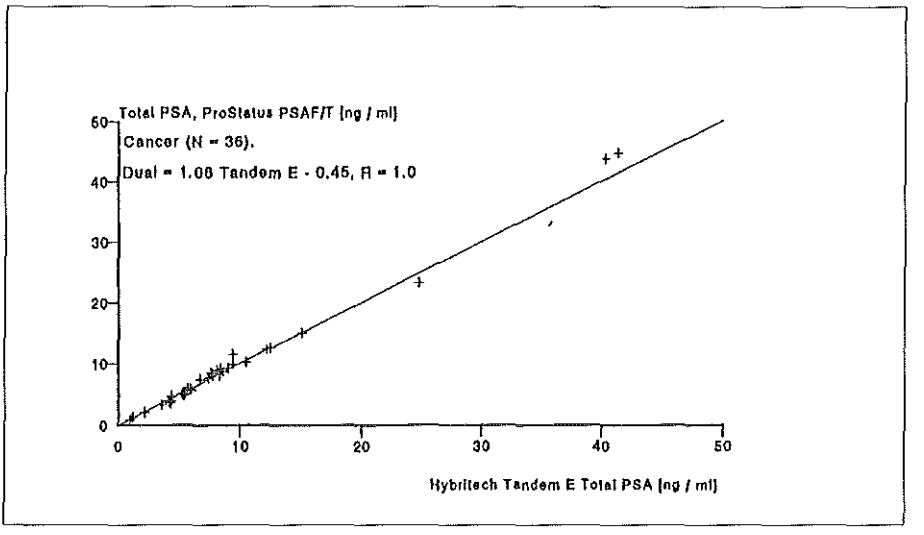


FIGURE 5C

Correlation between Abbott IMx (IMX) and DELFIA ProStatus-TM PSA Free/Total (Dual) assay for 31 participants with prostate carcinoma. Illustrated is the PSA range between 0 - 50 ng/ml. The line of equivalence is indicated.

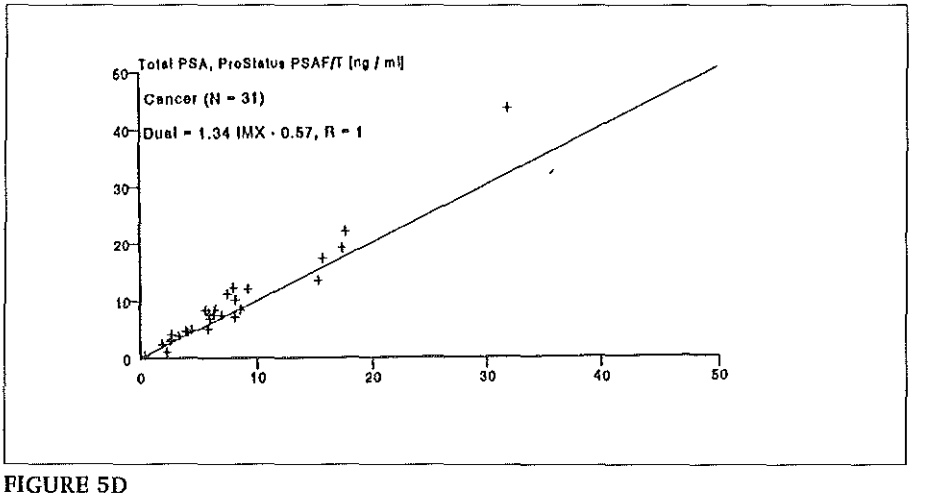


FIGURE 5D

Correlation between Hybritech Tandem E and DELFIA ProStatus-TM PSA Free/Total (Dual) assay for 36 participants with prostate carcinoma. Illustrated is the PSA range between 0 - 50 ng/ml. The line of equivalence is indicated.

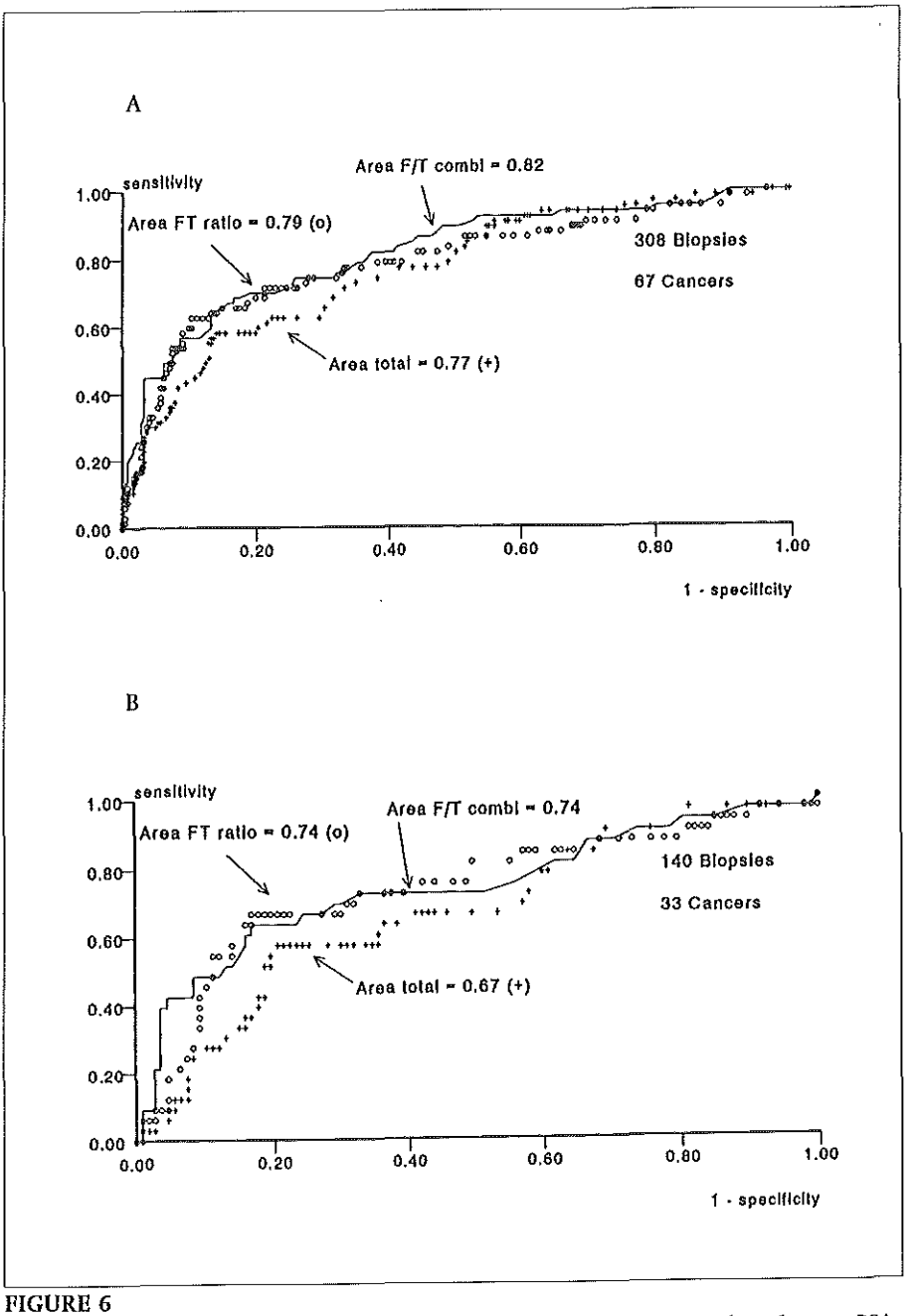
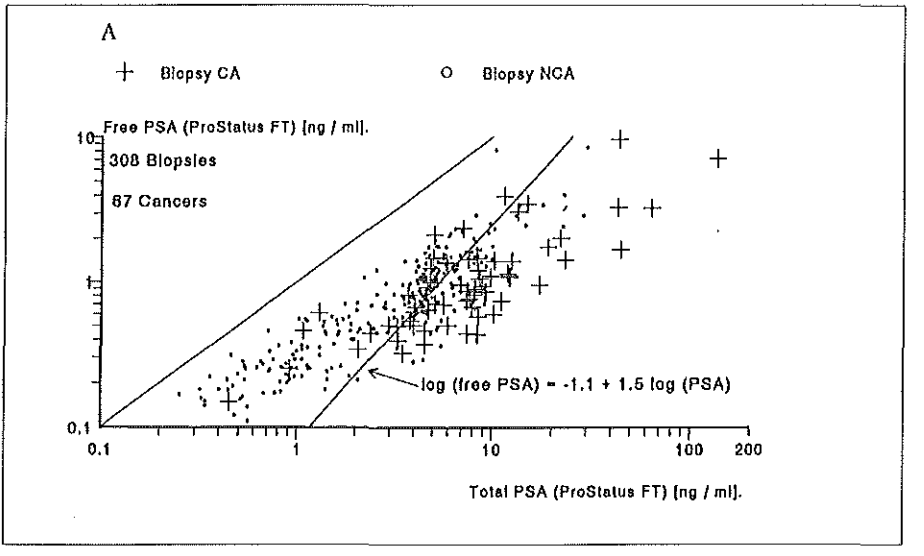


FIGURE 6

ROC curve for the combination of free and total serum PSA, the F/T ratio, and total serum PSA in the total serum PSA range of 0 - 200 ng/ml (A) (Standard Error = 0.03) and 4.0 - 10.0 ng/ml (B) (Standard Error = 0.05), illustrating their relative discriminating potential between benign and malignant disease.



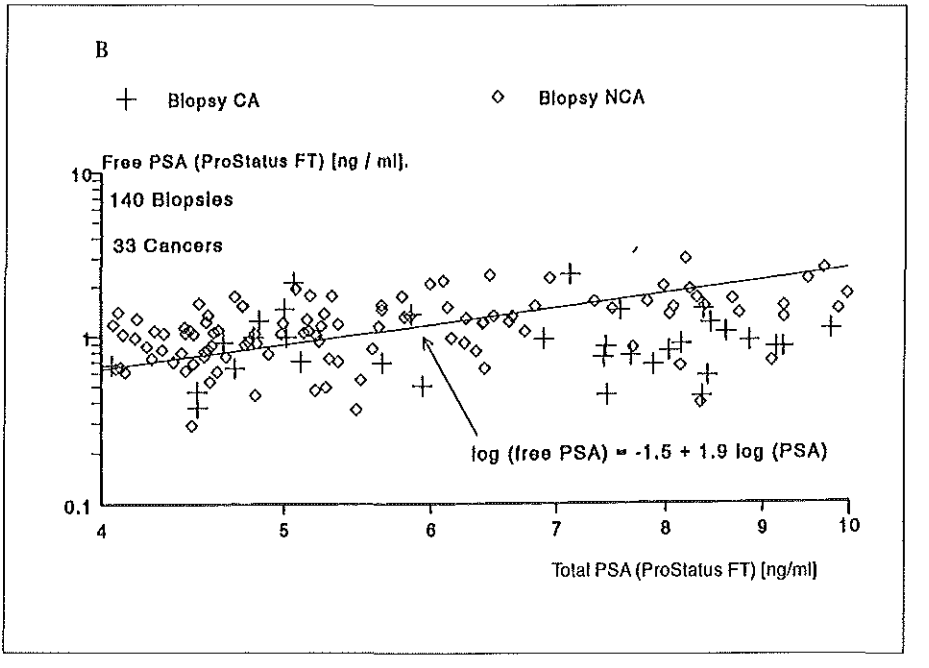


FIGURE 7

Relation between the total serum PSA and the free PSA using the ProStatus-TM PSA Free/Total assay for all men selected for prostate biopsy in the total serum PSA range of 0 - 200 ng/ml (A) and 4.0 - 10.0 ng/ml (B). Indicated are the lines representing the optimal discrimination between benign and malignant biopsy results by the best combination between free and total PSA.

Regarding the imprecision of both ProStatus assays we found coefficients of variation between 7.2 % (concentration 0.7 ng/ml) and 4.3 % (concentration 70.7 ng/ml) for total PSA, and between 10.3 % (concentration 0.17 ng/ml) for free PSA and 3.7 % (concentration 35.5 ng/ml) during 22 days, with 6 control samples supplied by the manufacturer. A comparable experiment for inter-assay precision with other materials for the Abbott IMx and Hybritech Tandem E revealed coefficients of variation between 3 and 6 %.

The biopsy results were used to assess the capability of the free serum PSA in combination with the total PSA to detect prostate carcinoma, as biopsy in this study was considered to illustrate the definite histological status of the individual. In FIGURE 6 A-B ROC curves demonstrate the relative discriminatory potential for detection of PCa of total serum PSA, the combination of free and total serum PSA ('combi'), and the F/T ratio for the total PSA range of 0 - 200 ng/ml (FIGURE 6 A: including 308 biopsies, of which 67 carcinomas), and for the total PSA range of 4.0 to 10.0 ng/ml (FIGURE 6 B: including 140 biopsies, of which 33 carcinomas). For the PSA range of 0-200 ng/ml, the area under the ROC curve is for total serum PSA 0.77, for the combination of free and total serum PSA 0.83, and for the F/T ratio 0.79. (S.E. 0.03). There was no significant difference between the ROC areas. In the PSA range of 4.0 - 10.0 ng/ml, the area under the ROC curve is for total serum PSA 0.67, for the combination of free and total serum PSA 0.74, and for the F/T ratio 0.74 (S.E. 0.05). For the range of total serum PSA of 7 ng/ml and more it was found that the F/T ratio had an identical potential for detection of PCa as the combination of free and total serum PSA. Expressed in the areas under the ROC curve these were both significantly better than serum PSA only (for F/T ratio 0.79, S.E. 0.06; for the combination of free and total 0.80, S.E 0.06; and for total PSA only 0.58, S.E. 0.07). FIGURE 7 A-B shows the relation between the total and the free serum PSA using the ProStatus-TM PSA Free/Total assay for men selected for prostate biopsy in the total serum PSA range of 0 - 200 ng/ml (A) and 4.0 - 10.0 ng/ml (B). The line indicated by the formula represents the threshold that, if used, would have resulted in an equal sensitivity and specificity in the material studied (this line was described under 'statistics' as the best predictor). It can be represented by the formula: log(free PSA) = 1.5 log(total PSA) - 1.1, which illustrates a sensitivity of 75 % and a specificity of 74 %. For the total serum PSA range of 4.0 - 10.0 ng/ml (FIGURE 7B) the equation of this combination is: log(free PSA) = 1.9 log(total PSA) - 1.5, which illustrates a sensitivity of 70 % and a specificity of 71 %. It can be seen that the distribution of cancers and non-cancers overlaps, also when the PSA range is restricted to the 'grey' area between 4.0 and 10.0 ng/ml. The F/T ratio at a sensitivity of 73 % and a specificity of 73 % is 0.18.

DISCUSSION

At this time serum PSA is the best single screening test available for prostate carcinoma. In cases of prostate carcinoma PSA is elevated, but values are overlapping with normal individuals (FIGURE 2). This analysis of 1726 participants of a screening study for PCa shows that the combination of free and total serum PSA discriminates sligthly better between benign and malignant conditions than total serum PSA only in the range of 4.0 - 10.0 ng/ml (FIGURE 6 B). Detection of PCa in this study currently is done by the combination of serum PSA, DRE, and TRUS. Detection rates in screening populations vary between 2 and 5 percent. In this study the detection rate was 3.8 %. The true value of the various screening modalities with respect to cancer detection and indication for biopsy will be evaluated with the first results of

the European Randomised Study of Screening for Prostate Cancer during the year of 1995.
The DELFIA ProStatus PSA assays measure total PSA in an equimolar fashion, but they use different monoclonal antibodies. The correlation between both assays is excellent (FIGURE 3 B). Comparison to the Hybritech Tandem E and Abbott IMx total PSA assays also shows excellent correlations. The regression equation between the IMx and ProStatus-TM PSA Free/Total is for this restricted number of carcinoma patients slightly different from that found in the non-cancer participants, but only 31 serum samples were used.

The ProStatus-TM PSA Free/Total assay furthermore measures the free PSA. In selected groups the F/T ratio discriminates well between benign and malignant prostatic disease. In FIGURE 2 is illustrated that the serum concentrations of the various circulating forms of PSA or their combinations have the potential of differentiating between benign and malignant disease. Stenman [1] measured the total serum PSA and the fraction of PSA complexed to ACT. In a selected group of 67 patients with various stages of PCa, 30 men with BPH, 10 healthy men, and 12 female controls, the sensitivity for PCa at a specificity level of 90 % rose from 61 to 66 % when serum PSA with serum PSA-ACT was compared. The ratio between free PSA and PSA-ACT (F/C ratio) had an even higher sensitivity of 78 %. Like Wood [10] he reported 40 - 60 % of PSA to be complexed with ACT, which is much lower than in later reports. Differences in assay design are thought to be responsible for these phenomena.
Christensson [4] showed in a selected population of 135 men with BPH and 66 men with PCa that the F/T ratio improved the specificity for PSA to detect PCa from 55 to 73 % at a sensitivity of 90 %. This level of sensitivity was reached by a cut-off value of total PSA of 5.0 ng/ml, and a F/T ratio cut-off value of 0.18. In contrast to Stenman, no correlation between the F/T ratio and the serum PSA was found in BPH ($r = 0.001$) or PCa ($r = 0.14$). The F/T ratio was used instead of the C/T ratio because of greater analytic precision and lower detection limits of the two assays used. This was illustrated in the report of Lilja [5], using the F/T or F/C ratio for the best of eight assays measuring free or complexed PSA. Assays detecting PSA complexed to ACT overestimated the real concentration of PSA-ACT in serum due to cross reaction to granulocyte derived cathepsin G. He therefore was in favour of assays measuring free PSA. Using ROC curves to illustrate the detectability of PCa of various assays, F/T and C/T values performed better than free, complexed or total PSA only.
The free PSA serum concentration in PCa is smaller than in BPH. Bjartell [11] found that the ACT production in carcinoma cells which produce PSA (especially in the well differentiated prostate carcinomas) is higher than in PSA producing BPH nodules. This may enhance the complex formation between PSA and ACT, and subsequently decrease free serum PSA. The reduction of free PSA may be influenced by a large BPH component in a prostate with PCa, which produces relatively much PSA, and therefore much free PSA. Relating the free PSA to the total PSA MIGHT possibly correlate the tumour load with the total prostate volume, and detect the relatively larger carcinomas best. This has to be analysed in larger numbers of untreated men with various stages and grades of prostate carcinoma.
So far, the ratios between the various types of PSA in serum are mathematical parameters which have been used for discriminating between benign and malignant disease. There is no theoretical base for preference of one of those ratios. We chose the relation between free and total serum PSA based on studies concerning the F/T ratio. The relation $\log(\text{free PSA}) = 1.58 \log(\text{total PSA}) - 1.24$ differentiated with equal

sensitivity and specificity between benign and malignant biopsies among which these parameters were tested, in the total serum PSA range of 0 - 200 ng/ml. There was no significant difference between ROC curves of this combination compared to the F/T ratio or total serum PSA, indicating that the free PSA did not add to the detection of PCa in this sample of the screening population. It remains to be seen that for a larger number these differences become statistically significant; it is doubtful that they become clinically important. The results reported in other studies [12] did not concern a screening population like in this study.
Also in the range between 4.0 - 10.0 ng/ml the difference between the ROC curves of the combination of free and total PSA, and total serum PSA was not significant (FIGURE 6B). At a sensitivity level of 0.70 and a specificity of 0.71, the F/T ratio was 0.18. This value has been described as a cut-off for discriminating between benign and malignant prostates [4], but data from the present study can not support this value: no biopsies were taken on indication of the F/T ratio below a PSA value of 4.0 ng/ml, and no increase of specificity was observed in the PSA range of 4.0 to 10.0 ng/ml.
In the range of total serum PSA of 7 ng/ml and more, it was seen that the F/T ratio had an identical potential for detection of PCa as the combination of free and total serum PSA. Expressed in the areas under the ROC curve these were both significantly better than serum PSA only. This unfortunately hardly is of clinical relevance, as prostate biopsies will always be taken for histological confirmation of the PCa in this PSA range. What is needed is an increase of specificity in a PSA range where relatively many biopsies have to be taken to detect one carcinoma.

CONCLUSION

The DELFIA ProStatus PSA assays are well correlated to the Abbott IMx ($r = 0.97$ for benign, $r = 0.99$ for malignant prostates) and Hybritech Tandem E assays ($r = 0.99$ for benign, $r = 1.00$ for malignant prostates), and to each other for measuring total serum PSA ($r = 0.96$ for benign, $r = 0.99$ for malignant prostates). The F/T ratio of serum PSA, as measured by the DELFIA ProStatus-TM PSA Free/Total assay, discriminates well between benign and malignant disease in selected patients. For purposes of screening for PCa however its use is limited, as it does not discriminate better than serum PSA does in the serum PSA range between 4.0 and 10.0 ng/ml. The best discrimination between benign and malignant biopsy results was obtained by a linear relation of the logarithmic values of free and total serum PSA. The value of the F/T ratio, in combination with DRE, TRUS, and derived parameters like PSAD and PSAT, to select participants of a screening study for prostate biopsy, remains to be analysed in this material. The relation between the F/T ratio and clinically important carcinomas also has to be assessed.

ACKNOWLEDGEMENT

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THE VALUE OF SCREENING TESTS IN THE DETECTION OF PROSTATE CANCER*

1. Results of a retrospective evaluation of 1726 men

Chris H. Bangma, Ries Kranse, Bert G. Blijenberg, Fritz H. Schröder

ABSTRACT

Objectives. The ratio between free and total PSA in serum (F/T ratio) was shown to improve the differentiation between prostate carcinoma and benign conditions in selected series of patients. In this study the F/T ratio was analysed for its ability to improve the specificity of total serum PSA, digital rectal examination (DRE), and transrectal ultrasonography (TRUS) for the detection of prostate cancer in an unselected screening population of men identified in the Rotterdam population.

Methods. In 1726 men between 55 and 76 years old 67 prostate carcinomas were detected by DRE, TRUS, and total serum PSA (Abbott IMx, Hybritech Tandem E). The DELFIA ProStatus-TM PSA EQM and ProStatus-TM PSA Free/Total assays (Wallac) were applied in retrospect to determine total and free serum PSA. Age, total prostatic and inner zone volume were taken into consideration.

Results. 67 carcinomas were detected of which two by TRUS and three by DRE alone. Total serum PSA was the most important single predictor for prostate cancer, followed by DRE. The F/T ratio increased the specificity of total serum PSA in the PSA range between 4.0 and 10.0 ng/ml, however, not significantly.

Conclusions. The combination of total serum PSA and DRE remains the standard for detection of prostate carcinoma in a screening population, but the exact indication of DRE with low PSA values (< 4.0 ng/ml) still has to be determined. Their specificity may be improved minimally by the F/T ratio. The threshold of the F/T ratio, and the PSA range for its application, remains to be assessed prospectively. The role of TRUS for detection of ultrasonic lesions and prostate volumetry is limited and not cost effective.

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INTRODUCTION

Screening on prostate carcinoma (PCa) has been performed from June 1994 onwards in the Rotterdam area as part of the European Randomised Study of Screening for Prostate Carcinoma to study the usefulness of early detection in the general population. Men between 55 and 75 years old are randomised to a control group or for screening by use of prostate specific antigen (PSA), digital rectal examination (DRE), and transrectal ultrasonography of the prostate (TRUS).

Selection of candidates for prostate biopsy is complicated by the fact that all screening modalities lack sufficient specificity. This results in a high ratio between the number of biopsies and the number of detected carcinomas. In most screening studies overall about 5 biopsies are needed to detect one carcinoma [1-5].

Several additional parameters, like prostate volume and age, have been used in an attempt to reduce this unfavourable ratio, without loss of sensitivity [6, 7].

Prostate specific antigen (PSA) can be found in serum in a free form or may be bound to various serum proteins, predominantly α-1-antichymotrypsin (ACT) [8, 9].

The detection of PSA depends on the identification of antigenic epitopes on the PSA molecule by labeled antibodies. Some of these epitopes may be (sterically) obscured by serum proteins, predominantly by ACT [10]. This introduces the possibility of discriminating between free (F) and complexed PSA, and of calculating the ratio between free and total PSA (F/T ratio). In selected groups the ratio between free and complexed PSA improves the differentiation between prostate carcinoma and benign conditions [11-13].

The concept of F/T ratio offers a new parameter for the early detection of PCa in addition to total serum PSA, digital rectal examination (DRE), and transrectal ultrasonography (TRUS). The question is raised whether the F/T ratio can increase the specificity of existing tests or their combinations in order to reduce the number of negative biopsies, or even replace a number of them to make screening less invasive and more cost-effective. To assess the value of the F/T ratio it should be compared to other parameters, like age, total prostate volume and transition zone volume, total PSA, DRE, and lesions seen by TRUS, which have been used to differentiate between normal and pathological prostates in screening populations. These parameters should be analysed in a representative sample of the male population, independent of the PSA level. The best estimate of the incidence of PCa in the general population would be obtained by submitting all participants to sextant prostate biopsies. However, this is not feasible. Assessment of screening modalities therefore will always be biased by the method of sampling, and by the method of PCa detection in the population studied.

PATIENTS AND METHODS

Materials

Serum samples, which had been stored at - 70° C, were used to determine total and free PSA by two DELFIA second generation PSA assays.

The DELFIA ProStatus-TM PSA Free/Total assay provides simultaneous dual label measurement of free and total PSA by using time-resolved fluorimetry of Europium (free PSA) and Samarium chelates (total PSA). The ProStatus-TM PSA EQM provides a highly sensitive single label (Europium) assay of total PSA. Both assays measure total PSA with the same reagent combination to give equimolar detection of PSA in free and complexed form. The detection limits for the dual label assay are < 0.01 ng/ml, and < 0.1 ng/ml for free and total PSA respectively, and < 0.01 ng/ml for the single label total PSA assay. Regarding the imprecision of both ProStatus assays we found

coefficients of variation between 7.2 % (at a serum concentration of 0.7 ng/ml) and 4.3 % (at a serum concentration of 70.7 ng/ml) for total PSA, and between 10.3 % (at a serum concentration 0.17 ng/ml) for free PSA and 3.7 % (at a serum concentration 35.5 ng/ml) during 22 days, with 6 control samples supplied by the manufacturer. The correlation coefficient (r) between the ProStatus assays, and both the Abbott IMx and the Hybritech Tandem E assays have shown to be better than 0.97 for men with and men without prostate cancer [14, 15].

Methods

Two sample collectives of men originating from the Rotterdam community were combined for this study: 903 participants of the European Randomised Study of Screening for Prostate Carcinoma (ERSPC), and 823 men of the Rotterdam Feasibility Study for Screening Prostate Carcinoma [16]. All men aged 55 to 77 years had been randomised for total serum PSA determination (Hybritech Tandem E for the ERSCP participants, Abbott IMx for the feasibility study), digital rectal examination (DRE) and transrectal ultrasonography of the prostate (TRUS). Blood sampling was done prior to further testing, and DRE was performed before TRUS by one of five well trained urological residents without knowledge of the PSA values. An 1846 Bruel and Kjaer 7.0 MHz biplanar ultrasound probe was used for diagnostic ultrasonography, and planimetric volumetry of the prostate gland and its inner zone [17]. Ultrasound guided sextant prostatic biopsies were taken in case of a suspect DRE and/or TRUS, and/or an elevated PSA over 4.0 ng/ml. All men with a negative biopsy, and those in whom no biopsy was taken, were considered free of prostate cancer.

The biopsy results were used to assess the capability of the F/T ratio in combination with the total PSA to detect prostate carcinoma, as biopsy illustrated the definite histological status of the individual.

Statistics

Continuous parameters were compared by means of the Mann Whitney U-test (a two-sided 0.05 level of significance was used). Linear univariate regression analysis of continuous parameters like total PSA, free PSA, total prostate volume, volume of the inner zone, and age was used to express the correlation between factors.

Predictors for a positive biopsy result were estimated as a function of these parameters, and of binairy parameters like DRE and TRUS, by means of logistic regression analysis. Logistic regression analysis was chosen as the distribution of the values of various parameters was not Gaussian. It was realised that several parameters may significantly correlate to each other. For all men that were biopsied these parameters were used as input in a backwards deletion logistic regression analysis. The output of this procedure is a predictor that specifies the chance on a positive biopsy result as function of the variables most relevant to this end. All variables with insignificant Wald statistics are deleted [18]. The relative intrinsic discriminatory potential of these predictors was assessed by comparing ROC curves [19]. For the predictor with the best properties (this is the largest area under the ROC curve) the predictive value was determined.

RESULTS

General characteristics of all men are shown in TABLE 1. In 1726 men 67 cancers were histologically diagnosed by 308 sextant prostate biopsies. TABLE 2 shows the ratio between the biopsies taken and the carcinomas detected (the Bx/PCa) for each of the screening modalities, or the four possible combinations. The Bx/PCa

TABLE 1

Age, prostatic volumes, and PSA values (DELFIA ProStatus-TM PSA EQM, DELFIA ProStatus-TM PSA Free/Total) of 1659 apparently benign prostates and 67 participants with prostate cancer.

	Benign			Prostate Cancer		
(units)	*number*	*median*	*range*	*number*	*median*	*range*
AGE (years)	1659	64	54-77	67	66.8	55.6-76.2
total prostatic VOLUME (ml)	1565	31	8-204	64	33	16-105
TOTAL PSA						
ProStatus-TM EQM	1659	1.3	0-35	67	7.2	0.4-165
ProStatus-TM F/T	1659	1.2	0-30	67	7.6	0.5-150
FREE PSA ProStatus-TM F/T	1659	0.33	0.01-8.4	67	0.89	0.15-15.6

TABLE 2

The ratio between the biopsies taken and the carcinomas detected (the Bx/PCa) for each of the screening modalities, or the four possible combinations

	number of biopsies	*number of PCa*	*Bx/PCa*
TRUS alone	53	2	26.5
DRE alone	29	3	9.7
PSA alone	126	22	5.7
TRUS + DRE	48	7	6.9
TRUS + PSA	11	4	2.8
DRE + PSA	11	6	1.8
TRUS + DRE + PSA	30	23	1.3
total	308	67	4.6

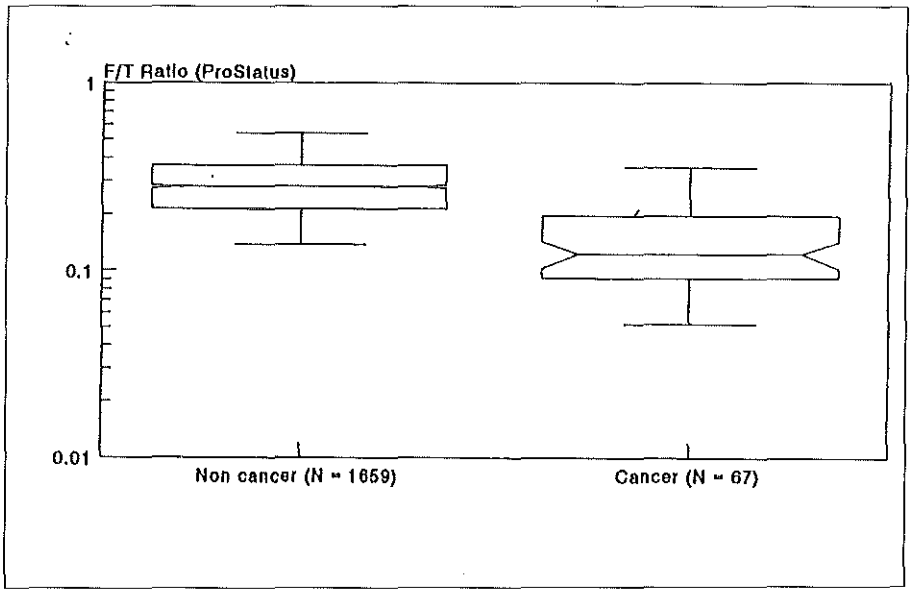

FIGURE 1
The PSA F/T ratio in 1659 benign and 67 prostate carcinoma patients, Box Whisker plots. $p < 0.05$.

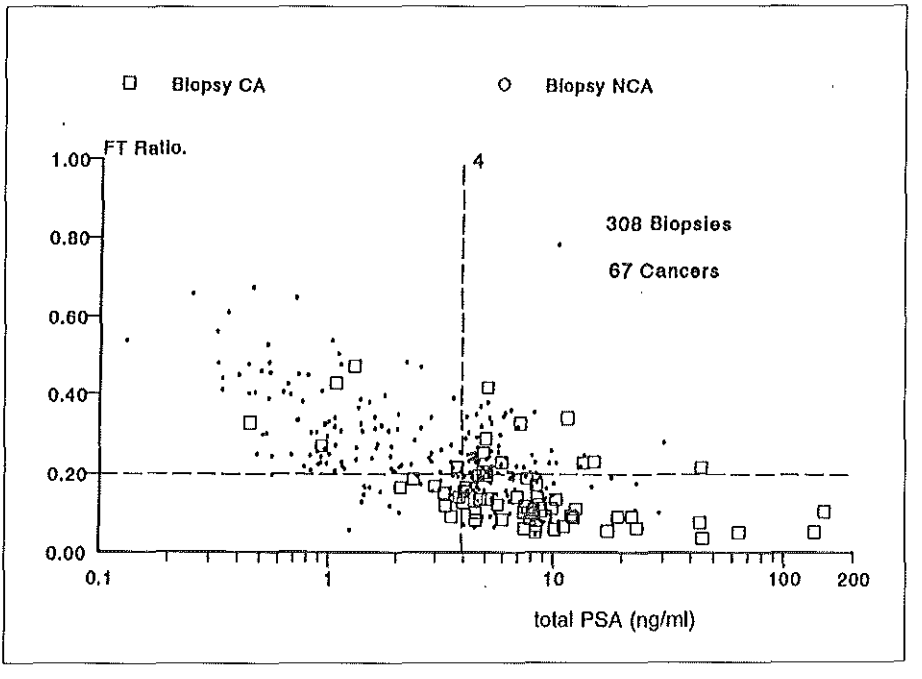

FIGURE 2
The relation between F/T ratio and the total PSA for 308 men who underwent a prostate biopsy; 67 carcinomas are indicated (CA = carcinoma, NCA = no carcinoma).

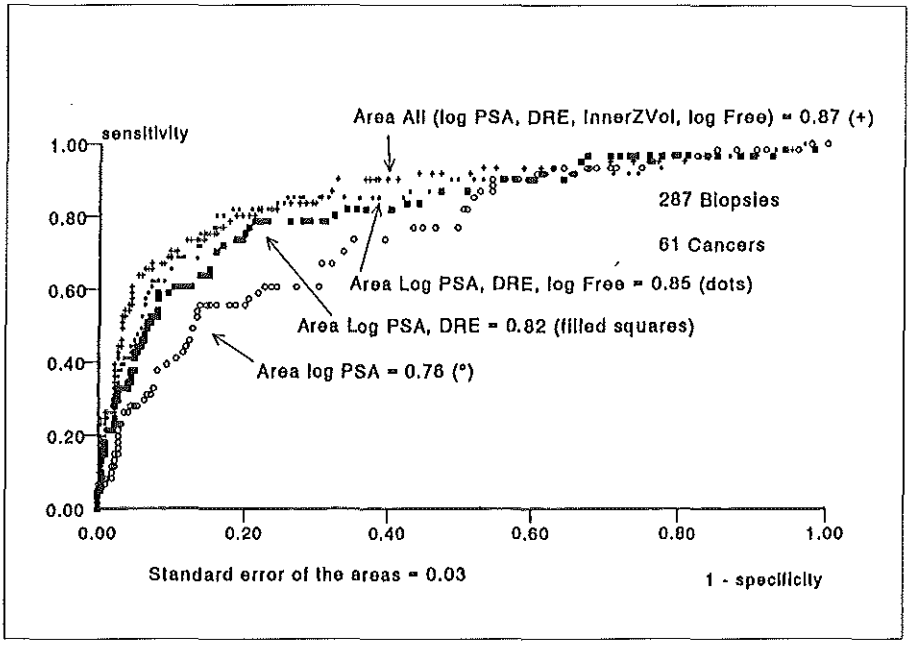

FIGURE 3
The relative effectiveness of diagnostics illustrated in ROC curves of 287 men who underwent prostate biopsies. Standard Error of the areas = 0.03.

illustrates the number of biopsies needed to detect one carcinoma, and is the reciproke of the positive predictive value of that screening modality. A suspicious TRUS alone showed the largest number of biopsies needed to detect one carcinoma, while the combination of a suspicious TRUS, an abnormal DRE, and a PSA value of 4.0 ng/ml or more was the most efficient in detecting PCa.
The values of F/T ratio measured by the ProStatus-TM Free/Total PSA assay in relation to men with and without cancer is illustrated in FIGURE 1 by Box Whisker plots. The box includes the results of 50 % of the participants, with the median value plotted inside. The notch represents the 95 % confidence interval of the median, and the middle vertical line the 90 % interval of all values. The median F/T ratio for 1726 benign participants was 0.28, which significantly differed from the F/T ratio of 0.12 of 67 men with a carcinoma.
In FIGURE 2 the relation between F/T ratio and the total PSA for 308 men who underwent a prostate biopsy is depicted.
The relative effectiveness of the diagnostic tests is illustrated in the ROC curves of FIGURE 3. These ROC curves show that, with a standard error of the areas of 0.03, the addition of DRE improved the detection of prostate carcinoma by total serum PSA significantly. Addition of free PSA improved detectability by one standard error. The best predictor of a positive biopsy was obtained by the combination of total PSA, DRE, inner zone volume, and free PSA.
For the range of total PSA between 4.0 and 10.0 ng/ml, including 140 prostate biopsies and 33 PCa's, the ROC curves of total serum PSA, and of the F/T ratio were constructed to illustrate their relative potential to predict a positive biopsy (FIGURE 4). The standard error of the areas under the curve was 0.05. No significant difference between the curves was obtained.

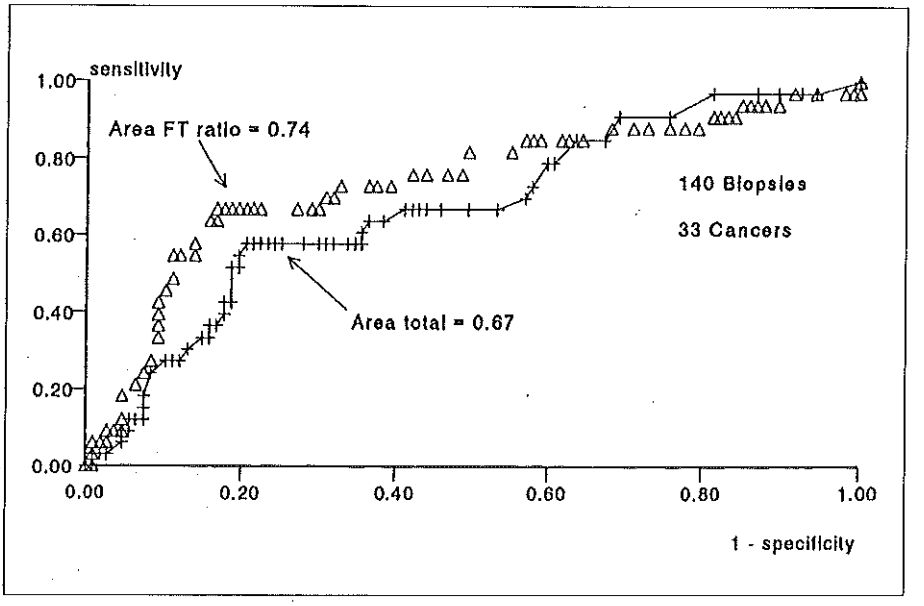

FIGURE 4

The relative effectiveness of diagnostics illustrated in ROC curves of 140 men who underwent prostate biopsies in the total serum PSA range between 4.0 and 10.0 ng/ml. Standard Error of the areas = 0.05.

In TABLE 3 the results of the linear univariate regression analysis is shown for 61 men with carcinoma, and 1542 without carcinoma. All correlations were significant. A high correlation existed between total and free PSA. Volumetric parameters correlated slightly better with free PSA compared to total serum PSA. For men with prostate cancer, the correlation between volumetric parameters and PSA was less compared to men without a carcinoma. Age was uniformely mildly correlated to all other parameters.

In TABLE 4 the results of logistic regression analysis of the parameters for predicting a positive biopy in 308 men are shown. Total serum PSA was the best single predictor for prostate carcinoma, followed by DRE. Free PSA and the ultrasonic volume of the inner zone of the prostate were significant as predictors. Age was the first parameter deleted in the backward stepwise elimination procedure, followed by total prostate volume and TRUS.

DISCUSSION

For the detection of PCa currently the combination of serum PSA, DRE, and TRUS is available. Detection rates in community based populations vary between 2 and 5 percent. In this study the detection rate was 3.3 %. A combination of total serum PSA, DRE, and TRUS as indicators for biopsy led to a subset of men in which 1.3 sextant prostate biopsies were needed to detect one carcinoma. The mean Bx/PCa ratio was 4.6. These ratios correspond very well to those found by Catalona et al. in a screening study of 6630 men evaluated by PSA and DRE [5]. In this study it was also found that the Bx/PCa ratio is relatively constant across age. Further clarifying information on the optimal use of the available screening tests will hopefully be an early result of the European Randomised Study of Screening for Prostate Cancer.

TABLE 3
Results of univariate regression analysis; p<0.01

Correlation	*LogFreePSA Total*	*Volume gland*	*Volume inner zone*	*Age*
n=1542 benign				
Log PSA	0.87	0.54	0.41	0.16
Log FreePSA		0.62	0.45	0.22
Volume Total gland			0.74	0.23
Volume inner zone				0.22
n=61 PC				
Log PSA	0.81	0.11	0.08	0.12
Log FreePSA		0.46	0.32	0.31
Volume Total gland			0.70	0.26
Volume inner zone				0.24

TABLE 4
Results of logistic regression analysis of independent parameters for predicting a positive biopy in 308 men, first step in backward deletion procedure

Variable	*B*	*S.E.*	*Wald*	*Significance*
LogPSA	5.84	0.98	35.12	0.00
LogFreePSA	-2.32	1.15	4.03	0.04
DRE	-0.93	0.24	14.97	0.00
VolumeTotal	-0.01	0.01	0.58	0.44
VolumeInner	-0.03	0.02	3.71	0.05
Age	0.04	0.04	1.06	0.30
TRUS	-0.18	0.22	0.63	0.42

B = predictor for a positive biopsy
S.E. = standard error of the predictor
Wald statistic = square of the ratio of the predictor to its standard error
Sig. = significance of Wald statistic, threshold 0.05

Serum PSA is the best single screening modality for prostate carcinoma available, as shown in TABLE 2, and TABLE 3. Logistic regression analysis could illustrate the value of various parameters which were no indicators for sextant biopy in this study (TABLE 4). Due to the high correlation between total serum and free PSA, much of the influence of free PSA as a predictor was reduced by total serum PSA. Due to the higher correlation between total or free PSA and the total gland volume, this volumetric parameter was replaced by the inner zone volume in the backward elimination procedure. A combination of the modalities of serum PSA and DRE, with other modalities, such as free PSA or the volume of the inner zone of the prostate, may add to the specificity and sensitivity of the detection, as illustrated by an increasing area under the ROC curve (FIGURE 3). DRE increased the area of total serum PSA by two standard errors, while the combination of DRE and free PSA increased the area by three standard errors. It is suggestive, although not statistically significant, that the application of the F/T ratio in addition to total serum PSA in combination with DRE for screening on PCa is useful. No optimal analysis could be performed in the total PSA range below 4.0 ng/ml, as biopsies were taken only on the indication of TRUS or DRE. For the total PSA range between 4.0 and 10.0 ng/ml however, no significant difference between the ROC curves was obtained in this study.

Ultrasonic volume of the inner zone (as a representation of the prostatic hyperplasia), only marginally adds to the prediction of a positive biopsy. Lesion detection by TRUS did not have significant predictive value in the logistic regression analysis. This means that the two carcinomas detected by TRUS (TABLE 2) could have been detected by use of the free PSA or the inner zone volume. The logistic regression does not illustrate the number of biopsies needed for the detection of those two carcinomas, but from TABLE 2 it can be seen that 26.5 biopsies per cancer found were needed to determine the histology of those hypoechogenic lesions. The use of TRUS for lesion detection and prostate volumetry in screening for prostate cancer is certainly not cost-effective. Application of the F/T ratio as an alternative might be justified.

CONCLUSION

The combination of total serum PSA and DRE remains the standard for detection of prostate carcinoma in a screening population. Their specificity may be improved minimally by the F/T ratio. The threshold of the F/T ratio, and the PSA range for its application, remains to be assessed prospectively. The role of TRUS for lesion detection and prostate volumetry is limited and not cost-effective. Whether DRE can be eliminated in a subset of men with low PSA values, remains to be studied in a larger series.

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THE VALUE OF SCREENING TESTS IN THE DETECTION OF PROSTATE CANCER*

2. A simulation of the role of the F/T ratio, age specific reference ranges, and PSA density

Chris H. Bangma, Ries Kranse, Bert G. Blijenberg, Fritz H. Schröder

ABSTRACT

Objectives. The ratio between free and total PSA in serum (F/T ratio) was shown to improve the specificity of total serum PSA for the detection of prostate carcinoma in selected populations. In this study the value of the F/T ratio for screening on prostate cancer was compared to that of age specific reference ranges for PSA, and PSA density by a simulation experiment.

Methods. In 1726 men between 55 and 76 years old 67 prostate carcinomas were detected by applying digital rectal examination (DRE), transrectal ultrasonography (TRUS), and total serum PSA. A simulation was performed in which an F/T ratio of 0.20 (ProStatus-TM PSA Free/Total), age specific PSA reference ranges, and a PSA density of 0.12 ng/ml/cc were used to study their capability to increase the specificity of total serum PSA in predicting prostate biopsy results.

Results. Using age specific PSA reference ranges and DRE as indicators for biopsy, a reduction of 37 % of biopsies would have been obtained, with a loss of detected cancers of 12 %. For the use of PSAD and DRE, these numbers were 28 % and 11 %, respectively. Application of a serum PSA of 4.0 ng/ml or more and an F/T ratio of 0.20 or less, and/or an abnormal DRE as indicators for biopsy would reduce the number of biopsies by 39 % and the number of detected cancers by 11 %. The Bx/PC ratio of these simulations varied between 3.3 and 3.6. Minimal loss of cancer detection of 3 % with a reduction in the number of biopsies of 17 % is obtained when TRUS is omitted from the screening protocol. Pre-screening by a total serum PSA value of 2.0 ng/ml would have reduced the number of biopsies by 30 %, and the number of cancers detected by 6 %.

Conclusions. The most cost-effective protocol for screening prostate carcinoma appears to be pre-screening by total serum PSA. The F/T ratio might be used to detect carcinomas in the PSA range below 4.0 ng/ml, but the best threshold remains to be assessed.

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INTRODUCTION

Screening for prostate cancer (PCa) is usually performed by prostate specific antigen (PSA) and digital rectal examination (DRE); transrectal ultrasonography (TRUS) can be added for ultrasonic lesion detection and prostate volumetry. Selection of candidates for prostate biopsy is complicated by the fact that all screening modalities lack sufficient specificity. This results in a high ratio between the number of biopsies and the number of detected carcinomas. In most screening studies overall about 5 biopsies are needed to detect one carcinoma [1-5].
Several additional parameters, such as prostate volume and age, have been analysed to reduce this ratio, without trying to limit the number of carcinomas [6, 7].
The free to total serum PSA ratio (F/T ratio) may represent a new parameter to increase the specificity of total serum PSA to detect PCa in selected patients [8-10] and in a screening population [11].
In this study a simulated case selection for biopsy was performed in a well defined screening population to illustrate the value of F/T ratio, age specific PSA reference ranges [12], and PSA density [13] for the detection of PCa.

MATERIALS AND METHODS

Serum sample collection and storage, PSA tests used, the study population, screening tools, and biopsy indications were described in chapter 12 of this dissertation [11].

Age specific reference ranges for PSA and F/T ratio were constructed [12] with the IMx and the ProStatus-TM PSA Free/Total assays, using the 95th percentile upper limit of values (mean value plus two standard deviations) in men without prostate cancer, to analyse their capacity to distinghuish between tumour and benign conditions. To allow comparison with available literature information, also the sera of 188 asymptomatic blood donors of 20 to 43 years old were assessed. Reference ranges for the age between 44 and 55 were extrapolated from these results. Grouping by age ranges of 5 years was used to allow for a more continuous spectrum compared to age ranges of 10 years [18].

Venn-diagrams were used to describe the number of biopsies performed, and the number of cancers detected in the population studied and in the simulation procedure. The Bx/PCa ratio was depicted between brackets. Entry in the various simulations was by a total serum PSA of 4.0 ng/ml and more, an abnormal DRE, an F/T ratio smaller than 0.20, a total serum PSA exceeding the constructed age reference value, and a PSA density value of 0.12 ng/ml/cc or more. The F/T ratio threshold of 0.20 was chosen, as this value was in between the median values of 67 men with and 1659 men without prostate carcinoma [11]. The PSAD threshold was chosen as recommended by Littrup et al., who optimalized their original threshold of 0.20 ng/ml/cc in 1991 to a value of 0.12 ng/ml/cc for use in a screening population [6]. Omitting lesion detection by TRUS in the various simulations excluded two men with a PCa, and limited the maximum number of detected carcinomas to 65. Also a PSA pre-screen value of 2.0 ng/ml was evaluated because of the expected cost-effectiveness. Results of the various simulations are summarized in a table, in which the standard error (S.E.) of the calculated reductions in biopsies and detected cancers are depicted [19].

RESULTS

General characteristics of all men are shown in TABLE 1. In 1726 men 67 cancers were histologically diagnosed by 308 sextant prostate biopsies. There was no significant difference between the median prostatic volume of men with and without prostate carcinoma. Therefore, no sampling advantage by biopsy for smaller prostates occurred [20].

TABLE 1

Age, prostatic volumes, and PSA values (DELFIA ProStatus-TM PSA EQM, DELFIA ProStatus-TM PSA Free/Total) of 1659 benign and 67 participants with prostate cancer.

	Benign			*Prostate cancer*		
(units)	*number*	*median*	*range*	*number*	*median*	*range*
Age (years)	1659	64	54-77	67	66.8	55.6-76.2
Total prostatic volume (ml)	1565	31	9-204	64	33	16-105
Total PSA						
ProStatus-TM EQM	1659	1.3	0-35	67	7.2	0.4-165
ProStatus-TM F/T	1659	1.2	0-30	67	7.6	0.5-150
Free PSA ProStatus-TM F/T	1659	0.33	0.01-8.4	67	0.89	0.15-15.6

TABLE 2

Age specific PSA reference ranges: indicated are the upper limits (all lower limits are equal to zero).

Age range	*40-49*	*50-54*	*55-59*	*60-65*	*65-70*	*70-75*
Oesterling	2.5	3.5[1]		4.5[2]		6.5
Abbott IMX	2.8	3.5	4.0	4.7	5.4	6.3
Prostatus FT total PSA	3.1	3.8	4.3	4.9	5.5	6.3
Prostatus FT free PSA	0.75	0.85	0.96	1.06	1.17	1.29

1 Age range 50-59.

2 Age range 60-70.

FIGURE 1 A shows the Venn diagram illustrating the distribution of the indications for prostate biopsy in 308 men with abnormal DRE, TRUS, and/or a serum PSA value of 4.0 ng/ml or more. In FIGURE 1 B the number of cancers found, and the Bx/PCa ratio (between brackets) is indicated. The combination of all three screening modalities led to the lowest, and most effective, Bx/PCa ratio of 1.3, while 26.5 biopsies were needed to detect one carcinoma when the indication for biopsy was a hypoechogenic lesion only. Overall the Bx/PCa ratio was 4.6.
TABLE 2 shows age specific reference ranges for PSA described by Oesterling [12] and those of the participants of this study, excluding those men with a detected PCa. The reference ranges include 95 % of the serum PSA values per age group for 1847 men (benign participants and donors) without detectable prostate carcinoma. The correlation coefficient between age and total PSA for all men including blood donors was 0.32, and 0.16 for men aged 50 years or older. For the free PSA (ProStatus-TM Free/Total) these correlations were 0.30 and 0.21 respectively.
In FIGURE 2 the detected prostate carcinomas are indicated in relation to the 95 percentile of the age reference ranges for PSA. Organ confined tumours (n = 59, clinically staged) are contrasted with non-organ confined tumours (n = 7), or carcinomas with unknown stage (n = 1). A large number of detected prostate carcinomas (39 %, 20 confined, 2 non-confined, 1 unknown staging) are found within the age specific reference ranges. The cases are rather equally distributed over the age groups involved.

F/T ratio

FIGURE 3 shows the simulated case selection for biopsy using a PSA value of 4.0 ng/ml or more, an abnormal DRE, and an F/T ratio of 0.20 or less as a criterium for biopsy. 494 men entered this simulation, and 263 were biopsied. Of 363 men who had an indication for biopsy based on the F/T ratio of 0.20 or less, only 132 were

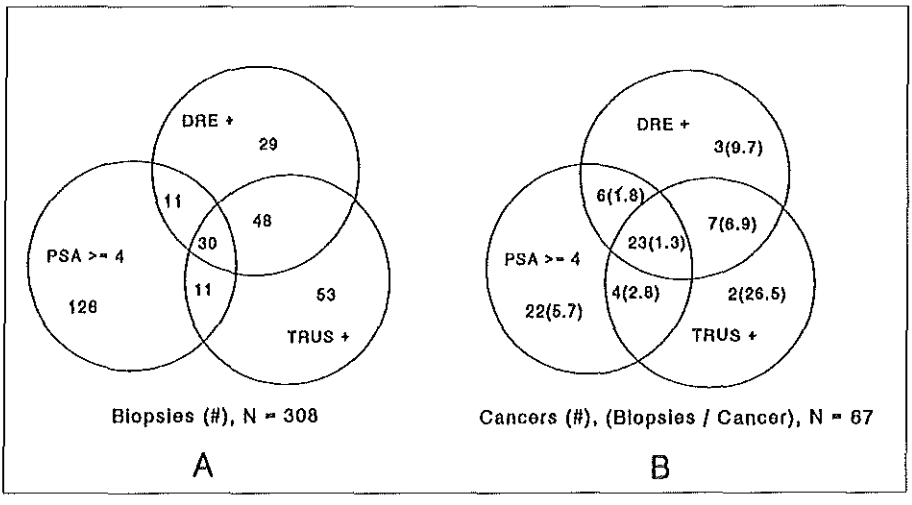

FIGURE 1

Venn diagrams illustrating the distribution of the indications for prostate biopsy in 308 men (A), and the number of cancers detected by DRE, TRUS, and a serum PSA value of 4.0 ng/ml or more (B); between brackets is noted the ratio between the number of biopsies performed, and the number of cancers detected (Bx/PCa ratio).

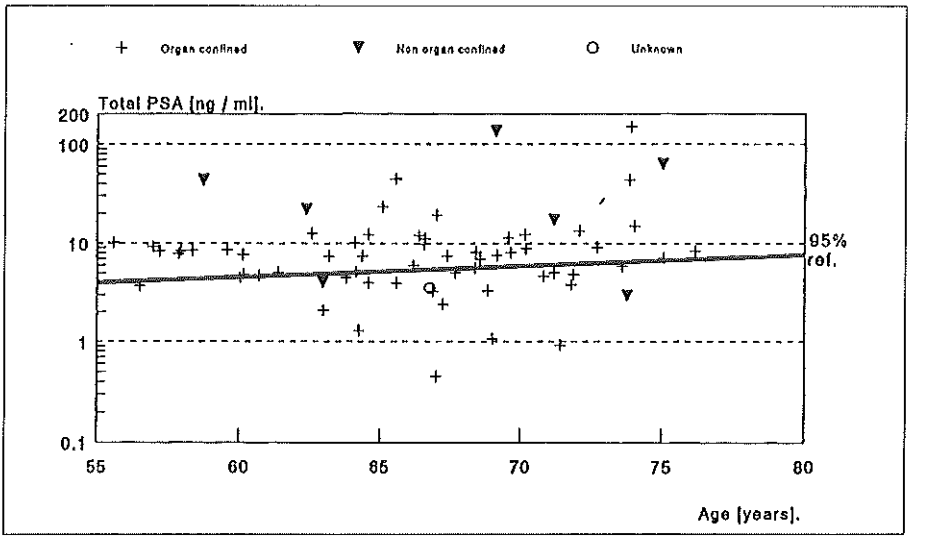

FIGURE 2

Relation between total PSA and age of 67 men with prostate carcinoma; the age specific reference range and clinical stage of 66 carcinomas are indicated (+ = organ confined PCa, ▼ = non organ confines PCa, 0 = unknown clinical stage).

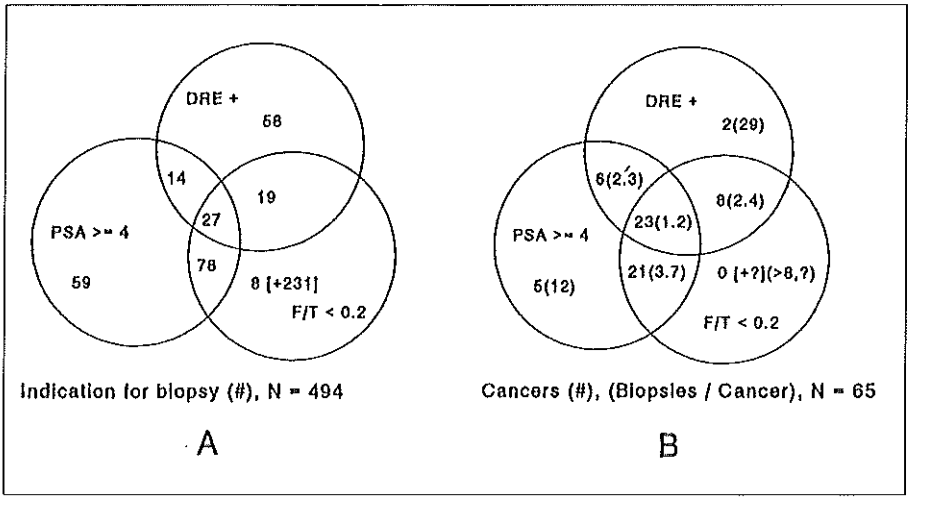

FIGURE 3 A-B

Venn diagrams showing simulated case selection for biopsy based on an abnormal DRE, a total serum PSA of 4.0 ng/ml or more, and an F/T ratio of 0.20 or less; between [ ] the number of men selected but without biopsy is noted (A). The number of positive biopsy results and the Bx/PCa ratio (between brackets) are depicted separately (B).

biopsied according to the study protocol (FIGURE 3 A). In the PSA range of 4.0 ng/ml and more, the specificity of PSA was increased by the application of the F/T ratio. Using the F/T ratio instead of the total serum PSA as an indicator for biopsy in this PSA range, 5 cancers would not have been detected (FIGURE 3 B). The number of biopsies decreased from 308 (FIGURE 1 A) to 196 (FIGURE 3 A), a reduction of 37 %. The overall number of detected carcinomas decreased with 5 + 2 = 7 (11 %), the Bx/PCa ratio was reduced from 4.6 to 3.3.

Age specific reference ranges

FIGURE 4 shows the simulation procedure using a PSA value of 4.0 ng/ml or more, an abnormal DRE, and age specific PSA reference ranges as a criterium for biopsy. 255 men entered this simulation. No participants would have been selected for biopsy based only on the age specific reference ranges, as all upper limits are above 4.0 ng/ml. 60 men were selected for biopsy only on the basis of a PSA of 4.0 ng/ml only (FIGURE 4 A). In this group 6 carcinomas were detected (FIGURE 4 B). So, if only age specific PSA reference ranges were used together with an abnormal DRE as indication for biopsy, 6 cancers would not have been detected. This would have limited the number of biopsies from 308 to 195, a reduction of 37 %, with a Bx/PCa ratio dropping from 4.6 to 3.3. The omission of TRUS and the use of age specific reference ranges would have induced a loss of 2 + 6 = 8 (12 %) unpalpable carcinomas. These 8 cancers were organ-confined.

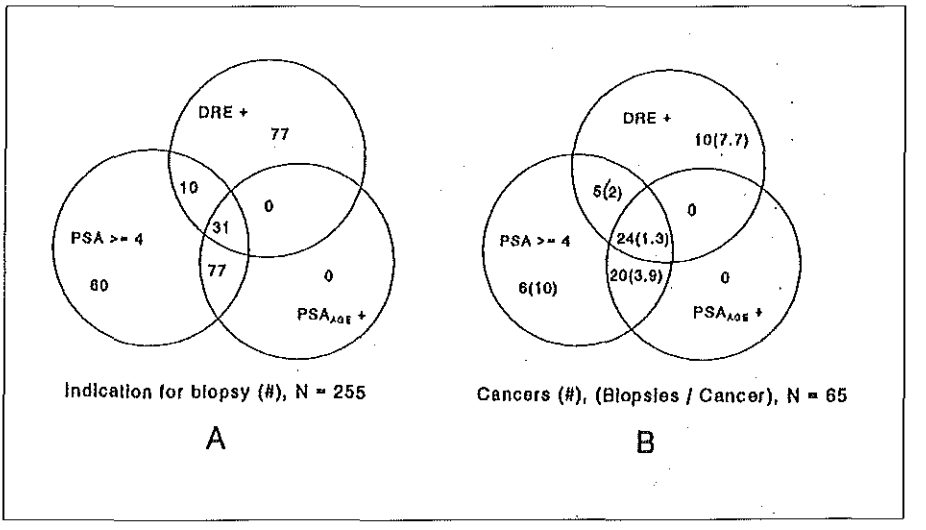

FIGURE 4 A-B

Venn diagrams showing simulated case selection for biopsy based on an abnormal DRE, a total serum PSA of 4.0 ng/ml or more, and a higher PSA value than the age specific reference range; between [ ] the number of men selected but without biopsy is noted (A). The number of positive biopsy results and the Bx/PCa ratio (between brackets) are depicted separately (B).

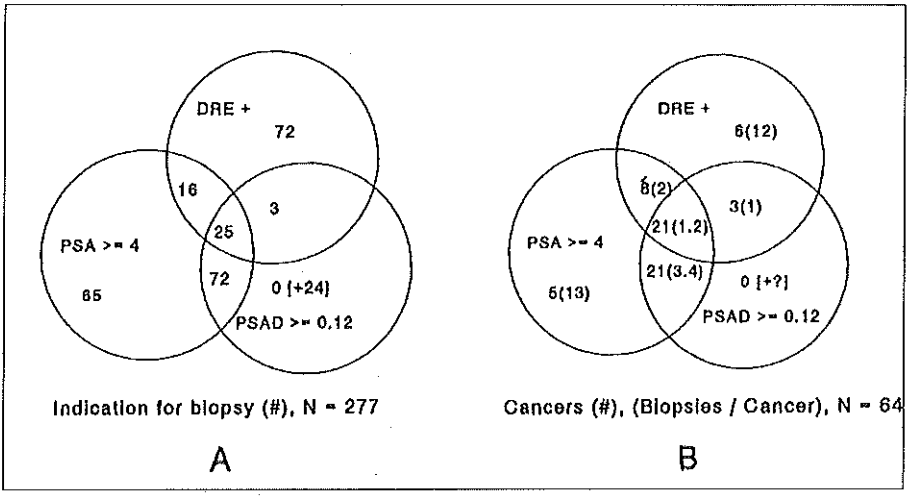

FIGURE 5 A-B

Venn diagrams showing simulated case selection for biopsy based on an abnormal DRE, a total serum PSA of 4.0 ng/ml or more, and a PSAD of 0.12 ng/ml/cc or more; between [ ] the number of men selected but without biopsy is noted (A). The number of positive biopsy results and the Bx/PCa ratio (between brackets) are depicted seperately (B).

PSA density

FIGURE 5 shows the simulated case selection for biopsy using a PSA value of 4.0 ng/ml or more, an abnormal DRE, and a PSA density of 0.12 ng/ml/cc or more as a criterium for biopsy. 277 men entered this simulation, and 253 were biopsied. In 66 of the 67 men with PCa the total prostate volume was measured; in 2 of them cancer was detected only on the base of a hypoechoic lesion. Therefore 64 cancers were included in FIGURE 5 B. Using PSAD and DRE as an indicator for biopsy, a reduction in the number of biopsies from 308 to 212 was seen (28 %), and a loss of 2 + 5 = 7 cancers (11 %).

If PSAD was used in the PSA range of 4.0 ng/ml or more, together with an abnormal DRE as indication for biopsy, 5 cancers would not have been detected (FIGURE 5 B). This would have limited the number of biopsies from 308 to 188, a reduction of 39 %, with a Bx/PC ratio dropping from 4.6 to 3.2. A loss of 2 + 5 = 7 (11 %) unpalpable carcinomas would occur. These 7 cancers were organ-confined.

PSA prescreen

Prescreening accounts for the selection of men before being submitted to screening for PCa by various screening tools, with the possible result of being selected for biopsy. A prescreen PSA value of 2.0 ng/ml as a threshold for DRE and TRUS would give a 70 % reduction in the number of TRUS investigations, at the price of 6 % loss of cancers. The number of biopsies (on indication PSA 4.0 ng/ml or more, and/or abnormal DRE or TRUS) would be reduced by 30 %. If also TRUS would be omitted, screening of men with a serum PSA of 2.0 ng/ml or more with DRE would not detect 6 % of cancers. The number of biopsies (on indication PSA 4.0 ng/ml or more, and/or abnormal DRE) would be reduced by 36 %.

TABLE 3

Results of simulated case selection for biopsy, based upon the results by screening with PSA, DRE, and TRUS in 1726 men, of whom 67 men with prostate cancer in 308 biopsies (upper) line

prescreen	*biopsy indicator*	*reduction of biopsies (%) (S.E)*	*loss of PCa (%) (S.E.)*	*overall Bx/PCa*
-	PSA>4, DRE, TRUS	-	-	4.6
-	PSA>4, DRE	17 (2.1)	3 (2.1)	3.9
PSA>2,	PSA>4, DRE, TRUS	30 (2.6)	6 (2.9)	3.4
PSA>2,	PSA>4, DRE	36 (2.8)	6 (2.9)	3.1
PSA-age	PSA>4, DRE, TRUS		39 (6.0)	
-	PSA-age, DRE	37 (2.8)	12 (4.0)	3.3
-	PSAD, DRE	28 (2.4)	11* (3.8)	3.6*
-	PSA>4 AND PSAD, DRE	39 (2.8)	11 (3.8)	3.2
-	PSA>4 AND F/T, DRE	37 (2.8)	11 (3.8)	3.3

PSA>4 : total serum PSA of 4.0 ng/ml or more
PSA-age : total serum PSA larger than age specific reference range
PSAD : PSA density 0.12 ng/ml/cc or more
F/T : Free/Total PSA ratio 0.20 or less
DRE : abnormal DRE result
TRUS : ultrasonic lesion detected by TRUS
* : real incidence of cancers unknown

Summary simulation results

The main effects of the simulated case selection for biopsy are summarized in TABLE 3. Significant differences between the various reductions of the number of biopsies were seen.

Concerning the loss of detected cancers, there was a significant difference between omitting TRUS, and the various additions of parameters to improve the specificity of a PSA value of 4.0 ng/ml and more.

It was calculated that at least 100 carcinomas would be needed to obtain a significant difference between a loss of 6 % and of 12 % of cancers [19].

DISCUSSION

In this screening population of 1726 men who all underwent total serum PSA measurement, DRE, and TRUS, most of the 67 carcinomas were found by sextant biopsies based on the indication of PSA and/or DRE. Only 2 carcinomas were found by TRUS alone. The overall Bx/PCa ratio was 4.6.

The simulated case selections for biopsy are limited by the fact, that all biopsies in the present study were taken on indication of total serum PSA, DRE, and/or TRUS. Data concerning additional parameters are never complete, unless all participants of the study are biopsied. However, the simulation procedure reasonably illustrates what may happen to the sensitivity and specificity of a combination of tests for the detection of PCa. The decrease in sensitivity has to be weighed against the increase in specificity.
The most cost-effective change of the present screening protocol would be to limit the number of TRUS procedures, or to omit TRUS completely. A prescreen PSA value of 2.0 ng/ml as a threshold for DRE and TRUS would give a 70 % reduction in the number of TRUS investigations, at the price of 6 % loss of cancers. The number of biopsies (on indication PSA 4.0 ng/ml or more, and/or abnormal DRE) would be reduced by 30 %. Screening all men by DRE and PSA only would reduce the number of biopsies by 17 % (FIGURE 1) with a loss of 3 % of cancers, and a Bx/PCa ratio of 255/65 = 3.9.

Two parameters were discussed which may improve the specificity of the combination of total serum PSA with DRE without the use of TRUS. The most cost-effective is the application of age specific PSA reference ranges. It was seen that this parameter decreases the sensitivity considerably: 12 % of carcinomas would have been missed, while the number of biopsies was reduced by 37 %. This is at variance with the study of Oesterling [7], in which 5.5 % of 1686 biopsies in an outpatient population of 2988 men of 60 years and older would have been avoided by the use of age specific reference ranges, and 19 of the 608 cancers (3 %) would not have been detected. In the study of Catalona et al.[5], who evaluated the effect of age specific reference ranges in a screening study of 6630 men of 50 years and older, an overall reduction in the number of biopsies of 6 % was obtained, with a loss of 8 % of palpable and non-palpable tumour detection. Both studies concern different study populations, without the use of TRUS for lesion detection.
The use of age specific reference ranges as a prescreen value for application of DRE and TRUS has to be discouraged, as 39 % of cancers would have been missed (FIGURE 2). These findings are in line with our previous observations [21], and those of Littrup et al.[6], who found a loss of 28 % of detectable cancers.

With a threshold of 0.20, the application of the F/T ratio would have induced a large number of additional biopsies, especially in the PSA range below 4.0 ng/ml. The use of F/T ratio for men with a total PSA of 4.0 ng/ml or more or an abnormal DRE, would miss 11 % of cancers, and reduce the number of biopsies with 37 %. According to Colberg et al.[22] there is a 7.9 % incidence of carcinomas in the PSA range between 2.9 and 4.0 ng/ml. It can be estimated by extrapolation that in the 328 men with a PSA range of 2.0 to 4.0 ng/ml in the present study 23 cancers would have been found by sextant routine biopsies, while actually 10 were detected. The value of the F/T ratio can be tested if one is willing to biopsy all men with a PSA of 2.0 ng/ml or more. The number of biopsies would increase from 308 to 506, with an overall change of Bx/PCa ratio of 4.6 to an estimated 506/80 = 6.3.

PSAD as an indicator for biopsy instead of a PSA value of 4.0 ng/ml or more, and in combination with DRE, has been reported to be more cost-effective than age specific PSA reference values [6]. In that evaluation of 2930 men recruited to a screening study, Littrup et al. recommended a 'tailored biopsy approach' using PSAD with a threshold of 0.12 ng/ml/cc in combination with DRE, in which the number of

biopsies was reduced with 15.7 % at the price of a loss of 3.6 % of the cancers. In the present simulation these numbers for the use of PSAD were larger, respectively, 28 % and 11 %. The larger number of undetected carcinomas might be due to the included effect of omitting TRUS for lesion detection, and the assumption that no cancers would have been detected by biopsies on the indication of PSAD only.

CONCLUSION

Minimal loss of cancer detection of 3 % with a reduction in the number of biopsies of 17 % is obtained when TRUS is omitted from a screening protocol in which a PSA value of 4.0 ng/ml and more, an abnormal DRE, and/or an abnormal TRUS are used as indicators for biopsy.
Age specific PSA reference ranges, an F/T ratio with a threshold of 0.20, and a PSAD with a threshold of 0.12 ng/ml/cc may all reduce the number of biopsies with approximately 35 % in this study, with a reduction in cancer detection of 11 %.
The value of F/T ratio for screening on prostate carcinoma has not yet been assessed optimally: especially in the PSA range below 4.0 ng/ml a threshold needs to be established for discrimination between benign and malignant disease.
The most cost-effective protocol for screening prostate carcinoma appears to be pre-screening by total serum PSA and exclusion of DRE and TRUS with PSA values of 2.0 ng/ml or less.

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14

THE FREE/TOTAL SERUM PSA RATIO FOR STAGING AND FOLLOW-UP OF PROSTATE CARCINOMA*

Chris H. Bangma, Ries Kranse, Bert G. Blijenberg, Fritz H. Schröder

ABSTRACT

Objectives. This study was performed to analyse the relation between the free/total PSA ratio and prostate cancer tumour stage and grade, compared to total serum PSA. Methods. In 128 patients clinical and pathological grade and stage were related to total serum PSA and F/T ratio. In 49 patients post-radical prostatectomy serum levels were assessed, and in 7 of those men PSA recurrence was compared to F/T ratio behavior in a graph.

Results. The total serum PSA parallels clinical staging of prostate cancer. The distributions of total serum PSA values and the F/T ratio were significantly different between benign and malignant disease (any stage), and between any T category and nodal disease. For serum PSA significant differences were seen between the distributions of men with locally confined (T1-2) and locally extended (T3) disease, and between all T categories and systemic metastatic disease; this was not so for the F/T ratio. The F/T ratio varies considerably in the PSA range of 0.3 ng/ml and less.

Conclusions. The F/T ratio has no additional value in clinical staging prostate carcinoma compared to serum PSA. The F/T ratio does not give clinically useful information compared to the total PSA in the immediate postoperative serum evaluation. The F/T ratio may be considered the result of cell differentiation, and not a parameter of tumour load.

INTRODUCTION

The clinical staging of prostate carcinoma is performed by physical examination, and additional radiologic procedures. Prostate specific antigen (PSA) has been helpful in a limited way to differentiate between clinically important and unimportant carcinomas [1], confined versus extended disease [2], or to predict pathologic stage [3]. New developments in serum PSA assays offer the possibility to distinguish free circulating PSA molecules, and those complexed to serum proteins, of which α-1-antichymotrypsine (ACT) is the predominant [4,5]. In selected groups the ratio between free and total PSA improves the differentiation between prostate

* *Submitted.*

carcinoma (PCa) and benign conditions [6-8]. It is not known whether the F/T ratio may improve differentiation between various grades and stages of prostate cancer. This study was performed to analyse the relation between the free/total PSA ratio and prostate cancer tumour stage and grade, and to assess whether the F/T ratio is better in differentiating various stages than total serum PSA is. Furthermore its value in patients after radical prostatectomy was analysed, and in the follow-up of a limited group of patients with untreated prostate cancer.

PATIENTS AND METHODS

For this study 128 patients with an untreated prostate carcinoma were selected, of which 67 from a screening population, and 61 from an outpatient clinic. Histology was confirmed by ultrasound guided prostate biopsy. Clinical stage was assessed by digital rectal examination and bone scan according to the 1992 TNM classification. Abdominal computer tomography was performed on indication, usually when suspicion on positive pelvic lymphnodes was raised by increased serum PSA levels. Grading was done by the biopsy according to the Anderson classification. Of all participants deep frozen serum (- 70 degrees Celsius) was used to determine total serum PSA and the free to total serum PSA ratio by the DELFIA ProStatus-TM PSA Free/Total assay in retrospect. This assay has been evaluated extensively [9-11]. Its detection limits are < 0.01 ng/ml, and < 0.1 ng/ml for free and total PSA, respectively.

In 12 of these 128 men serial determinations with a minimal follow-up of 60 days and two or more serum samples were obtained. Five of them had lymphnode positive disease. The results of their PSA determinations and F/T ratios were used to illustrate graphically the level and variation along time. In 49 men with clinical confined prostate carcinoma pelvic lymphnode dissection was performed, followed by radical prostatectomy. The pathologic stage and grade were analysed separately from their clinical stage. Also post radical prostatectomy sera in 37 of these patients were assessed. In case the PSA remained undetectable during the follow-up (Abbott IMx), only the last available serum sample was reevaluated. In 7 patients direct postoperative or delayed PSA recurrence occurred. Three of them showed clinical metastatic disease (bone scan) during this follow-up. In those men all available serum samples were included for analysis, and the results were used to illustrate graphically the level and variation along time.

To illustrate the distribution of various PSA values, Box Whisker plots were used. The box includes the results of 50 % of the participants, with the median value plotted inside. The middle vertical line represents the 90 % interval of all values. The values for the total serum PSA and the F/T ratio of 1726 men without carcinoma, all participants of the Randomized Study of Screening for Prostate Carcinoma [11], were used for comparison.

Continuous parameters were compared by means of the Mann Whitney U-test, and a two-sided 0.05 level of significance was used.

RESULTS

TABLE 1 shows the distribution of grade and clinical stage among 128 participants of the study. Men with lower T categories had lower grades compared to men with higher T categories or metastatic disease.

FIGURE 1 A shows Box Whisker plots of the total serum PSA for 123 men with various clinical stages of prostate carcinoma, broken up for local disease (T1-3 N0M0), lymphnode metastatic disease (T1-4 N+M0), and systemic metastatic disease (T1-4 NxM1). Also a diagram of the 1659 men without prostate carcinoma is given.

TABLE 1
Distribution of grade and clinical stage among 128 participants.

Stage		*T1*	*T2*	*T3*	*T4*	*N+*	*M+*
Grade	1	16	25	4	-	3	2
	2	8	29	16	2	6	2
	3	2	9	10	1	5	4
unknown		1				1	1
all		26	64	30	3	15	9

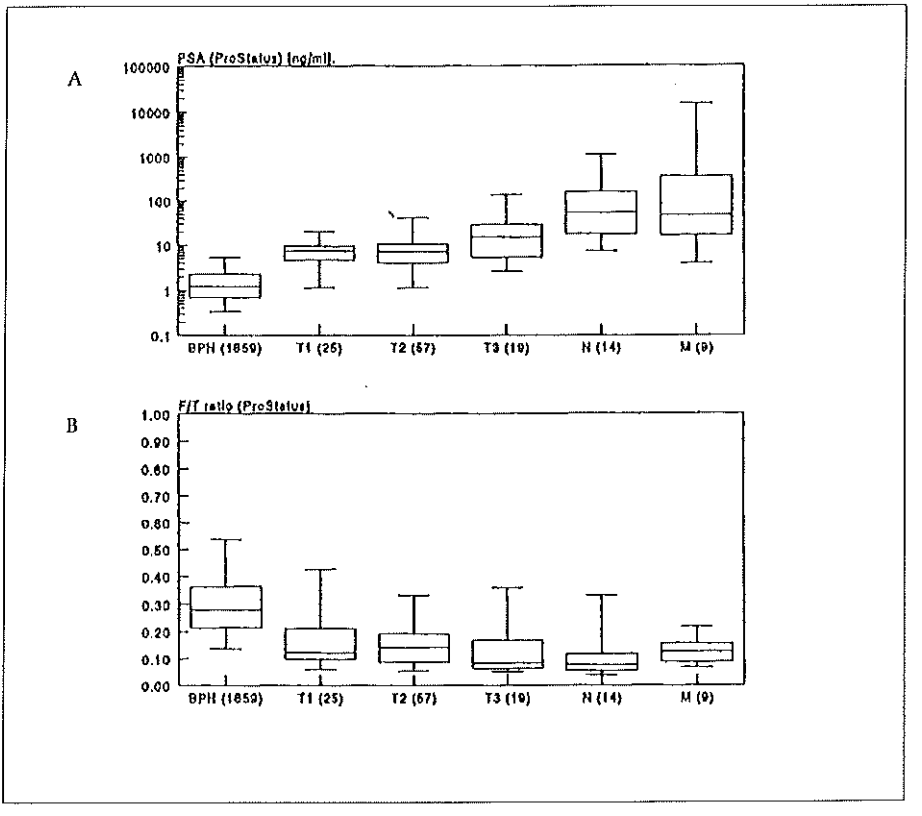

FIGURE 1
Box Whisker plots of the total serum PSA (A) and F/T ratio (B) for 124 men with various clinical stages of prostate carcinoma: T1, T2, T3 (all N0M0), N+ (T1-4 N+M0), M+ (T1-4 NxM1), and of 1659 men without prostate carcinoma.

FIGURE 1 B shows Box Whisker plots of the F/T ratio of the same group. It can be seen that considerable overlap between stages exists regarding total serum PSA as well as F/T ratio. Remarkably, the most explicit difference is appreciated between benign and malignant disease. The median value for the F/T ratio of benign men was 0.28. For the distributions of total serum PSA values and the F/T ratio, significant differences were found between benign and malignant disease (any stage), and between any T-category and nodal disease. There was NO significant difference between systemic metastatic disease and any of the T-categories for the F/T ratio, but regarding total serum PSA this was only so for the T3-category ($p = 0.06$). Redistribution of patients was done in FIGURE 2 A-B, in which clinically prostate confined disease (T1-2) and locally extended disease (T3-4) were grouped. Total serum PSA (A) and the F/T ratio (B) are depicted in Box Whisker plots. Significant differences were found between the distributions of locally confined and locally extended disease for PSA values ($r = 0.02$), but not for the F/T ratio ($r = 0.08$). FIGURE 3 A-B shows the distribution of total serum PSA (A) and the F/T ratio (B) for various grades in 122 men with a prostate carcinoma. Also a Box Whisker plot for 1659 men without prostate carcinoma is given. There was a significant difference between benign and malignant disease for both total serum PSA and the F/T ratio. For the distributions of total serum PSA and for the F/T ratio among men with prostate carcinoma, a significant difference between the various grades was obtained regarding G1 versus G2 or G3, but NOT between G2 and G3.

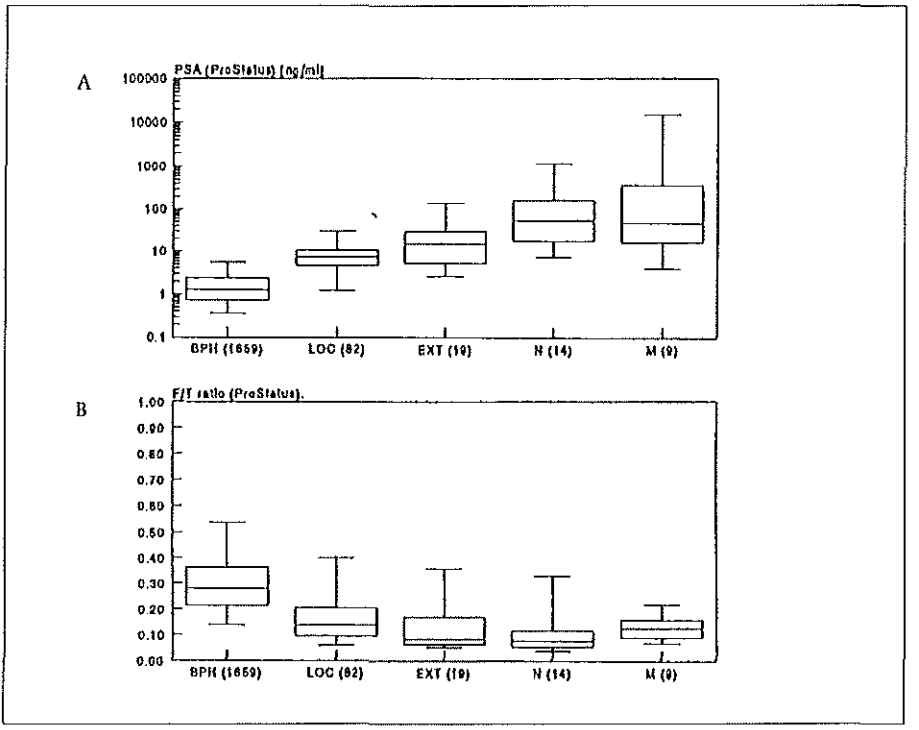

FIGURE 2

Box Whisker plots of total serum PSA (A) and F/T ratio (B) for 124 men with various clinical stages of prostate carcinoma: stages T1-2 (all N0M0) are represented by LOC, and clinical stages T3-4 (all N0M0) by EXT.

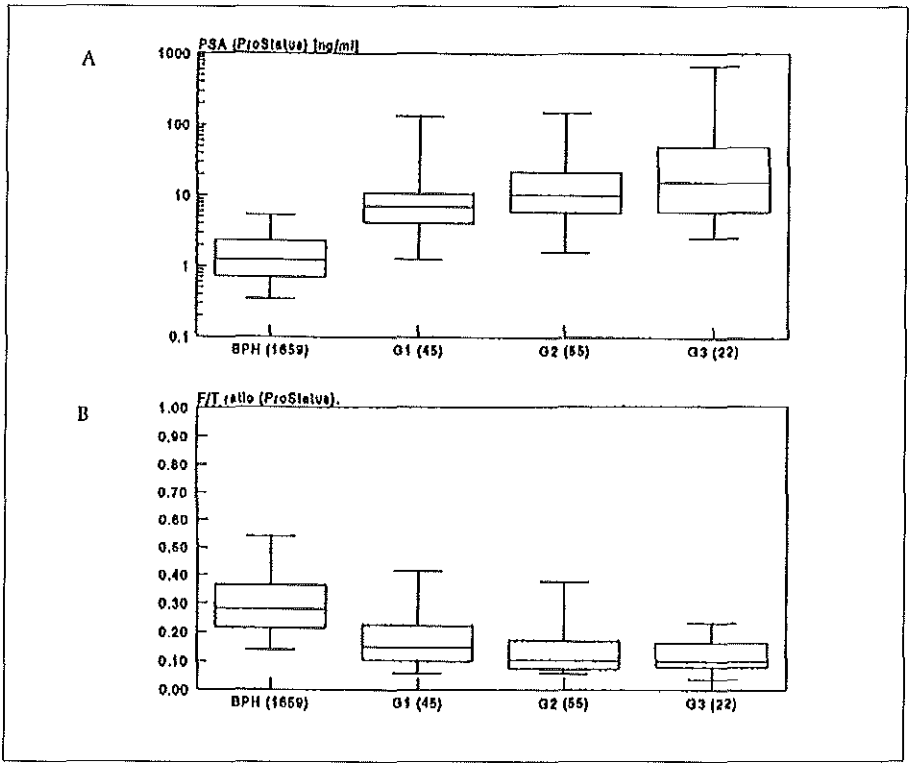

FIGURE 3
Box Whisker plots of total serum PSA (A) and the F/T ratio (B) for various grades in 122 men with a prostate carcinoma, and for 1659 men without prostate carcinoma.

FIGURE 4 illustrates the distributions of the F/T ratio for the various pathologic stages (A) and grades (B) of 49 men who underwent radical prostatectomy is given. Two men appeared to have nodal micrometastases in the paraffin embedded sections after a negative peroperative frozen section of the lymphenodes. In this group of 49 men clinical understaging was seen in 23 patients, and overstaging in 1 patient. Only a limited number of patients with extended disease was found, so no statistical procedures were performed to show differences between various stages and grades. The Box-Whisker plots illustrate distributions of the F/T ratio similar to clinical stage and grade.
FIGURE 5 shows the serial PSA determinations of 12 men with untreated prostate carcinoma. Five of them had histologically proven lymphnode metastases, and 7 clinically confined disease. Their follow-up varied between 2 and 18 months. The performance of the total serum PSA over time is compared to F/T ratio. Patient number 1, 10, and 12 had Grade 3 prostate carcinoma, patient number 5 and 7 Grade 1, and the other patients Grade 2 disease. Metastatic disease showed overall higher PSA values than confined disease. Graphs of the F/T ratio did not show clinical useful information compared to total serum PSA.
In 44 patients who underwent radical prostatectomy 126 pre- and postoperative PSA values were reassessed. In 37 asymptomatic men with normal physical examination

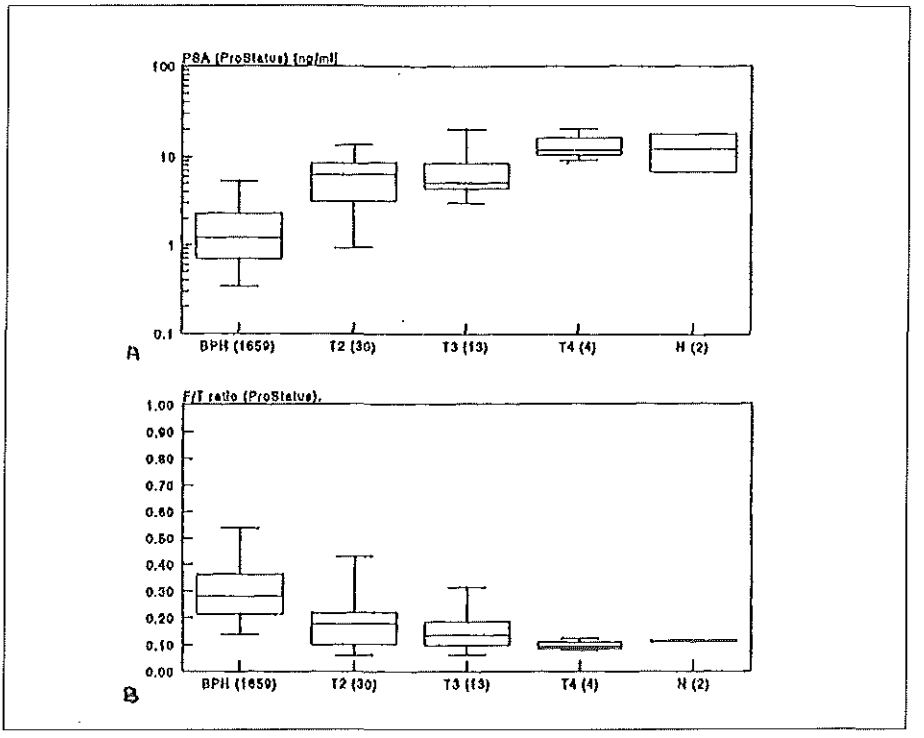

FIGURE 4
Box Whisker plots of F/T ratio for various pathological stages (A) and grades (B) in 49 men after radical prostatectomy.

37 sera, drawn between 3 and 12 months postoperatively, had become less than 0.1 ng/ml (Abbott IMx). The total PSA measured by the ProStatus-TM PSA Free/Total varied between 0.01 and 0.16 ng/ml, and free PSA varied between 0.01 and 0.09 ng/ml. In 14 of these 37 sera the free PSA was undetectable (< 0.01 ng/ml), and the F/T ratio was not calculated. For the other 23 sera the F/T ratio varied between 0.34 and 0.01.
In 7 patients postoperative PSA values remained more than 1.0 ng/ml, indicating residual disease. In three of them metastatic disease was diagnosed later on by bone scan. FIGURE 6 shows their serial total PSA measurements (ProStatus), and the F/T ratio. The F/T ratio remained less than 0.15, except for two patients (number 4 and 5), who showed metastatic disease later on.

DISCUSSION

In this study of 123 clinically staged patients the F/T PSA ratio was compared to total serum PSA for its ability to stage prostate carcinoma. It was seen that total serum PSA distinguished better between confined and non-confined disease compared to the F/T ratio. However, total serum PSA as well as the F/T ratio varied considerably among stages and grades.
The interpretation of PSA among various stages in this study is biased by the uneven distribution of grades (TABLE 1). Almost 80 % of Grade 1 tumours is confined to the prostate (low volume), while this is, respectively, 50 % and 30 % for Grade 2 and 3

tumours. In similar groups of selected clinical patients such distributions are not uncommon. The number of patients is too small to analyse for grade among various stages. These distributional effects may be responsable for the finding that the F/T ratio differed significantly between men with local disease versus men with nodal metastases, while no significance was seen between men with local disease versus men with systemic metastases (FIGURE 1B).
Staging by PSA only [12] or in combination with biopsy grade [3] has shown previously, that a considerable overlap exists between various stages of the disease. For clinical purposes often the most important differentiation is that between

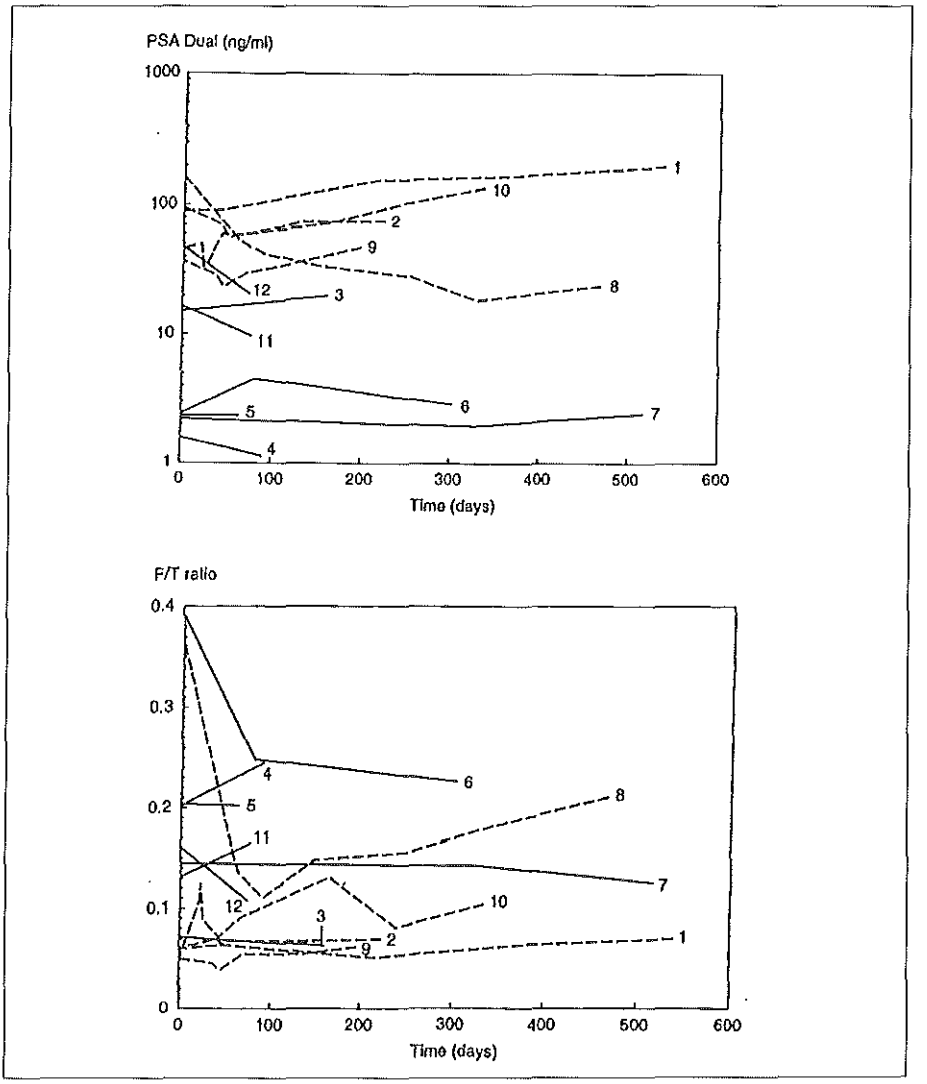

FIGURE 5 A-B
Serial measurements of F/T ratio and total serum PSA for men with clinically confined PCa (N = 7, continous lines) and men with lymphnode metastases (N+M0, N = 5, dashed lines).

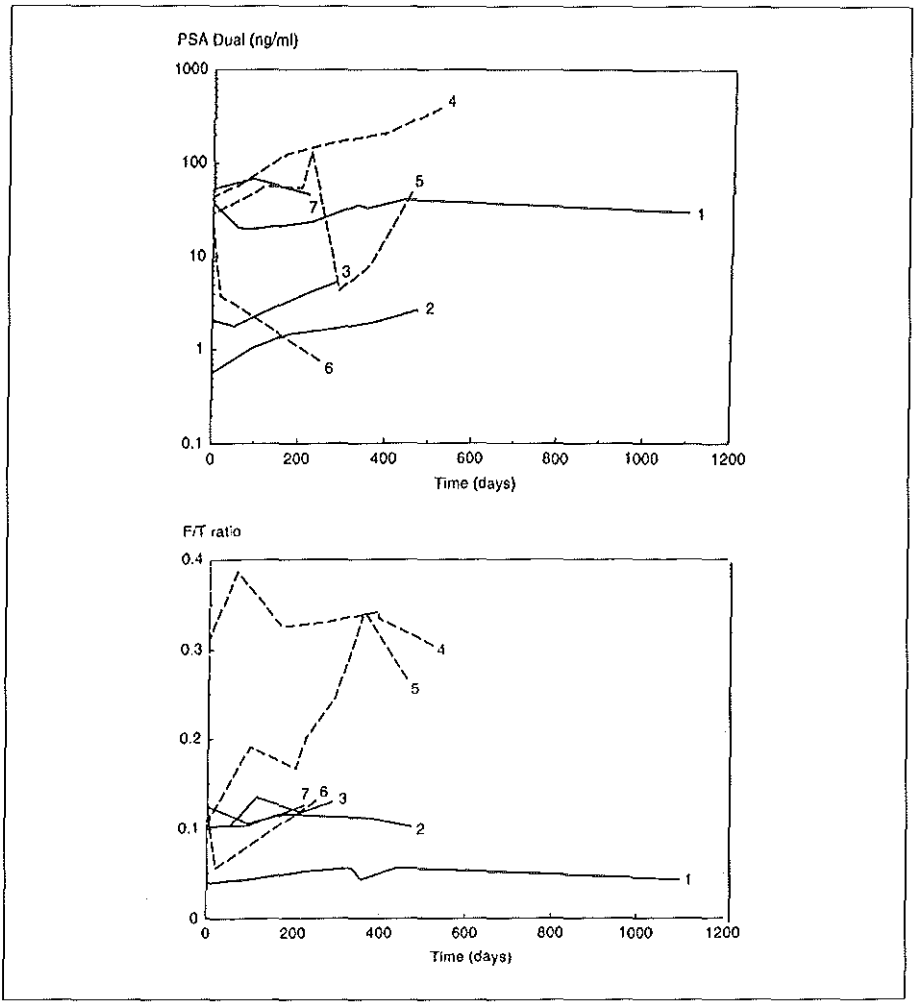

FIGURE 6

Serial measurements of postoperative total serum PSA and F/T ratio in 7 patients with residual disease after radical prostatectomy (continuous lines N = 3 bone scan positive, dashed lines N = 4 no evidence of metastasis).

locally confined and metastatic disease, to be able to stratify patients for lymphnode dissection.

Tumour volume is the predominant factor influencing serum PSA. In this series it has not been possible to compare PSA to tumour volume by means of measurements of pathology specimens. Therefore stage probably represents tumour volume best in this material.

The median total serum PSA increased with increasing stage (FIGURE 1 A) and grade (FIGURE 3 A), like in other studies. Partin et al. reported on the correlation between grade and total serum PSA: an overall positive correlation between pathological Gleason score and serum PSA was obtained (r = 0.30) in 350 men who underwent radical prostatectomy for clinically localized carcinoma [3]. When corrected for

tumour volume, a negative correlation (r = -0.37) was calculated. Regarding the F/T ratio it can be seen that its median value does not parallel stage or grade as well as PSA does (FIGURE 1, 3). The F/T ratio differentiates well between benign and malignant disease [10], but not between local and metastatic disease, or locally confined and locally extended disease (FIGURE 1B, 2B).

The F/T ratio is a mathematical ratio, and its application is based on the observation that free and complexed PSA forms appear in different concentrations in men with prostate carcinoma compared to men without. The biochemical pathway of the complexation of free PSA to ACT is not clear. It is unlikely that the ratio between free and bound PSA forms is determined only in the serum. In all patients, whether with or without carcinoma, an excess of ACT is available in the serum. There is no obvious reason why the equilibrium in the reaction between free PSA and ACT is different between these two sets of men.

Before PSA enters the circulation, it has to diffuse through a prostate-blood barrier, which theoretically may be different between benign and malignant disease. As in tumours a relative hyperaemia due to neovascularisation may exist, and relatively less epithelial cells are in direct contact to an exocrine glandular lumen, the amount of PSA leaking to the circulation may be larger than in benign disease, resulting in a higher total serum PSA. If the prostate-blood barrier in case of malignancy would be disturbed, it is not likely that the free PSA concentration in blood would be lower than in benign prostates: the smaller molecule of free PSA (circa 33.000 Dalton) is expected to diffuse relatively more easily to the circulation than the larger molecule of PSA bound to ACT. This is a simplification of a complicated process influenced by the physical and electrochemical characteristics of the components. However, it supports the suggestion that the F/T ratio is determined by intracellular processes. Björk et al.[13] reported that in well differentiated prostatic epithelial tumour cells ACT can be shown by immunohistochemical staining, while this is less so in the glandular acini in nodules of benign prostatic hyperplasia. Both groups of cells stained well for PSA. In poorly differentiated (high Gleason score) prostate cancer cells a double immunostaining technique for PSA and for ACT showed a larger variation in staining intensity and overall less frequent staining compared to well differentiated (low Gleason score) tumour cells. Also the balance between PSA and ACT staining was more variable. In situ hybridization techniques detected ACT and PSA transcripts in the cytoplasm of the cancer cells, but no transcripts for ACT were found in BPH nodules. This supports the idea that the formation of ACT, and PSA complexed to ACT is increased in tumour cells, most likely due to an alteration in cell metabolism.

Also from clinical studies there are several arguments to speculate on an intracellular mechanism which is responsible for the F/T ratio. In a study of 1726 men, of whom 67 with prostate cancer, we reported on significant increases of total serum PSA, free PSA, and PSA complexed to ACT (PSA-ACT) in men with prostate cancer, compared to normal men [10]. As a result the F/T ratio was decreased in those men with prostate cancer, indicating that the relative concentration of PSA-ACT had been increased more than the free PSA concentration in serum. This might be due to the intracellular overproduction of the PSA-ACT, as indicated by the mentioned histochemical observations. In another study of the same population we reported on the correlation between various forms of PSA, ultrasonic volume parameters, and age (TABLE 2) [12]. Free PSA showed a larger correlation (r = 0.62) to the ultrasonic prostate gland volume than total serum PSA had (r = 0.54), especially for men with a prostate cancer (r = 0.46 versus r = 0.11). This means that the volume of the normal tissue in a gland with a malignancy influences the value of the free

TABLE 2
Results of univariate regression analysis; $p < 0.01$ [12]

Correlation	*LogFreePSA Total*	*Volume gland*	*Volume inner zone*	*Age*
n=1542 benign				
Log PSA	0.87	0.54	0.41	0.16
Log FreePSA		0.62	0.45	0.22
Volume Total gland			0.74	0.23
Volume inner zone				0.22
n=61 PCa				
Log PSA	0.81	0.11	0.08	0.12
Log FreePSA		0.46	0.32	0.31
Volume Total gland			0.70	0.26
Volume inner zone				0.24

PSA more compared to total serum PSA. The intracellular overproduction of PSA-ACT and its subsequent leaking to the blood might be the main reason why the total serum PSA is increased. The ratio between the various forms of PSA in the serum might not correspond to their intracellular ratio, as the contribution of PSA from normal cells of the diseased prostate gland alters the relative proportions. The distribution of F/T ratio values of prostates containing a malignancy is thus expected to overlap the distribution of F/T ratio values of normal prostates.
Illustrations of serial measurements of the F/T ratio in untreated prostate carcinoma did not show obvious differences between curves of metastatic and confined disease, while this was clearly observed in the curves of total serum PSA (FIGURE 5). This illustrates the idea that the F/T ratio is not supportive for clinical staging.
Further clinical applications of the F/T ratio were sought in the detection of recurrences after radical prostatectomies, but are not found in this series. The F/T ratio in the total PSA range below 0.3 ng/ml and the detection limit of 0.1 ng/ml (ProStatus) was too variable. Due to small variations in the free or the total PSA, the F/T ratio may fluctuate enormously. After radical prostatectomy PSA levels should be undetectable. Minimal levels of detection of serum PSA normally may range among different assays from < 0.3 ng/ml to < 0.1 ng/ml. The undetectable total serum PSA levels measured by the Abbott IMx assay corresponded to PSA levels less than 0.3 ng/ml measured by the ProStatus F/T assay.
An increase of PSA is evidence of residual disease or recurrence. We considered 7 patients with a total serum PSA of 1.0 ng/ml or more (IMx) to have recurrent/residual disease after radical prostatectomy. It was seen that the F/T ratio in most cases was

less than 0.15, which may support the diagnosis of a malignant source of the raise in serum PSA. The limited number of patients does not allow for further conclusions, but differences in clinical staging were not obvious from the differences in graphs between biochemical and clinical recurrences.

CONCLUSION

The F/T ratio has no additional value in clinical staging of prostate carcinoma compared to serum PSA. The F/T ratio does not give clinically useful information compared to the total PSA in serial measurements for the follow-up of untreated carcinoma. In the serum evaluation after radical prostatectomy the F/T ratio is not an adequate marker for recurrence.
The F/T ratio may be considered the result of cell differentiation, and not a parameter of tumour load.

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SUMMARY AND CONCLUSIONS

Prostate cancer in most European countries is the second most frequent cancer in males and the second most frequent cause of cancer death. Prostate specific antigen is an important marker which relates to many aspects of this disease. It has been shown that PSA is helpful in the early diagnosis of prostate cancer and in this respect is more sensitive compared to the other available tests, namely rectal examination and transrectal ultrasonography. PSA is also helpful in staging of locally confined disease. It can be used to identify or exclude local extension of disease, if combined with T-category and grade of differentiation determined on biopsy. The same parameters also give an indication of the presence of lymphnode metastases, which may prevent unnecessary and invasive staging procedures in certain groups of patients with favourable prognostic factors and a low PSA value. PSA is less suitable as a marker for metastatic disease. Progression of untreated prostate cancer in various stages can be monitored by PSA. The true value of the marker in this respect is still underexplored. It may be possible that PSA will be shown to differentiate effectively between progressive and non-progressive disease. In this respect, it could become an essential tool to identify those patients that may not require treatment at all. PSA is also a useful marker for therapy response. An elevation of PSA after radical prostatectomy indicates local or metastatic progression, which will occur within 1-2 years. PSA is an androgen dependent enzyme and decreases under endocrine treatment. It is unexplained why in spite of its endocrine dependent character, PSA rises with endocrine independent progression of prostate cancer.

For the determination of serum PSA various immunoassays may be used. The variety of their design, and the developing concept of PSA complexation by serumproteins, have led to the world-wide confusing situation in which, so far, no universal serum PSA standards have been accepted. This has been illustrated in chapter 2.4 (Part 1), in which six different PSA assays were used to describe the dilemmas with which the physician may be confronted in nowadays clinical practice. The PSA determinations of different laboratories of an identical patient may contradict each other due to wide variability in results by different assays. For the usual range of PSA between 0 and 20 ng/ml the actual difference between PSA results may be up to approximately 85 %, even if no biological factor is of influence. Differences between assays were also seen after radical prostatectomy. The variability of PSA detectability might lead to different conclusions in an identical patient. In case of detectability the physician might presume residual disease or recurrence, while the inability to detect PSA induces the opposite. These results are unlikely to cause practical problems for as long asymptomatic PSA recurrence remains untreated. The overlapping distribution of serum PSA values between benign and malignant disease increases the confusion, as no absolutely normal, or with respect to cancer 'safe', PSA values can be given. The interpretation of PSA values can only be done when combined with the patients history and physical examination by digital rectal examination.

Early detection of prostate carcinoma might provide a way to detect confined prostate carcinoma in a treatable stage. The combined application of PSA and DRE appeared more effective for detection than each of these alone. Transrectal ultrasonography as a tool for detection of ultrasonic lesions did not attribute to these great extent to detectability, but added in conducting guided prostate biopsies of hypoechogenic lesions and at random. Furthermore it offered the possibility of volumetry of the prostate, and subsequent adjustment of the serum PSA for that fraction of PSA which is predominantly produced in adenomas. The reliability of transrectal ultrasonic volumetry of the prostate gland and its innerzone, harbouring the adenomatous tissue, therefore had to be analysed.

In chapter 3 the in-vivo and in-vitro reproducibility of 5 mm step section planimetry, and various methods using caliper measurements to calculate the estimated volume, are described. For the total gland the in-vivo intra-observer variation was up to 11 % for planimetry, and 19 % for the prolate spheroid method. The mean differences between two observers were less than 3 %, and not significantly different from zero. The standard deviation of the mean differences between observers was for total planimetric volume 13 %, and for total prolate spheroid volume 16 %. This means that for serial measurements of the prostate gland a subsequent planimetric change of 26 % or more can be considered as a real change. For volumetry of the inner zone the in-vivo intra-observer variation was up to 10 % for planimetry, but 27 % for the prolate spheroid method. The mean difference between two observers was for the planimetric volume 7 %, and statistically not different from zero; for the prolate spheroid volume, however, this was 32 %, and statistically different from zero. The standard deviation of the difference between two observers was 20 %. Therefore a change in planimetric volume of the inner zone on a subsequent measurement should be 40 % or more to be considered significant.
Because of these results planimetry is regarded as the volumetric method of choice for follow-up of patients by TRUS. The variation of this method is apparantly considerable, and an effort has been made to explain this by a computer model of planimetric measurements of the prostate. In this model the shape of the prostate is simplified to the ellipsoid, which also is the base of the volumetric formulas using caliper measurements. In chapter 4 this model analyses various effects of interference between the transrectal ultrasound probe and the prostate gland. These are described as the salami-effect, the capsizing-effect, and the first-step-effect. The combined error of the salami-effect with the step-section technique is in our model 12 % at maximum, and relativily larger in shorter ellipsoids. The capsizing-effect caused only 2-7 % loss of volume, predominantly in longer ellipsoids. Missing steps in the first step effect induced a loss of volume up to 11 %. This was applied to an in-vivo study, and it was seen that missing steps occured in 15 % of the study population. It is therefore advised to check the position of the transrectal probe in the longitudinal plane at the moment of starting planimetry.
The availability of several methods of volumetry within the population of the Rotterdam feasibility study for screening prostate cancer created the possibility to analyse these methods with each other on a large scale. It provides information concerning alternatives for the standard, but time consuming, method of planimetry. As long as the place of prostate volumetry with respect to calculating PSA density values is not definite, the accuracy of the various volumetric methods is of interest. In chapter 5 total prostate volume and inner zone volume were measured in 716 men by semiplanimetric and caliper formula methods, and compared with step section planimetry as the golden standard. The elliptic volume

(width x height x length x 0.52) was more accurate than the prolate spheroid volume (width x width x height x 0.52) of the total gland, as the correlation coefficient between total elliptic volume and planimetry was higher compared to prolate spheroid volume (0.89 versus 0.83), and the standard deviation of the mean volumetric difference smaller. The mean total prolate spheroid volume resembled the mean total planimetric volume better than elliptic volume did, as the mean volumetric difference was smaller. For measurement of the inner zone volume the prolate spheroid volume was more accurate than the elliptic volume. The correlation coefficient between length and planimetric volume was similar to that of width and height, which accounted for more accuracy of the elliptic volume than of prolate spheroid volume in larger prostates. This was in fact also observed in the in-vivo study of chapter 4 concerning missing steps in planimetry. The elliptic formula method parallels planimetry for incidental volumetric measurements of the total gland between different individuals, for example in preoperative evaluation or screening studies.

Ultrasonic volumetry normally is used in the calculation of volume adjusted PSA values, and numerous reports concluded that in selected outpatient populations these values could differentiate better between prostate carcinoma and benign disease than serum PSA only. We therefore applied these calculations to our community based population, and hoped that these adjusted PSA values could improve differentiation especially in the grey area of serum PSA range up to 10 ng/ml. In addition to already known calculations of whole gland PSA-density (PSAD) and PSA-density of the inner zone (PSAT), a mathematical method is described in chapter 6 to obtain optimal adjustment with both total gland and inner zone volumes in the same formula. Therefore extrapolation of inner zone volumina measurements was performed to calculate the volume of the peripheral zone, and to estimate its contribution per ml to the serum PSA. For each method of volumetry a formula was derived, which calculated the expected PSA, estimated from the volumetric measurements, compared to the actual serum PSA. These formulas are specific for this study population. However, this optimal method of volume adjusted PSA was only sligthly better than just serum PSA in differentiating between cancer and non-cancer. This was illustrated by Receiver Operator Characteristic curves, including all volumetric methods and age as parameters. For practical purposes in a screening protocol, volume adjusted PSA values did not appear useful. Volume adjusted values combine the analytical and biological variation of both PSA and prostate volume, and this might be the main cause of its marginal improvement of cancer detection in community based populations compared to serum PSA.

In Part 4 a different way to study serum PSA determinations for detection of PCa is shown. When screening studies mature, more information will be obtained concerning serum PSA determinations over time. Instead of single values of PSA, serial measurements will be analysed for the detection of prostate carcinoma. From a mathematical point of view, changes of PSA measurements of an individual patient are best interpreted in relation to former measurements, and therefore as a percentage of the baseline value. In that way PSA increase can be expressed as PSA doubling time.
In practice, also absolute serum PSA increases (as illustrated by the PSA velocity of ng/ml/year) seem to be useful to discriminate between cancer and non-cancer. Data are premature, and so far PSA doubling time nor PSA velocity have shown to be effective for predictions in individuals. Cross sectional studies and limited cohort

studies of community based populations have shown that serum PSA shows an annual increase of approximately 2 % in normal men. Next to this, there are several reports indicating that it does not seem to be useful to compare PSA measurements annually. A single baseline PSA determination offers more indication for hidden malignant disease than an early repeated one, and the normal annual serum PSA increase does not overshoot the analytic variance of 5-10 % of the PSA assay. It therefore seems reasonable to repeat serum PSA determinations once every four years, as is done in the European Randomised Study of Screening for Prostate Carcinoma. Little is known concerning serum PSA in respect to the natural history of prostate carcinoma. The question whether serum PSA parallels or even precedes clinical tumour progression in groups of patients and in individual cases stays centrally. This is illustrated for patients with confined prostate carinoma in chapter 9. So far no individual prediction can be done between PSA increase and clinical progression. This study has the obvious limitation of a selected patient population with only grade 1-2 carcinomas, a short follow-up, and the rather subjective end-point of clinical progression parameter measured by digital rectal examination. In a similar study in our hospital the PSA doubling time differed between groups of patients staged T1-4N1M0 with and without metastatic progression on the bone scan. Mean PSA doubling time in men with prostate carcinoma staged T1-4NxM0G1-2 appeared to be 59 months, in T1-4N1M0 42 months, and in T1-4N1M1 13 months.

Part 5 discusses the use of serum PSA in combination with preoperative Anderson biopsy grade to predict peroperative frozen section results of pelvic lymphnodes independent of clinical staging. Based on this prediction, and accepting a 5 % chance on positive frozen section results, 1 in 214 patients (0.4 %) would have undergone radical prostatectomy for micrometastases in the lymphnodes. This in addition to the 4.7 % of patients in whom the frozen section results were false negative compared to the definite histology. In the clinical situation, in which frozen section always is done peroperatively to decide on proceeding to radical prostatectomy, the prediction is time saving. After the bilateral pelvic lymphnode dissection, which is routinely performed for adequate staging, no time has to be wasted to await the frozen section results in at least 17 % of the patients. The prediction is also helpful in planning these patients for the operation room time schedule.

In Part 6 the value of the fractions of the various forms of PSA in serum is discussed. For selected groups of patients the ratio between free PSA or PSA complexed to α-1-antichymotrypsin (ACT) to the total serum PSA had shown to contribute to the differentiation between benign and malignant prostates. In chapter 11 the free to total ratio (F/T ratio) is determined by the second generation DELFIA ProStatus PSA F/T and EQM assays in a screening population of 1726 men between 55 and 74 years, including 67 men with a prostate carcinoma detected by DRE, TRUS, and PSA. The median F/T ratio value for men without prostate carcinoma was 0.28, and for men with prostate carcinoma 0.12. The F/T ratio improved the specificity of total serum PSA for the detection of prostate cancer only minimally: in the PSA range of 10.0 ng/ml and more the specificity was increased, however in the PSA range between 4.0 and 10.0 ng/ml there was no significant benefit. The optimal differentiation between benign and malignant disease in this study was not obtained by the F/T ratio, but by a more complex mathematical relation between free and total PSA.
In chapters 12 and 13 the role of various parameters for the detection of the carcinomas in the same group of men is analysed. The most important determinants

for a positive biopsy were total serum PSA and DRE, whereas TRUS as a single screening modality detected only 6 % of the carcinomas. In a simulated case selection for biopsy the F/T ratio, the PSA-density, and age specific PSA reference ranges were tested for their ability to improve the specificity of PSA for the detection of prostate cancer. Increasing the specificity aims to reduce the number of biopsies and/or the number of time consuming and cost-ineffective screening procedures. Age specific PSA reference ranges compensate for the increase of serum PSA with increasing age in men with normal prostates. In combination with DRE in the simulation, these would reduce the number of biopsies with circa 35 %. However, also the number of carcinomas detected would decrease with 11 %. Identical results were seen, when the F/T ratio of 0.20 or lower, or a PSA-density of 0.12 ng/ml/cc or more would have been used in addition to a PSA value of 4.0 ng/ml or more, or an abnormal DRE as indicators for biopsy. The loss of cancer detection was between 3 and 6 % when TRUS was omitted completely as a screening modality, or when a prescreen PSA value of 2.0 ng/ml would have been used. The reduction in biopsies would have been 17 % and 30 %, respectively. The value of the F/T ratio for the PSA range below 4.0 ng/ml could not be assessed adequately, as not all men were biopsied. It was estimated that a threshold of 0.20 in the PSA range of 2.0 ng/ml and more would induce an increase in the number of biopsies of 64 %.
In chapter 14 the F/T ratio is compared to serum PSA for its ability to differentiate between various stages and grades in 128 men with untreated prostate cancer. The distribution of F/T ratio values did not differ significantly between local and metastatic disease, in contrast to those of PSA values. In a small group of these men, and in men with a PSA recurrence after radical prostatectomy serial measurements were illustrated. Furthermore, in 37 men without PSA recurrence after radical prostatectomy the sera were analysed. Overall, the F/T ratio did not show any advantage over the use of total serum PSA. The F/T ratio appears to be the result of the intracellular metabolism, which is altered in case of a malignancy. The abundant formation of intracellular PSA complexed to ACT seems to play a central role in the difference of the F/T ratio between benign and malignant prostates. So far, differentiating between benign and malignant disease is the most important application of the F/T ratio.

This thesis discussed the use of PSA and TRUS for the detection and follow-up of prostate carcinoma. Emphasis was laid on how to use serum PSA for these purposes, and various efforts to improve this use. This thesis therefore is a reflection of the continuous developments in this field of investigation. The main value of the separate studies is for the early detection of prostate cancer. With the present standards of prostate volumetry and serum PSA assays, the effectiveness of the biopsy protocol in screening community based populations can only very marginally be improved by volume adjusted PSA values, or determination of the F/T PSA ratio.

The general physician is being confronted with the question whether a patient is helped by knowing his number (of PSA). Is it useful to measure serum PSA?
In assessing men with urinary complaints serum PSA will be measured, though only a minority of the men with a prostate carcinoma is symptomatic. However, missing a prostate cancer may have serious (including legal) consequences. It is always needed to perform a physical examination including a digital rectal examination. Suspicious results have to be investigated by an urologist to exclude prostate carcinoma.

Due to public awareness about PSA, the individual patient might force the general physician to determine serum PSA, even in the absence of complaints. The physician needs to know that serum PSA cannot exclude prostate carcinoma, but that a low value in the absence of suspicious DRE makes the presence of a clinically important prostate cancer highly unlikely. Much doubt may exist concerning the interpretation of the DRE [1], which makes referal to an urologist advisable. Approximately 15 % of detected carcinomas in a community based population is palpable and has a serum PSA below 4.0 ng/ml.
Case-finding through the measurement of PSA in asymptomatic men by the general physician or by insurance companies introduces a way of screening which is presently firmly criticized in The Netherlands as long as the European Study of Screening for Prostate Carcinoma has not been completed.
It is not known yet whether early detection of prostate cancer improves the health status of the asymptomatic men in the community.

PSA and TRUS will develop in the near future. Various PSA-isomolecules and serum protein complexes will be assessed. Ultrasonically derived parameters of images of the prostate will be evaluated for their tissue specificity. New techniques, like polymerase chain reaction (PCR), to detect and follow prostate carcinoma by serummarkers are available. It is likely that it remains a busy time.

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NEDERLANDSTALIGE SAMENVATTING

PROSTAAT SPECIFIEK ANTIGEEN EN ECHOGRAFIE VOOR DE DETECTIE EN HET VOLGEN VAN PATIËNTEN MET PROSTAATCARCINOOM

Prostaatcarcinoom is in de meeste Europese landen het op een na meest voorkomende kwaadaardige gezwel bij mannen, en de op een na meest voorkomende oorzaak van overlijden ten gevolge van kanker.
Prostaat specifiek antigeen, afgekort PSA, is een eiwitprodukt van de epitheliale cellen van de prostaat. Het wordt uitgescheiden met het zaad, en laat dit na enkele minuten vervloeien. Onafhankelijk daarvan lekt een klein gedeelte naar de bloedbaan. Het PSA-gehalte in het bloed kan in het laboratorium uit een bloedmonster bepaald worden. De PSA-concentratie in het bloed kan ten gevolge van goedaardige condities, zoals prostaatvergroting, verhoogd zijn, maar zo'n verhoging kan ook bij prostaatkanker voorkomen. Het PSA wordt daarom een tumorindicator genoemd, zonder dat het specifiek is voor prostaatkanker. Met behulp van het PSA en het voelen van de prostaat (rectaal toucher) kan men prostaatkanker op het spoor komen.
Soms kan men deze afwijkingen in de prostaat zichtbaar maken door middel van echografie. Hierbij wordt met ultrageluidsgolven via een sonde in de endeldarm de prostaat zichtbaar gemaakt (transrectale ultrasonografie, afgekort: TRUS). Niet alleen het weefsel-aspect kan worden bekeken, maar ook de afmeting van de prostaat kan gemeten worden, hetgeen kan helpen bij bepaalde klinische beslissingen. Schatting van het volume met echografie is nauwkeuriger dan met het rectaal toucher.
Kwaadaardige afwijkingen kan men alleen met zekerheid vaststellen door een punctie uit de prostaat te verrichten op geleide van het echobeeld, en het weefsel onder de microscoop te onderzoeken. De bevindingen die leiden tot het verrichten van zo'n biopsie dienen zo specifiek mogelijk te zijn, zodat zo min mogelijk mannen zo'n onderzoek ondergaan voor een goedaardige afwijking. Aan de andere kant is het uiteraard wenselijk zo min mogelijk carcinomen onopgemerkt te laten.
In dit proefschrift wordt met name behandeld hoe de bestaande middelen om prostaatkanker op te sporen gebruikt kunnen worden om mannen voor een biopsie te selecteren.

In hoofdstuk 1 (deel 1) wordt een overzicht gegeven over de productie van PSA, hoe het gemeten kan worden, en wat dat voor de praktijk betekent. Veel daarvan staat ook in het Nederlands in Appendix 1. PSA is gevoeliger dan het rectaal toucher of de echografie in het opsporen van een prostaatcarcinoom. Bij een bestaand carcinoom is PSA in beperkte mate in staat de uitgebreidheid van het gezwel aan te geven. Zelfs in combinatie met andere gegevens is er echter nooit een volledige zekerheid te geven over het stadium van tumorgroei en de mogelijkheid om het gezwel bij operatie geheel weg te nemen. In bepaalde gevallen is een hoge waarschijnlijkheid

voor het bestaan van uitzaaiingen te geven. Na een volledige prostaatverwijdering behoort het PSA gehalte in bloed zo laag te zijn dat het met normale laboratorium tests niet meer waarneembaar is. Als de kwaadaardigheid echter soms na jaren weer de kop op steekt, dan is het meestal het eerst in het bloedonderzoek te merken. Bij onbehandeld prostaatkanker loopt het PSA-gehalte meestal gelijk op met de groei van het carcinoom. Ten gevolge van hormonale behandeling voor prostaatkanker daalt het PSA-gehalte veelal, omdat de produktie van PSA beïnvloed wordt door mannelijke hormonen. Voor de bepaling van het PSA in bloed zijn verscheidene tests beschikbaar. Deze berusten alle op het principe dat het PSA herkend wordt door een antistof tegen een gedeelte van het PSA-molecuul, en vervolgens na herkenning een merkstof aangehangen krijgt om de hoeveelheid van deze combinatie vast te stellen. De variatie in uitslagen tussen verschillende tests wordt weergegeven in de figuren in Appendix 1.
In hoofdstuk 2 worden de problemen besproken die deze tests met zich meedragen. Vanwege allerlei factoren is het tot nu toe niet gelukt om een wereldwijde eenvormige test te maken, of om (bloed)monsters te produceren die als ijking voor de PSA-bepaling kunnen dienen. Daarbij komt, dat door nieuwe inzichten over het PSA ontwikkelingen van enkele jaren geleden al weer sterk verouderd zijn. Het blijkt dat een deel van het PSA in bloed zich bindt aan andere eiwitten, waardoor een gedeelte van het PSA door enkele testen minder, en een gedeelte zelfs helemaal niet waar te nemen is. De mogelijkheid om de verhouding te meten tussen het vrije ongebonden PSA, en het gebonden gecomplexeerde PSA (de F/T ratio) levert echter ook een voordeel op, omdat het in bepaalde situaties de gevoeligheid om prostaatkanker vast te stellen vergroot. Bij het beoordelen van het PSA is het derhalve van belang te weten met welke PSA-test men van doen heeft, om zodoende de bevindingen bij onderzoek van de patiënt goed te kunnen interpreteren.

Opsporing van prostaatcarcinoom in een vroeg stadium zou kunnen leiden tot eerdere en meer effectieve behandeling. Het is gebleken dat het combineren van PSA-gegevens met het rectale onderzoek effectiever is dan elk van beide onafhankelijk om mannen te identificeren die verdacht zijn voor het hebben van prostaat kanker. Het echo-onderzoek is hierbij veel minder van belang, en wordt voornamelijk gebruikt om de naald, die gebruikt wordt voor de prostaatbiopten, nauwkeurig te kunnen richten. Verder kan echografisch het prostaatvolume gemeten worden. Grote prostaten maken meer PSA dan kleiner, en om een hoog PSA-gehalte in bloed ten gevolge van kanker te onderscheiden van een hoge productie door veel normaal prostaatweefsel, wordt het PSA gecorreleerd met het prostaatvolume. De kwaliteit van volumemetingen (volumetrie) met behulp van de transrectale echografie, behoort dan wel bekend te zijn. Aspecten daarvan werden door ons onderzocht en in de praktijk getest, wat in deel 2 beschreven staat. Bij de volumemetingen werd zowel de grootte van de hele prostaat als de grootte van het 'binnengebied' van de prostaat bepaald. Dit 'binnengebied' ziet er bij echografie wat donkerder en grover van structuur uit. In dit gebied treft men bij operatie en onder de microscoop meestal goedaardige prostaatvergroting (Benigne Prostaat Hyperplasie) aan. Het is gebleken dat dit binnengebied gemiddeld meer PSA vormt dan het gebied dat aangeduid wordt met de 'perifere zone', die zich meer aan de buitenzijde van de prostaat bevindt. Dit perifere gebied is goed af te tasten bij rectaal toucher, en hierin komen de meeste carcinomen voor.
In hoofdstuk 3 wordt de herhaalbaarheid van de volumemetingen onderzocht. De nauwkeurigheid (dit is: hoe precies een meetinstrument iets kan meten) en herhaalbaarheid (dit is: hoe goed verscheidene achtereenvolgende metingen van

hetzelfde object op elkaar lijken) is uitstekend voor metingen aan balonnetjes in een waterbak (in vitro). In de praktijk van een echografische prostaatmeting ligt dit iets anders, omdat er allerlei storende invloeden zijn. Bijvoorbeeld: de vorm van de prostaat is niet precies rond, zodat er afrondingsfouten gemaakt worden; er onstaan bewegingsartefacten ten gevolge van de ademhaling, of kleine positieveranderingen van het lichaam. Ook laat het echobeeld zich door verschillende onderzoekers niet hetzelfde interpreteren, omdat de grenzen van de prostaat met het omliggende weefsel niet overal even duidelijk zijn. Het prostaatvolume kan in principe op twee verschillende manieren gemeten worden. Bij de planimetrische methode wordt de prostaat in evenwijdige plakjes van 5 mm dik onderverdeeld, en wordt het oppervlak van elk van die plakjes bepaald op het echobeeld. Het volume wordt dan berekend door elk van die oppervlaktes met de plakdikte te vermenigvuldigen, en op te tellen. Bij de tweede methode wordt de vorm van de prostaat vereenvoudigd tot die van een ellipsoid. Daarvan kan het volume berekend worden door gebruik te maken van de lengte, de hoogte, en de breedte, die uit het echobeeld gemeten worden.
Van deze twee methoden bleek uit ons onderzoek de planimetrie het meest geschikt voor volumetrie. Voor elk van beide methoden bleek dat er gemiddeld geen verschil bestond tussen de metingen van twee onderzoekers, die het volume van dezelfde reeks prostaten bepaalden. Bij volumemeting van het binnengebied bleken alleen de metingen met de planimetrische methode niet significant verschillend tussen de onderzoekers. De variatie van de verschillen tussen twee onderzoekers was voor de planimetrische methode het kleinst. Met deze variatie kon berekend worden dat een verschil tussen twee opeenvolgende metingen van de gehele prostaat tenminste 26 % moest zijn om als een waar verschil opgevat te kunnen worden. Voor het binnengebied was dit tenminste 40 %. Indien de metingen door dezelfde onderzoeker verricht werden, was een significant verschil tussen opeenvolgende metingen iets kleiner, maar nog steeds tenminste 22 % voor planimetrie van de hele prostaat, en tenminste 20 % voor het binnengebied.
Vanwege de gemeten verschillen werd vervolgens onderzocht hoe groot de invloed zou zijn van bepaalde storende effecten zoals beweging. Hiertoe werd een computermodel ontworpen, waarbij de prostaat teruggebracht werd tot de vorm van een ellipsoid. Drie mogelijk storende effecten werden benoemd en bestudeerd: het salami-effect, het kapseis-effect, en het eerste-stap-effect, alle visueel weergegeven in hoofdstuk 4.
Bij het salami-effect werd de invloed van een incidentele constant gekantelde positie van het vlak van de planimetriemeting ten opzichte van de optimale lengterichting gesimuleerd. Er werd een maximaal verlies van 11 % in volume waargenomen. Bij het kapseis-effect werd draaiing van de echoprobe ten opzichte van de prostaat gedurende de metingen nagebootst. Hierbij werd een maximaal verlies van 7 % in volume geconstateerd. Bij het eerste-stap-effect werd nagegaan in hoeverre het later herkennen van de prostaat in het echobeeld bij de eerste planimetrische bepaling van invloed was op de volumebepaling. Ook hierbij werd een verlies in volume gemeten, met name als er door het te late herkennen een planimetrische stap verloren ging. Bij een kleine groep mannen werd geschat hoe vaak het verliezen van een planimetrische stap tot vermindering van het planimetrisch volume leidde: dit bleek in 15 % het geval te zijn. De beschreven effecten kunnen verantwoordelijk zijn voor de variatie in volumemetingen van de prostaat, maar zijn niet eenvoudig te verbeteren.

Ofschoon de planimetrie de meest geschikte methode bleek voor volumemetingen van de prostaat, is deze ook de meest tijdrovende, en niet met elk echoapparaat mogelijk. Uit de gegevens van de Rotterdamse haalbaarheidsstudie voor bevolkingsonderzoek op prostaatkanker werden daarom in hoofdstuk 5 gegevens gebruikt over volumemetingen met de andere methode. Daarbij werd gebruik maakt van lengte (L), hoogte (H), en breedte (B). Berekening van het volume (V) geschiedt dan door de formule: V = L x H x B x 0,52 (methode 1) of door V = B x B x H x 0,52 (methode 2, waarbij aangenomen wordt dat de lengte en breedte even groot zijn). De berekende volumes werden vergeleken met het planimetrische volume van de hele prostaat. Het verschil tussen het gemiddelde volume van methode 2 en dat van de planimetrie was kleiner dan tussen methode 1 en planimetrie. Echter, de variatie van het verschil tussen de metingen en de planimetrie was veel groter voor methode 2 dan voor methode 1. Methode 1 gaf een constant kleiner volume weer ten opzichte van planimetrie, en bleek door het gebruik van de lengtemeting meer te lijken op de planimetrie dan methode 2. Methode 1 is goed bruikbaar voor het bestuderen en onderling vergelijken van eenmalige bepalingen van het prostaatvolume in grotere groepen mannen.

Met behulp van echografisch bepaalde volumemetingen kan het serum PSA-gehalte gecorrigeerd worden voor de grootte van de prostaat. Voor geselecteerde studiegroepen van poliklinisch behandelde patiënten bleek de zo berekende PSA-dichtheid (het PSAD, dit is het PSA-gehalte gedeeld door het prostaatvolume) een betere indicatie te geven voor het bestaan van prostaatcarcinoom dan het PSA-gehalte alleen. Daarom werd in deel 3 onderzocht of de PSA-dichtheid ook in een ongeselecteerde groep van mannen zonder klachten (afkomstig uit de haalbaarheidsstudie voor bevolkingsonderzoek op prostaatcarcinoom) meer informatie zou kunnen geven dan PSA alleen. Hierbij werd voor optimale correctie van het PSA ook een parameter samengesteld, die, naast het gehele volume, het volume van het binnengebied van de prostaat in de berekening betrok. Er werd gekeken naar mannen met een PSA-gehalte tot 10 ng/ml (nanogram per milliliter). In deze groep wordt in ongeveer 1 op de 5 biopten een carcinoom gevonden. Bij mannen met een hogere waarde is dat veel vaker het geval, namelijk in ongeveer de helft van het aantal biopten, zodat bij hen altijd een reden voor biopsie bestaat. Het zou aantrekkelijk zijn een manier te vinden waardoor het aantal (overbodige) biopsieën voor een goedaardige prostaataandoening (vier van de vijf in de groep met een PSA-gehalte kleiner dan 10 ng/ml) teruggedrongen kon worden. Bij de analyse werd gebruik gemaakt van zogeheten ROC-curves, die op elk punt van de grafiek een combinatie van de gevoeligheid en de specificiteit weergeven. Het bleek dat geen van de gecorrigeerde PSA-waarden een betere voorspeller was voor de aanwezigheid van prostaatcarcinoom dan het serum PSA-gehalte alleen. Ook in de groep van mannen die reeds geselecteerd waren voor het ondergaan van een biopsie werd geen verbetering van de specificiteit gezien.

Een andere manier om naar PSA te kijken is om een serie PSA-waarden van één patiënt in verloop van de tijd met elkaar in verband te brengen, in plaats van alleen één waarde op één moment te gebruiken (deel 4). Bij groeiende biologische processen, zoals gezwellen, kan het volume van het proces gelijkmatig toenemen over een bepaalde tijdsperiode, maar ook exponentieel. Indien een gezwel PSA zou produceren, zoals bij het prostaatcarcinoom, dan zou de vorming van het PSA parallel kunnen verlopen aan de toename van het volume. De toename van het PSA-gehalte in bloed zou daardoor bij gelijkmatige groei met een vaste hoeveelheid per periode

toenemen, maar bij exponentiële groei ook exponentieel kunnen verlopen. Deze toename kan uitgedrukt worden in het absolute aantal ng/ml per tijdseenheid, als het om een gelijkmatige lineaire vermeerdering gaat. Bij exponentiële toename kan dit beschreven worden door het percentage PSA wat er over een bepaalde tijd toegevoegd wordt, of het vermelden van de tijd die nodig is om het PSA-gehalte te verdubbelen. Bij patiënten zonder prostaatcarcinoom neemt het PSA globaal 2 tot 3 % per jaar toe. Bij patiënten met een prostaatcarcinoom lijkt dit meer te zijn, maar de variatie van de PSA-bepaling is zodanig, dat het vooralsnog niet mogelijk is om voor een individu aan de hand van zijn PSA-verandering over de periode van één jaar met enige zekerheid een carcinoom vast te kunnen stellen. De hoogte van de eenmalige PSA-bepaling blijkt daarover meer informatie te geven. Herhaling van de PSA-bepaling lijkt in de groep van mannen zonder klachten pas zinvol na een periode van ongeveer 4 jaar.
Er is weinig bekend over de relatie tussen het PSA-gehalte en het natuurlijk beloop van het onbehandeld prostaatcarcinoom. Hierbij staat de vraag centraal of het PSA-gehalte de voortgang van het ziekteproces volgt, of dat er zelfs een stijging van het PSA-gehalte voorafgaat aan de verergering van de symptomen en de uitbreiding van het carcinoom bij onderzoek. Bij patiënten met uitzaaiing naar de lymfklieren in het bekken bleek de verdubbelingstijd van het PSA-gehalte korter bij diegenen die ook uitzaaiingen naar andere plaatsen kregen, vergeleken met die mannen waarbij dit niet het geval was. In hoofdstuk 9 wordt een onderzoek beschreven waarbij een groep mannen met een beginstadium van prostaatkanker onderzocht wordt. Bij al deze 29 mannen werd een carcinoom vastgesteld dat zich alleen in de prostaat leek te bevinden. Om allerlei redenen ondergingen zij geen operatie of andere therapie. Zij werden over een periode van gemiddeld 39 maanden gevolgd. Ofschoon bij rectaal toucher werd vastgesteld dat bij 16 van hen een toename van het kwaardaardige proces alleen in de prostaat was opgetreden, verliep dit niet parallel aan toename van het PSA-gehalte of aan metingen van het echografische prostaatvolume. Hieruit bleek dat de toename van het PSA-gehalte niet gebruikt kan worden als vervanging van het rectaal onderzoek bij mannen die onder controle zijn voor een prostaatcarcinoom dat alleen behandeld wordt op het moment dat er symptomen zijn.

In deel 5 wordt een praktische toepassing gegeven van de PSA-bepaling bij patiënten, die (in tegenstelling tot de groep van patiënten beschreven in hoofdstuk 9) een verwijdering van de prostaat moeten ondergaan voor een carcinoom dat zich tot de prostaat beperkt. Hierbij is het doel het verkorten van de operatieduur. Bij een dergelijke operatie wordt begonnen met het verwijderen van de lymfklieren in het kleine bekken om te zien of er geen onverwachte uitzaaiingen zijn. Tijdens de operatie wordt gewoonlijk een microscopisch onderzoek van de weggehaalde lymfklieren verricht. Indien er sprake is van uitzaaiingen, dan wordt de prostaat niet verwijderd, omdat de uiteindelijke overlevingsduur daardoor niet meer beïnvloed wordt. Het verrichten van het microscopisch onderzoek vraagt enige tijd, en betekent een gedwongen oponthoud voor het operatieteam. Daardoor kan de beschikbaarheid van de operatieruimte en het personeel in het gedrang komen, met als gevolg dat er minder operaties gepland kunnen worden, en wachttijd ontstaat voor deze verrichting.

In een serie van 214 patiënten, die allen deze operatie ondergingen, bleek dat het PSA-gehalte voorafgaande aan de operatie in combinatie met het resultaat van het weefselonderzoek uit het prostaatbiopt in 17 % van de patiënten in deze groep kon

voorspellen dat er geen uitzaaiingen waren. Indien men op deze voorspelling af zou gaan, dan zou bij 1 van de 214 patiënten toch de prostaat verwijderd zijn ondanks de aanwezigheid van metastasen in de lymfklieren. Ook bij de microscopische beoordeling van de lymfklieren tijdens de operatie wordt in vergelijking met het definitieve microscopische onderzoek na de operatie in een aantal gevallen foutief aangegeven dat er geen uitzaaiingen zijn. In deze serie was dat in 10 patiënten het geval (dit is 4.7 %).

Onderzoek bij mannen op de polikliniek waarbij eerder een prostaatkanker gediagnostiseerd was, bracht aan het licht dat de verhouding tussen het vrije en het gebonden PSA in bloed (de eerder genoemde F/T-ratio) een aanvulling was op de bepaling van het totale PSA-gehalte bij de herkenning van prostaaatcarcinoom. In deel 6 wordt nagegaan of dit ook geldt voor mannen die meedoen aan een bevolkingsonderzoek. In zo'n groep mannen is de verhouding tussen mannen met en mannen zonder een prostaatcarcinoom veel kleiner dan bij bezoekers van een urologische polikliniek. Daardoor kunnen factoren die wel bijdragen aan de herkenning van een carcinoom in een polikliniekpopulatie, ondergesneeuwd worden door de grote aantallen normale uitslagen in een steekproef uit de normale bevolking. De F/T-ratio bleek, net als de door het prostaatvolume gecorrigeerde PSA-waarden, zo'n ondergesneeuwde factor te zijn. Dit blijkt uit de resultaten beschreven in hoofdstuk 11.

Bij het onderzoek van 1726 mannen tussen 55 en 77 jaar, die een representatieve steekproef uit de Rotterdamse bevolking vormden, werden met behulp van serum PSA, rectaal onderzoek, en transrectale echografie, 67 prostaatcarcinomen (dit is 3.9 %) gevonden. Door achteraf met behulp van een nieuw ontwikkelde PSA-test (de DELFIA ProStatus PSA F/T assay) de F/T-ratio vast te stellen, kon de waarde van deze factor bepaald worden in het PSA-bereik van 4.0 ng/ml en hoger. Het bleek dat de bijdrage van de F/T-ratio aan de selectie van mannen voor een prostaatbiopsie slechts zeer gering was. De waarschijnlijkheid op een carcinoom nam weliswaar toe bij mannen met een totaal serum PSA van meer dan 10 ng/ml, maar in het intermediaire PSA-gebied tussen 4.0 en 10.0 ng/ml werd geen winst geboekt wat betreft de specificiteit. In deze groep mannen maakte de lineaire verhouding tussen vrij en totaal serum PSA (de F/T ratio) overigens niet het beste onderscheid tussen mannen met en zonder een prostaatcarcinoom, maar bleek deze relatie mathematisch meer complex.
In hoofdstuk 12 en 13 wordt aan de hand van dezelfde groep mannen getoond dat de detectie van de carcinomen voornamelijk gebaseerd is op het PSA en het rectaal toucher, en niet op de transrectale echografie. Verder worden de verschillende methoden om het serum PSA te corrigeren met elkaar vergeleken. De F/T-ratio, de correctie van het PSA-gehalte met behulp van het prostaatvolume (de PSA-dichtheid), en leeftijdsgebonden referentiewaarden van PSA werden hiervoor gebruikt. Uit de literatuur is bekend, dat het PSA-niveau waaronder 95 % van de PSA-waarden bij normale mannen valt, met het stijgen van de leeftijd hoger wordt. Bij mannen op jongere leeftijd zou men op grond van het overschrijden van dit niveau bij een lager PSA-gehalte tot een prostaatbiopsie beslissen dan bij mannen op een oudere leeftijd. Het gebruik van deze PSA-corrigerende factoren heeft twee oogmerken: het kan het aantal biopsieën terugdringen wat nodig is om een carcinoom te ontdekken, en het kan het gebruik van andere screenings-modaliteiten wellicht overbodig maken. Beide kunnen ook gevolgen hebben voor de financiële aspecten van bevolkingsonderzoek.

In hoofdstuk 13 wordt gebruik gemaakt van een simulatie waarbij mannen mede op grond van de genoemde PSA-corrigerende factoren een biopsie ondergingen, en waarbij aan de hand van de reeds bekende uitslag van de biopsieën de waarde van de verschillende factoren bepaald werd. Het bleek dat door toepassing van de leeftijdsgebonden PSA-categorieën in combinatie met het digitaal onderzoek het aantal biopsieën met ongeveer 35 % verminderd zou kunnen worden, maar dat tegelijkertijd 11 % van de carcinomen niet ontdekt zou worden. Hetzelfde deed zich voor als de F/T ratio of de PSA-dichtheid werd gebruikt in plaats van transrectale echografie voor correctie van het PSA-gehalte van 4.0 ng/ml of meer. Een duidelijk minder verlies aan carcinoom detectie deed zich voor als men echografie bij de screening achterwege zou laten, of alleen die mannen zou onderzoeken met echografie en digitaal onderzoek die een PSA-gehalte van 2.0 ng/ml of meer hebben. Daarbij zou het aantal biopsieën dalen met 17 tot 30 %, hetgeen een forse vermindering van de kosten van het screeningsonderzoek zou kunnen betekenen. Zoals al gebleken was uit hoofdstuk 11, was het niet mogelijk met behulp van de F/T ratio het aantal biopsieën, genomen op basis van een PSA-waarde van 4.0 ng/ml of meer, te verminderen zonder het aantal ontdekte carcinomen te reduceren. In het PSA-gebied lager dan 4.0 ng/ml zou men de waarde van de F/T ratio eigenlijk het best kunnen onderzoeken door alle mannen een biopsie te laten ondergaan.

In hoofdstuk 14 wordt de F/T ratio vergeleken met het totaal serum PSA bij het gebruik daarvan voor andere doeleinden dan het onderscheid tussen goed- en kwaadaardig. Om een indruk te krijgen van de uitgebreidheid (stagering) en differentiatie (gradering) van een reeds gediagnostiseerd carcinoom, en voor het ontdekken van achtergebleven en terugkerend tumorweefsel na een prostaatoperatie, speelt het PSA een belangrijke rol. Bij 128 mannen met een onbehandeld prostaatcarcinoom bleek, zoals reeds eerder waargenomen, het totaal PSA in serum tussen mannen met diverse stadia te overlappen. Dit was ook het geval voor de F/T ratio, waarbij de F/T ratio geen beter onderscheid maakte tussen de diverse groepen dan PSA.
Bij analyse van 39 patiënten na een radicale prostatectomie werd gevonden dat de F/T ratio geen verbetering opleverde voor de diagnose van tumorrecidief vanwege de variatie van de F/T ratio in het PSA-gebied kleiner dan 0.3 ng/ml. Ook het verloop van de F/T ratio over een periode bij onbehandeld prostaatcarcinoom of na een radicale prostatectomie bleek geen bruikbare informatie te geven in kleine patiëntengroepen in vergelijking met het verloop van het serum PSA. Aan de hand van deze resultaten werd gespeculeerd over het concept van de F/T ratio. In tegenstelling tot bijvoorbeeld de PSA-dichtheid is het niet duidelijk wat de F/T ratio eigenlijk voorstelt, behalve een rekenkundige grootheid waarmee voorspellingen ten aanzien van prostaatcarcinoom gedaan kunnen worden. Op grond van een aantal argumenten wordt geconcludeerd dat de F/T ratio niet zozeer een relatie heeft met het totale tumorvolume in het lichaam, maar meer met veranderingen van de stofwisseling in de tumorcel. De voornaamste betekenis van de F/T ratio is vooralsnog het onderscheid tussen goedaardige en kwaadaardige prostaataandoeningen.

Deze dissertatie besprak de toepassing van verschillende PSA-tests en de combinatie van het PSA-gehalte met transrectale echografische volumebepalingen van de prostaat voor het herkennen van prostaatcarcinoom, en het vervolg daarop. Met name de ontwikkeling en toepassing van gecorrigeerde PSA-waarden werden belicht. De belangrijkste betekenis van de verschillende artikelen ligt in de toepassing van deze wetenschap in de studie van vroege herkenning van prostaatcarci-

noom onder de bevolking. Met de huidige stand van zaken rond volumetrie van de prostaat en de bepaling van het PSA-gehalte wordt de herkenning van prostaatcarcinoom in een steekproef van de normale bevolking maar marginaal verbeterd door gecorrigeerde PSA-waarden. Het totale serum PSA-gehalte, aangevuld met het rectale onderzoek, zijn tot nu toe de beste voorspellers op de aanwezigheid van een prostaatcarcinoom. Verder onderzoek zal moeten uitwijzen wat de eigenschappen zijn van de carcinomen die ontdekt worden, en of vroege herkenning daarvan goed is voor de volksgezondheid. Het is immers nog niet bewezen dat gemiddeld genomen de kwaliteit en duur van leven verbeterd worden door de vroege herkenning en behandeling van prostaatcarcinoom bij mannen die daarvan geen symptomen hebben.

Met name de huisarts zal zich steeds moeten afvragen of de patiënt erbij gebaat is zijn PSA-gehalte te meten en te weten. Voor de beoordeling van plasklachten bij mannen formuleerde de Commissie Kwaliteit van de Nederlandse Vereniging voor Urologie richtlijnen ten aanzien van diagnostiek [1]. Ofschoon klachten, (zoals geevalueerd in de ICSS symptoomscore) in lichte mate gecorreleerd zijn met de grootte van de prostaat, hebben mannen met een prostaatcarcinoom niet meer klachten dan mannen zonder prostaatcarcinoom. Het is derhalve moeilijk na te gaan of de klachten bij mannen met een prostaatcarcinoom veroorzaakt worden door de aanwezigheid van hun carcinoom, dan wel door een benigne component van de prostaat. Behandeling van de klachten zal zich dan ook richten op de behandeling van het carcinoom. Het is daarom redelijk om bij de diagnostiek van plasklachten de mogelijkheid van het bestaan van een prostaatcarcinoom te onderzoeken. In dat kader is het verrichten van een PSA-bepaling gerechtvaardigd. Ook het verrichten van een rectaal onderzoek is noodzakelijk om de prostaat te kunnen beoordelen. Wanneer naar aanleiding van deze onderzoeken de verdenking bestaat dat er een prostaatcarcinoom aanwezig is, dan moet dat verder uitgezocht worden door een uroloog. Vanwege de langzamerhand algemene bekendheid met PSA onder de bevolking, kan de huisarts door de patiënt gevraagd worden zijn PSA-gehalte te bepalen, zelfs als er geen klachten zijn. De informatie om deze vraag te beoordelen dient beschikbaar te zijn voor de huisarts. Een PSA-bepaling kan de aanwezigheid van een prostaatcarcinoom niet uitsluiten, maar een lage waarde in combinatie met normale bevindingen bij een rectaal onderzoek maakt de kans op een carcinoom erg klein. Er bestaat bij artsen veel onzekerheid over de bevindingen bij rectaal toucher [2], zodat moet gelden: verwijs bij twijfel naar een uroloog. Immers, ongeveer 15 % van de prostaatcarcinomen gevonden bij onderzoek van de normale bevolking zijn geassocieerd met een verdacht rectaal onderzoek ondanks een PSA-gehalte kleiner dan 4.0 ng/ml. Bij deze vorm van 'case finding' dient men zich te realiseren dat men via een achterdeur screening op prostaatkanker introduceert, waarvan de waarde nog niet bekend is. De voorstelbare motivatie van verzekeringsgeneeskundigen om in het kader van risicoberekening het PSA-gehalte te bepalen bij hun cliënten, moet derhalve afgekeurd, en wellicht als onethisch beschouwd worden.
Er zijn verscheidene PSA-testen op de markt, die verschillende uitslagen kunnen geven van hetzelfde bloedmonster. De arts, die gebruik maakt van de PSA-bepaling, is verantwoordelijk voor het interpreteren van de resultaten van het door hem/haar aangevraagde onderzoek, en moet op de hoogte zijn van de betekenis van de verschillende 'normaal'-waarden die per test aangegeven worden. Het klinisch laboratorium dient zich hierbij te onthouden van uitspraken omtrent de kans op een kwaadaardigheid bij een bepaalde uitslag, omdat die kans niet bepaald wordt door het PSA-gehalte alleen. Het is voor de laboratoria van belang om de ontwikkelingen

rond de standaardisatie van de PSA-bepaling in de gaten te houden.

PSA-bepalingen en transrectale echografie zullen nog allerlei ontwikkelingen doormaken. Verscheidene moleculaire verschijningsvormen van het PSA in bloed zullen onderzocht worden. Van echografische parameters zal bekeken worden of ze specifiek voor prostaatcarcinoom genoemd kunnen worden. Nieuwe technieken die in het bloed circulerende tumorcellen kunnen waarnemen zijn reeds aanwezig. Voor PSA-geïnteresseerden zal het een drukke tijd blijven.

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APPENDIX 1[1] (to Chapter 2)

VARIABILITEIT VAN UITSLAGEN VAN PROSTAAT SPECIFIEK ANTIGEEN (PSA) MET 6 BEPALINGSMETHODEN*

Chris H. Bangma, Bert G. Blijenberg, Fritz H.Schröder

SAMENVATTING

Doel: Het verschaffen van een indruk over en de oorzaak van de variabiliteit van PSA-bepalingen voor klinisch relevante patientenpopulaties.
Plaats: Academisch Ziekenhuis Rotterdam.
Opzet: descriptief.
Materiaal en methode: Klinische en klinisch-chemische aspecten van het PSA werden beschreven aan de hand van de literatuur. Voor drie patientenpopulaties bestaande uit participanten in een screeningspopulatie, patienten waarbij poliklinisch een prostaatcarcinoom vastgesteld werd, en patienten na een radicale prostatectomie, werd een beperkte studie opgezet, waarbij het PSA bepaald werd met behulp van zes verschillende analysetechnieken.
Resultaten: Met de Hybritech Tandem-R bepaling als gekozen standaard bestond er tussen verschillende analysetechnieken rond een PSA-waarde van 4.0 ng/ml een spreiding van 3.3 tot 7.2 ng/ml. Rond een waarde van 10.0 ng/ml bedroeg de spreiding 8.7 tot 18.5 ng/ml. Na radicale prostatectomie bleken de verschillende analyse-technieken slechts in 5 van de 15 patienten volledig met elkaar in overeenstemming te zijn.
Conclusie: Referentiewaarden voor PSA dienen met grote voorzichtigheid gebruikt te worden gezien de overlap tussen patienten met benigne en maligne prostaat-pathologie, en de variabiliteit van het PSA en de analysetechnieken.

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Opgenomen in Medline onder: Bangma C.H., Blijenberg B.G., Schröder F.H.; Variability of values of Prostate Specific Antigen determined with 6 methods

INLEIDING

Gedurende de afgelopen jaren geniet het Prostaat Specifiek Antigeen (PSA) een toenemende belangstelling onder huisartsen, specialisten, klinisch-chemici, en epidemiologen. Het PSA beloofde vanaf het begin een unieke en selectieve marker te zijn voor het prostaatcarcinoom. De incidentie van prostaatcarcinoom in Nederland neemt toe, en zal, in navolging van de USA, waarschijnlijk binnen korte tijd het meest frequente carcinoom onder de mannelijke bevolking zijn. Een groeiend aantal publicaties uit de urologische wereld over PSA beschrijft de mogelijkheden op het gebied van het herkennen en vervolgen van prostaatcarcinoom. De PSA-bepaling behoorde daardoor al snel tot het arsenaal van de uroloog, maar ook tot dat van de huisarts. Referentiewaarden voor PSA dienen echter met grote voorzichtigheid gebruikt te worden. De aard van het PSA, de manier van klinisch-chemische bepaling, en de aard van de prostaatpathologie brengen dit met zich mee. In dit artikel worden deze elementen toegelicht, en geïllustreerd met voor de praktijk belangrijke patientenpopulaties.

PSA voor de praktijk

Het PSA is een eiwitsplitsend produkt (protease) dat in de epitheliale prostaatcellen aangemaakt wordt, en in hoge concentratie in het semen uitgescheiden wordt om het te vervloeien (1, 2). Biochemisch is het PSA een glycopeptide met een halfwaarde tijd tussen de 2,2 en 3,3 dagen (3, 4). Bij -20 C is het PSA-gehalte in serum stabiel over zeer lange tijd, zodat het voor onderzoek bewaard kan worden (5, 6).
PSA circuleert in het serum voor een klein gedeelte in een vrije inactieve vorm, maar voornamelijk in een aan eiwitten gebonden vorm. De fractie van de gebonden vorm bedraagt 40 tot 90 procent, afhankelijk van de bepalingsmethode (7, 8, 9). Het α-1-antichymotrypsine is de belangrijkste complex-vormer (10). De vrije fractie is wisselend, en er zijn aanwijzingen dat deze fractie kleiner is bij prostaatcarcinoom (9, 10). Het mechanisme dat de verhouding tussen gebonden en vrije vorm bepaalt, is niet bekend. Deze verhouding kan wel de specificiteit van PSA voor de detectie van prostaatcarcinoom verhogen zonder verlies van sensitiviteit bij waarden van PSA tussen 4 en 20 ng/ml (4).
Zoals bij een groot aantal eiwitbepalingen in serum het geval is, vertoont het PSA-gehalte in bloed ook fluctuaties. Er bestaat een algemeen erkende 24-uurs variatie zonder standaardritme, en een mogelijk hormonaal beïnvloede seisoensvariatie. Deze variaties storen de bepalingen niet in relevante mate (11, 12, 13). Ejaculatie en rectaal toucher lijken geen invloed te hebben op de hoogte van het serum PSA (14), maar transrectale echografie (TRUS), prostaatpuncties, en transurethrale manipulaties hebben dit zeker wel: het is aangewezen een venapunctie voor PSA te verrichten voordat men overgaat tot een van deze onderzoeken.

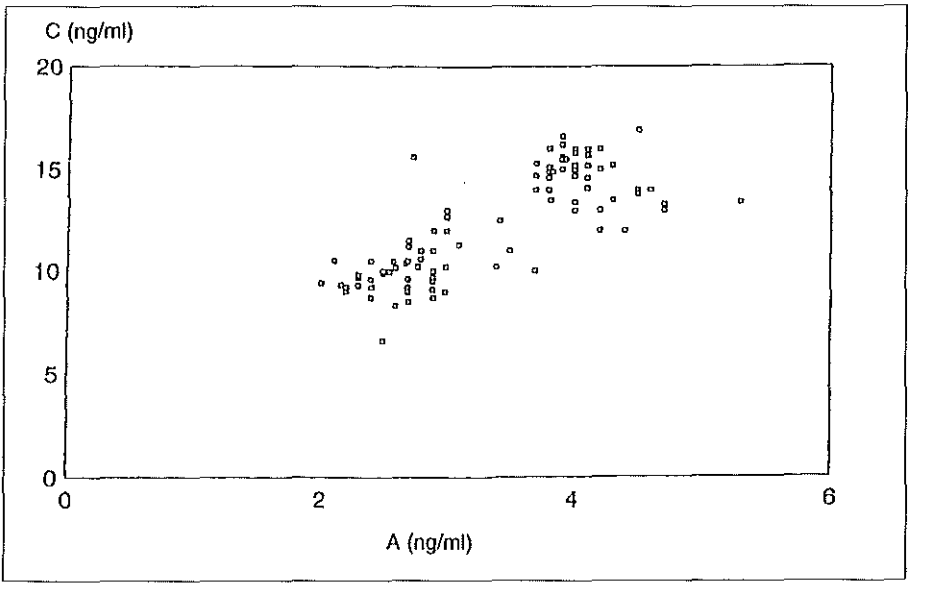
Bij benigne prostaat hyperplasie kunnen de verschillende componenten van de prostaat onafhankelijk van elkaar toenemen. Wanneer de epitheliale fractie vergroot, zal het serum PSA stijgen (15), echter niet altijd evenredig met het prostaatvolume, omdat de stromale component niet altijd recht evenredig met de glandulaire component toeneemt. Ook is het mogelijk dat een verhoogde doorbloeding, zoals bij ontsteking het geval is, en een verhoogde celafbraak bij necrose of infarcering een onvoorspelbare verandering van het PSA geven.

Ook in geval van een prostaatcarcinoom kan er sprake zijn van een verhoogd serum PSA. Vooralsnog is er geen mogelijkheid om het PSA afkomstig uit maligne cellen te onderscheiden van goedaardige productie, ofschoon het mogelijk anders reageert in

het serum, namelijk door een vermeerderde eiwit binding. Verschil in differentiatie graad van de tumorcellen draagt bij aan een variabel PSA per volume-eenheid tumorweefsel (16). Een PSA-verhoging treedt eerder op dan een verhoging van het Prostaat Zure Phosphatase, en heeft deze bepaling dan ook geheel verdrongen in de diagnostiek (17). De toepassingen voor het gebruik van het serum PSA zijn velerlei, en elke toepassing beslaat een eigen bereik van PSA-waarden. In combinatie met andere modaliteiten zoals het rectale toucher, transrectale echografie, en transrectale prostaatbiopsie, worden PSA en de toename van PSA per jaar (PSA-velocity) in Nederland gebruikt in het kader van een studie die tot doel heeft de waarde van vroege opsporing van prostaatcarcinoom te onderzoeken (18). In de urologische praktijk is PSA een onderdeel van de diagnostiek van symptomatische mannen. Bij prostaatcarcinoom vormen het PSA en de verdubbelingstijd (19) markers voor het beloop na een ingestelde therapie, zoals hormonale en radiotherapie, en operatieve resectie. Na een radicale prostatectomie voor een gelocaliseerde tumor dient het PSA niet detecteerbaar te zijn. Zeer kleine hoeveelheden PSA of een toename daarvan kunnen wijzen op een locaal recidief of metastasering (20, 21). Bij gemetastaseerde tumoren kunnen de PSA-waarden echter oplopen tot nabij 1000 ng/ml.

Spreiding en standaardisatie van de PSA-bepaling

In Nederland worden op vrijwillige basis regelmatig laboratoriumbepalingen onderworpen aan een kwaliteitscontrole. Aan de enquetes van de Landelijke Werkgroep Bindingsanalyse (LWBA) van de Stichting Kwaliteitscontrole Ziekenhuislaboratoria (SKZL) doen op dit moment ruim 100 laboratoria mee. Zoals gebruikelijk worden bij deze enquetes een tweetal monsters ter analyse aangeboden. Een voorbeeld van de resultaten van een recent gehouden enquete over de bepaling van serum-PSA is weergegeven in figuur 1. Hierin worden de resultaten van de PSA-



FIGUUR 1

Resultaat PSA bepaling in serummonster A en C ten bate van de enquete Landelijke Werkgroep Bindingsanalyse in 90 laboratoria (met toestemming LWBA).

bepaling van twee verschillende serummonsters A en C in 90 verschillende Nederlandse laboratoria weergegeven. Alle in de figuur gepresenteerde resultaten zijn verkregen met methoden die door een tiental leveranciers op de Nederlandse markt gebracht worden. Hierbij dient aangetekend te worden dat verschillende leveranciers meer dan één modificatie uitbrengen. In werkelijkheid is het aantal in Nederland leverbare PSA-kits groter. Enkele modificaties zijn derhalve niet verwerkt. Een vergelijkbare situatie doet zich in Duitsland voor. Bij de laatste Duitse tumormarkerenquete (TM 2/93) gaven de beide enquetemonsters gemiddeld als resultaat te zien: 5.2 en 2.9 ng/ml, met als standaard afwijking 1.9 respectievelijk 1.0 ng/ml. Het aantal deelnemers bedroeg 249, en het aantal gebruikte methoden 14.

De grote spreiding van de resultaten is toe te schrijven aan twee belangrijke oorzaken. Om te beginnen is er de spreiding als gevolg van toevallige fouten bij de verschillende analyses. Deze bedraagt bij iedere SKZL-enquete, zelfs met de meest nauwkeurige en goed gestandaardiseerde bepalingstechnieken, ten minste enkele procenten. Een grotere bijdrage aan de variatie bij de PSA-bepaling wordt echter geleverd door het ontwerp van de methodes van de verschillende leveranciers. Zonder in deze context al te diep in te gaan op de immunochemische reacties die aan de bepaling van PSA ten grondslag liggen, moet in ieder geval vermeld worden dat er in het algemeen sprake is van analyse volgens het zogenaamde 'sandwich-principe'. Hierbij 'vangt' het ene antilichaam, gekoppeld aan een vaste drager, het PSA, waarna een detectiereactie volgt van dit complex met het tweede antilichaam waaraan de label gekoppeld is. Deze detectie is mogelijk op basis van een enzymatische reactie, de meting van radio-activiiteit, of van fluorescentie. De gebruikte antilichamen, meestal van monoclonale oorsprong, kunnen van leverancier tot leverancier verschillen, evenals de combinaties die toegepast worden. Er zijn inmiddels tenminste 30 antilichamen tegen PSA bekend, al zijn deze niet alle even goed bruikbaar bij de hier beschreven analyses. De variatie in de uitslagen ontstaat doordat de diverse antilichamen verschillend reageren met het PSA-molecuul dat ofwel vrij voorkomt ofwel gebonden is. Dit geldt niet alleen voor het te analyseren serummonster, maar ook voor de calibrator waarmee in de commerciele kit de ijking wordt verricht. Voor deze calibratoren maken de verschillende leveranciers gebruik van een aantal media waarvan de meest gebruikte zijn serum van manlijke of vrouwelijke donoren en runderalbumine-oplossingen. Het eraan toegevoegde PSA is in het algemeen niet duidelijk gekarakteriseerd. Al met al een onbevredigende situatie, niet alleen in biochemische zin, maar ook in klinische, omdat er bij de analyse van patientenmonsters verschillen kunnen optreden. .
Een poging om uit deze impasse te geraken is ondernomen in december 1992 op de Stanford Conference on International Standardization of PSA Assays. In de aanwezige groep van experts en vertegenwoordigers van de World Health Organization, en de pharmaceutische industrie, kon vooralsnog geen communis opinio bereikt worden, enerzijds vanwege nog onvoldoende biochemisch inzicht, maar anderzijds ook vanwege gevestigde commerciele belangen. De verwachting is echter wel dat in de loop van 1994 een pragmatische oplossing voorgesteld zal worden. In de praktijk betekent het een en ander nu dat leveranciers zich veelal richten of reeds gericht hebben op de commerciele Hybritech Tandem-R PSA-bepaling, die in de jaren tachtig als eerste op de markt werd gebracht en de referentiewaarden in de literatuur bepaalde.

Referentiewaarden
De hierboven genoemde gegevens gaan voorbij aan de meest gestelde vraag over

PSA: wat is normaal, en vanaf welke waarde moet er onderzoek gedaan worden naar een prostaatcarcinoom totdat het tegendeel bewezen is ? Gezien het voorgaande is het antwoord niet eenduidig te geven. Veelal wordt verwezen naar referentiewaarden in de literatuur. Hierbij wordt minder dan 4,0 ng/ml normaal geacht, tussen 4,0 en 10,0 is licht verdacht op prostaatcarcinoom, meer dan 10,0 is abnormaal verhoogd. Het getal 4.0 is afkomstig van een studie beschreven door Myrtle (22). Voor een populatie van 860 gezonde asymptomatische manlijke vrijwilligers vond hij in 99 % van de gevallen een serum-PSA lager dan 4.0 ng/ml. Strikt genomen nam hij ook de leeftijd in beschouwing. Voor mannen jonger dan 40 jaar werd een bovengrens van 2.5 ng/ml gevonden, terwijl voor mannen ouder dan 40 jaar de bovengrens 4.0 ng/ml bleek te zijn. (Recent beschreven Oesterling en medewerkers (23) een nadere uitwerking van deze leeftijdsafhankelijkheid. Zij vonden in een screeningspopulatie als referentie waarden: 40-49 jaar: 0.0 - 2.5 ng/ml; 50-59 jaar: 0.0-3.5 ng/ml; 60-69 jaar: 0.0-4.5 ng/ml; 70-79 jaar: 0.0-6.5 ng/ml.)

Uit screeningsstudies van Brawer (24) en Labrie (25) bleek dat ongeveer 26 % van de participanten met een PSA tussen 4 en 10 ng/ml een prostaatcarcinoom had, terwijl bij een PSA groter dan 10 ng/ml dit steeg tot 50 %. Bij benigne prostaat hyperplasie (BPH) werd in 9 klinische studies gevonden dat gemiddeld het PSA in 21 % boven 4.0 ng/ml, en in 10 % boven 10.0 ng/ml ligt (26). Bij patiënten die een klinisch gelocaliseerd prostaatcarcinoom hadden bleek uit de gecombineerde resultaten van 8 studies dat in 77 % een PSA groter dan 4 ng/ml gevonden werd. Slechts 43 % van de prostaatcarcinoompatiënten had een PSA-waarde van meer dan 10 ng/ml. Het is duidelijk dat patiëntenpopulaties uit klinische studies niet vergeleken kunnen worden met screeningspopulaties. Er bestaat aanzienlijke overlap tussen de patiënten met BPH en met een prostaatcarcinoom in elk PSA-interval. Wanneer de bovengrens voor normaal op 4.0 ng/ml gesteld wordt, dan blijkt ongeveer 30 % van de patiënten met een op andere wijze gevonden prostaatcarcinoom een 'normaal' PSA te hebben.

PATIËNTEN EN METHODE

Om een indruk te geven van de plaats van de PSA-bepaling bij de diagnostiek van het prostaatcarcinoom werd door ons een beperkte studie opgezet met een drietal verschillende patiëntenpopulaties. In alle gevallen werd het PSA uit 1 serummonster bepaald met 6 verschillende analysetechnieken, die ter beschikking werden gesteld door desbetreffende leveranciers. Alle 6 methoden kwamen voor in de bovengenoemde LWBA-enquete: zij zijn de in Nederland meest toegepaste. Het betreft de Abbott IMx PSA (Abbott, Amstelveen), de DPC Immulite PSA (Diagnostic Products Corporation, Apeldoorn), de TOSOH AIA-1200 PSA (Eurogenetics, Moordrecht), de Phamacia Delfia PSA (Kabi Pharmacia, Woerden), de Corning ACS 180 PSA (Ciba-Corning Diagnostics, Houten), en de Hybritech Tandem-R PSA (Eli Lilly Hybritech, Nieuwegein). Alle monsters werden in enkelvoud bepaald volgens voorschrift van de fabrikant. Voor vergelijking van de PSA-waarden werd de Hybritech Tandem-R als standaard gekozen. Uit de gevonden PSA-waarden werd voor elke bepaling een regressielijn berekend (27). In tabel 1 worden enkele praktische gegevens met betrekking tot de gebruikte PSA-reagenscombinaties weergegeven. De vergelijkingen met Hybritech zijn afkomstig van de fabrikant (Delfia), uit de literatuur (IMx (28), ACS 180 (29), verkregen (AIA-1200: Dr.M.A.Blankenstein, Academisch Ziekenhuis Utrecht), of zelf bepaald (Immulite). In de beide laatste gevallen was er geen sprake van een uitvoerig onderzoek (76 resp. 51 analyses). De gegevens met betrekking tot de referentiewaarden zijn afkomstig van de verschillende bijsluiters. Hybritech vermeldt ook een leeftijdsafhankelijkheid (22).

TABEL 1

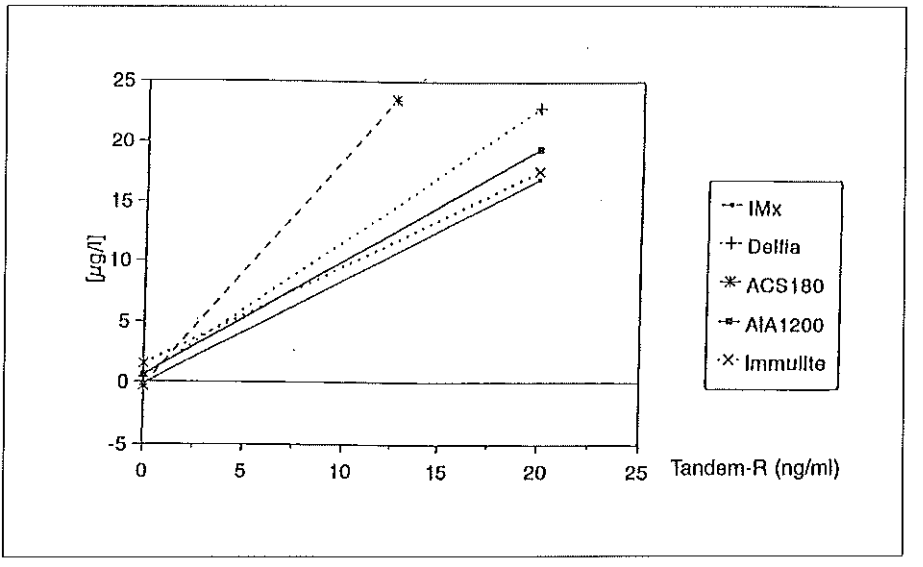
Praktische gegevens aangaande verschillende PSA analysetechnieken.

Bepaling (ng/ml)	*Detectielimiet (ng/ml)*	*Referentiewaarden (x = Hybritech)*		*Correlatie Hybritech*
		< 40 jaar	*> 40 jaar*	
Delfia	< 0.1	0.7-2.0	1.2-7.0	y = 1.10 x - 0.24
AIA-1200	0.1	0.5-1.9	0.5-3.1	y = 0.99 x - 0.10
IMx	< 0.1	0-4.0	0-4.0	y = 0.97 x +0.20
ACS 180	0.1	0.7-4.0	1.3-5.0	y = 1.21 x +1.11
Immulite	< 0.1	niet bekend		y = 0.89 x +0.95
Tandem-R	< 0.1	0-4.0	0-4.0	

De eerste studiepopulatie bestond uit nieuwe poliklinische patienten waarbij vanaf januari 1993 een histologisch bewezen prostaatcarcinoom werd ontdekt. Van elke patient die voor het eerst de polikliniek urologie bezocht, werd bij aanvraag van het PSA een extra serummonster afgenomen, en bewaard in een serumbank bij -70 graden Celsius. Gedurende 5 maanden werden 19 patienten met een prostaatcarcinoom en een PSA-waarde tussen 0 en 20 ng/ml geselecteerd. De resultaten van de PSA-analyses gaven een goede spreiding te zien over dit gebied.
De tweede studiegroep bestond uit een populatie van mannen tussen 55 en 75 jaar die vanaf eind 1991 participeerden in het Rotterdamse screenings onderzoek in het AZR naar prostaatcarcinoom (18). Uit deze groep van mannen werden voor ons onderzoek at random 20 participanten geselecteerd, waarvan het PSA rond de 4 ng/ml bedroeg.
De derde studiegroep bestond uit patienten die een radicale prostatectomie hadden ondergaan voor een gelocaliseerd prostaatcarcinoom (pT2-3N0M0) tussen februari 1990 en maart 1993. Uit deze groep werden at random 15 patienten gekozen. Het geanalyseerde serummonster werd 4 tot 41 maanden, gemiddeld 16, na de operatie afgenomen.

RESULTATEN

De PSA-waarden van de 19 poliklinisch gedetecteerde prostaatcarcinomen werden met elkaar vergeleken door van elke analysetechniek de regressielijn te bepalen ten opzichte van de gekozen standaard, de Hybritech Tandem-R waarde. In figuur 2 worden de regressielijnen grafisch weergegeven. Het in de literatuur veel aangegeven PSA-getal 4.0 (Hybritech Tandem-R) vertoont een spreiding van 3.3 tot 7.2 ng/ml tussen de diverse analysetechnieken. Dit geldt eveneens voor het getal 10.0: de spreiding bedraagt 8.7 tot 18.5 ng/ml. Voor de ACS 180 is het mogelijk, indien gewenst, een correctiefactor te gebruiken ten opzichte van de Hybritech. Deze hebben wij echter niet toegepast.
De PSA-waarden van 20 screeningspatienten werden vergeleken in tabel 2: met het oog op hun geringe spreiding werd het gemiddelde vermeld.



FIGUUR 2

Grafische weergave van regressielijnen van de analyses met de prostaatcarcinoomsera.

In formule (y = Hybritech Tandem-R):

IMx = 0.86 y - 0.14 (r = 0.98)
Delfia = 1.13 y + 0.39 (r = 0.99)
ACS = 1.88 y - 0.31 (r = 0.99)
AIA = 0.95 y + 0.57 (r = 0.99)
Immulite = 0.81 y + 1.48 (r = 0.98)

TABEL 2

Spreiding in resultaten van 6 PSA analysetechnieken verkregen uit sera van 20 participanten van de Rotterdamse screeningspopulatie.

	gemiddelde (ng/ml)	*minimum-maximum* (ng/ml)
Tandem-R	2.5	0.7 - 5.8
IMx	2.7	1.0 - 4.8
Delfia	3.2	1.2 - 6.4
ACS	4.6	1.1 - 8.6
AIA	2.8	0.9 - 5.6
Immulite	3.4	1.0 - 6.3

TABEL 3

Frequentie van positieve en negatieve detectie van PSA bij 15 patienten na radicale prostatectomie voor 6 verschillende analysetechnieken

	detectie						
negatief	6	5	4	3	2	1	0
positief	0	1	2	3	4	5	6
frequentie	4/15	5/15	2/15	1/15	1/15	1/15	1/15

Van 15 patienten na radicale prostatectomie werd in tabel 3 genoteerd hoe vaak overeenstemming bestond tussen de analysetechnieken wat betreft detecteerbaarheid van het PSA. Indien detecteerbaar bedroeg de spreiding 0.1 tot 0,5 ng/ml. Bij 5 sera bleken de analysetechnieken volledig met elkaar in overeenstemming te zijn. In 6 gevallen week er 1 af, in 3 gevallen 2, en in 1 geval was detectie in de helft positief. Een van de technieken gaf in vier gevallen een positieve detectie aan, waar de meerderheid negatief was.

DISCUSSIE

Teneinde de toepassing van de PSA-bepaling toe te lichten werden voor de praktijk relevante patientenpopulaties gekozen. In deze groepen speelt het gros van de medische beslissingen zich af, waarbij het PSA-meetgebied in het algemeen beperkt is tot 0 - 20 ng/ml. Afgezien is hierbij van de beschrijving van andere patientenpopulaties. Voor het belang van PSA-bepaling voor patienten met een prostaatcarcinoom, dat hormonaal of met radiotherapie behandeld wordt, zij verwezen naar recente overzichtsartikelen (30, 31, 32). De grootte van de gekozen groepen is gelimiteerd omdat zij alleen dienen als voorbeeld.

Het ligt met name niet in de bedoeling om producten te vergelijken, aangezien dit ook elders verricht is (28, 29, 33). De analysetechniek die het meest frequent gebruikt of geciteerd wordt, hoeft niet in alle gevallen het meest geschikt te zijn. Hierbij dient ook vermeld te worden dat onze huidige kennis van zaken van immunochemische bepalingstechnieken helaas nog niet toelaat dat, al zijn de antilichamen en de standaarden geuniformeerd, de resultaten van de analyses met patientensera precies gelijk te krijgen zijn bij de verschillende bepalingstechnieken (34, 35).

In onze beperkte studie bleken 3 tot 7 van de 19 prospectief verzamelde carcinomen een PSA kleiner dan 4.0 ng/ml te hebben, en 1 tot 4 een PSA onder de 2,5 ng/ml. 6 tot 11 patienten met een carcinoom hadden een PSA boven 10,0 ng/ml. Figuur 2 en tabel 2 dienen als ondersteuning met patientenmateriaal. Zowel uit de variatie onder de analysetechnieken, als uit de frequentie van de carcinomen beneden of boven een referentiewaarde, blijkt het gevaar van het verheffen van een PSA-waarde tot 'normaal'.

Detectie van serum-PSA na radicale prostatectomie duidt op een recidief dan wel een niet-radicale resectie van het carcinoom. Het PSA is het vroegst optredende signaal

hiervoor (36, 37). Indien asymptomatisch heeft dit veelal geen therapeutische consequenties. Echter bij de clinicus kan de wetenschap van de aanwezigheid van carcinoom leiden tot intensievere controle en diagnostiek. Voor de patient is deze wetenschap, naast de eventuele diagnostiek, een ernstige belasting. Zoals blijkt uit tabel 3 is voorzichtigheid derhalve geboden bij de beoordeling van het zeer lage of niet detecteerbare serum-PSA.

CONCLUSIE EN AANBEVELINGEN

PSA is een waardevolle bepaling voor prostaat specifieke pathologie. Een standaard analysetechniek ontbreekt vooralsnog. In de praktijk wordt echter veelal op pragmatische gronden de Hybritech Tandem-R als zodanig gebruikt. Consequentie hiervan is dat alleen al uit biochemisch oogpunt geen vast omschreven referentiewaarden te geven zijn. Consensus hierover wordt niet voor 1995 verwacht. In de praktijk blijken geen absoluut discriminerende PSA-waarden te bestaan tussen patienten met of zonder een prostaatcarcinoom. De literatuur omtrent referentiewaarden van PSA dient met name in het licht van de gekozen studiepopulatie gelezen te worden. Voor detectie van prostaatcarcinoom blijven het rectaal toucher en de transrectale echografie onontbeerlijk.

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APPENDIX 2 (to chapter 4)

DESCRIPTION OF THE COMPUTER MODEL USED FOR SIMULATION OF ERRORS IN PLANIMETRIC VOLUMETRY OF THE PROSTATE

In this model the shape of the prostate is simplified to that of an ellipsoid. This ellipsoid is situated in a three dimensional space, which is described by three perpendicular axes. Every point on the surface of the ellipsoid is described by three parameters x_{1-3}, of which x_1 corresponds to the projection on the y axis, x_2 to the x-axis, and x_3 to the z-axis. The ultrasonic probe is translated along the x-axis, while rotation of the ellipsoid occurs along the z axis. The ultrasonic probe observes a cross-section of the ellipsoid in a two dimensional plane defined by the y- and z-axis.

The general description of an ellipsoid with height=$2r_1$, length=$2r_2$, and width=$2r_3$ is:

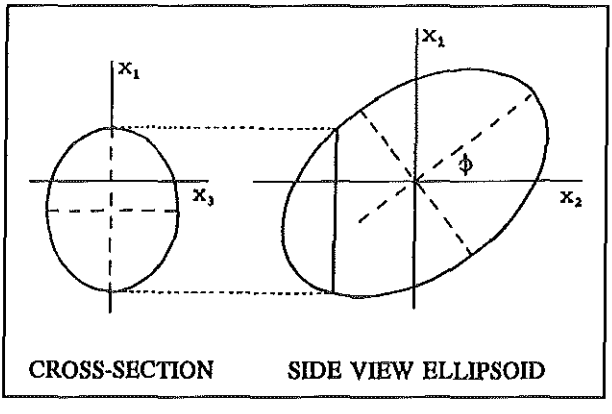
$$\frac{x_1^2}{r_1^2} + \frac{x_2^2}{r_2^2} + \frac{x_3^2}{r_3^2} = 1 \qquad [1]$$

Calculation of the area of the cross-section of an ellipsoid rotated on an angle ϕ is done by using:

$$a_{11} = \cos\phi \quad a_{12} = -\sin\phi$$
$$a_{21} = \sin\phi \quad a_{22} = \cos\phi \qquad [2]$$

The rotation of the ellipsoid, the x_3 or z-axis is the axis of rotation, is described with:

$$x_1' = a_{11}x_1 + a_{12}x_2$$
$$x_2' = a_{21}x_1 + a_{22}x_2 \qquad [3]$$



Substituting in [1] gives

$$M_1x_1^2 + M_2x_2^2 + 2M_{12}x_1x_2 + M_3x_3^2 = 1 \qquad [4]$$

with

$$M_1 = \frac{a_{11}^2}{r_1^2} + \frac{a_{21}^2}{r_2^2}$$

$$M_2 = \frac{a_{12}^2}{r_1^2} + \frac{a_{22}^2}{r_2^2} \qquad [5]$$

$$M_{12} = \frac{a_{11}a_{12}}{r_1^2} + \frac{a_{21}a_{22}}{r_2^2}$$

$$M_3 = \frac{1}{r_3^2}$$

Making a cross-section perpendicular on the x_2 or x-axis at a fixed value for x_2 gives the description of the following ellips:

$$M_1(x_1 + R_{12})^2 + M_3x_3^2 = R \qquad [6]$$

with

$$M_{12} = \frac{M_{12}x_2}{M_1} \qquad [7]$$

$$R = 1 + \frac{M_{12}X_2^2}{M_1} - M_2x_2^2$$

R_{12} describes the position of the center of the ellips moved up or down along the x_2 or x-axis.
The height and width for the ellips are given by

$$2\sqrt{\frac{R}{M_1}} \quad \text{and} \quad 2\sqrt{\frac{R}{M_3}} \qquad [8]$$

Therefore the area of the cross-section is:

$$PA = \pi R \sqrt{\frac{1}{M_1M_3}} \qquad [9]$$

Finding the tangent planes of the ellipsoid at R=0 or

$$x_2 = \pm \sqrt{\frac{M_1}{M_1M_2 - M_{12}^2}} \qquad [10]$$

The numerical integration of the planimetric volume, as provided by the software of the Bruel and Kjaer 1846 ultrasonic equipment, is done using

$$\sum_{\text{all i}} \frac{1}{3} \left(PA(i) + PA(i-1) + \sqrt{PA(i) \times PA(i-1)}\right) \times \text{Stepsize} \qquad [11]$$

in which PA(i) is an area of the cross-section measured during the planimetric procedure. The square root is included to improve the numerical result in case the two areas involved are different; e.g. if PA(i)=0 the formula reduces to the volume of a cone with base PA(i-1).
Stepsizes are defined by the cradle in which the ultrasonic probe is fixed, and are 5 mm in length.

Simulation of planimetry at a fixed angle, the salami effect.

For the simulation of planimetric volumetry of an ellipsoid, which is rotated in relation to the axis of the ultrasonic probe (or x-axis) over a fixed angle α, the following considerations are made:
The ideal starting position for the probe x_{start} is calculated as the x_2-value of the tangent plane at one end of the ellipsoid, indicating a zero-area just to be seen. From there on the probe is moved along the x_2 or x-axis. Using stepsizes, at each position of the ultrasonic probe the area PA(i) is calculated by formula [9]. The last tangent plane gives the value of x_{end} and defines the ultimate probe-position. The number of steps n is given by

$$n = \text{Int}\left\{\frac{x_{end} - x_{start}}{\text{Stepsize}}\right\} \qquad [12]$$

The Int-function returns the integer part of the number within brackets. The planimetric volume is calculated using the summation formula [11] described above.

Simulation of planimetry for a capsizing ellipsoid, the capsizing effect.

For the simulation of planimetric volumetry of an ellipsoid, which continuously capsizes from the first to the last measurement, the following considerations are made:
At the starting position the ellipsoid is rotated at its maximal angle α_{start}. During the measurement the ellipsoid rotates to a position with angle α_{end}, which is defined as zero. x_{start} and x_{end} are found as the x_2-values for the two tangent planes using these two angles.
From x_{start} the probe is moved along the x_2-axis.
The rotation of the ellipsoid is taken to be proportional to the distance the probe has moved from the ideal starting position. The angle is calculated by:

$$\alpha(x_2) = \alpha_{start} + \frac{\alpha_{end} - \alpha_{start}}{x_{end} - x_{start}}(x_2 - x_{start}) \qquad [13]$$

Using stepsizes, at each position of the ultrasonic probe the area PA(i) is calculated by formula [9]. The planimetric volume is calculated by summation, formula [11].

Simulation of planimetry for a rotating ellipsoid including all PA(i) $\geq A_{min}$, the first step effect.

During the simulation of the first step effect, the movement of the ellipsoid is similar to the capsizing effect described above.
The position of the first step x_{first} at the angle α_{first} is found using an iterative procedure to solve the equation $PA(i)=A_{min}$. From thereon the areas PA(i) are found and summed to give the volume. Only areas not smaller than A_{min} are included.

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DANKWOORD

Aan wie eigenlijk niet ?
Op de afdeling Urologie van het Dijkzigt Ziekenhuis was het klimaat aangenaam voor het ontstaan en ontwikkelen van ideeën en onderzoek. Qais Niemer vertelde mij in het najaar van 1992 alles van transrectale echografie en volumetrie. De samenwerking werd onderdeel van onderzoek naar reproduceerbaarheid. Geen idee was te exotisch om uit te proberen. Mijn oudste vriend Evert Jan Hengeveld raakte verwikkeld in de schijnbewegingen van de prostaat bij echografie. De link naar het PSA werd door Ronald Nooter in de kleine uurtjes uit zijn personal computer geperst.
Met de hulp van de medewerkers van de polikliniek ontstond tijdens de stage aldaar met mysterieuze rode stickers een serumbank van patiënten met onbehandelde prostaatpathologie. Dankzij Bert Blijenberg, hoofd van het klinisch laboratorium, werd de betekenis van deze bank duidelijk. PSA begon ook zijn werk te beheersen vanwege het belang daarvan voor de vroegdetectie van prostaatcarcinoom. Zijn enthousiasme voor samenwerking met een clinicus met belangstelling voor laboratoriumbepalingen was bepalend voor het schrijven van enkele 'essays on assays'. Ik ben bijzonder blij dat hij mijn co-promotor wilde zijn.
Tegelijkertijd suggereerde mijn promotor professor Schröder dat, naast al het onderzoek over diagnostiek, er toch ook een klinisch probleem in de studie verwerkt zou worden. PSA-veranderingen bij onbehandeld prostaatcarcinoom boden een mogelijkheid hiervoor. De ingewikkelde statistiek die hierbij behoort kan alleen maar afkomstig zijn van het kritische en zorgvuldige brein van Wim Hop. Professor Schröder, wiens naam ik nooit correct kon typen wegens een gebrek in mijn simpele tekstverwerkingsprogramma, zette de puntjes op de o. In zijn niet aflatende gedrevenheid kreeg ik van hem de mogelijkheid om onderzoek naar gebonden en vrij PSA te verrichten. Dit bleek eerder een nieuw begin dan een eindpunt van dit boekje.
Gelukkig dat Ries Kranse er was. Zijn nuchterheid en soberheid vormen een genot voor samenwerking. Hij verdient tenminste een eigen telefoonnummer op het trialbureau. I am most thankful to Kim Pettersson, the PSA-specialist from Turku University (Finland), who provided an excellent scientific input.
Els Forman hielp met de tekstverwerking, zodat alle letters gerangschikt op papier kwamen. Ninska Deen zag toe op het Engels. Professor Grobbee, professor Prins, en professor Laméris lazen het concept. Ik dank hen voor de opbouwende kritiek.
Met het VU-busje van mijn oma in de kamer, en de medische studie-aantekeningen van mijn opa in mijn boekenkast, werd deze dissertatie geschreven aan het bureau van mijn grootvader Daddy, die ons altijd voorhield: 'Er is nog nooit een slagersjongen aan werken dood gegaan'. Zijn discipline kreeg ik vast via mijn moeder. Met mijn Grotemoeder kunnen wij nog warme herinneringen uitwisselen. Het doet mij genoegen mijn respect en waardering uit te kunnen drukken voor mijn vader en voor mijn 'heit', die mijn beide paranimfen zijn. Ook zij gingen mij voor.
Mijn echtgenote Marise kan nauwelijks enige hinder ondervonden hebben van het schrijven van mijn proefschrift: haar dissertatie over het meten van prestatiegerichtheid verscheen een maand eerder. Hetgeen niemand verwondert. We lopen al jaren achter elkaar aan.