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DEVELOPMENT AND VALIDATION OF METHODS FOR DETECTION OF PROSTATE CANCER

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Academic Dissertation

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I   Finne P, Auvinen A, Koistinen H, Zhang W-M, Määttänen L, Rannikko S, Tammela TLJ, Seppälä M, Hakama M and Stenman U-H. Insulin-like growth factor-I is not a useful marker of prostate cancer in men with elevated levels of prostate-specific antigen. J Clin Endocr Metab. 2000;85:2744–47.

II   Finne P*, Zhang W-M*, Auvinen A, Leinonen J, Määttänen L, Rannikko S, Tammela TLJ, and Stenman U-H. Use of the complex between prostate-specific antigen and α_1-protease inhibitor in screening for prostate cancer. J Urol. In press 2000.

III Finne P, Auvinen A, Aro J, Juusela H, Määttänen L, Rannikko S, Hakama M, Tammela TLJ, and Stenman U-H. Who should be biopsied in prostate cancer screening? Estimation of prostate cancer risk on the basis of total and free prostate-specific antigen, prostate volume and digital rectal examination. Submitted for publication.

IV   Finne P, Finne R, Auvinen A, Juusela H, Aro J, Määttänen L, Hakama M, Rannikko S, Tammela TLJ, and Stenman U-H. Predicting the outcome of prostate biopsy in screen-positive men by a multilayer perceptron network. Urology. 2000;56:418–22.

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2 ABBREVIATIONS

A2M α_2-macroglobulin
ACT α_1-antichymotrypsin
ANN   artificial neural network
API α_1-protease inhibitor
AUC   area under the curve
BPH   benign prostatic hyperplasia
CI   confidence interval
DRE   digital rectal examination
hK2   human kallikrein 2
IGF-I   insulin-like growth factor I
IGFBP-3   insulin-like growth factor binding protein 3
LVQ   learning vector quantization
MLP   multilayer perceptron
PSA   prostate-specific antigen
ROC   receiver-operating characteristic
SD   standard deviation
TRUS   transrectal ultrasound
TZ   transition zone

3 INTRODUCTION

Screening for prostate cancer by measuring the serum concentration of prostate-specific antigen (PSA) is recommended health policy in the USA. Final scientific evidence that prostate cancer screening reduces mortality in prostate cancer is still lacking. Ongoing randomized screening trials will hopefully reveal whether screening is beneficial. The prostate cancers detected by PSA screening have been shown to mostly be clinically relevant and confined to the prostate gland, and thus potentially curable by radical treatment. A major problem of using serum PSA for screening is the high frequency (about 70%) of false positive test results caused mainly by benign prostatic hyperplasia (BPH) but also by prostatic inflammation and other prostatic diseases. This causes expenses for the society and unnecessary anxiety for the men with falsely elevated serum PSA concentrations. Several methods for reducing the number of false positive PSA results have been developed. Of these, the proportion of free PSA has been most promising and by using this method 20–30% of the false positive PSA results can be identified. However, a larger reduction of the number of false positive PSA results is desirable.

This study was undertaken to evaluate existing methods and to develop new and better ones for reduction of the number of false positive test results in PSA based screening for prostate cancer.

4 REVIEW OF THE LITERATURE

4.1 Epidemiology of prostate cancer

4.1.1 Incidence and prevalence

Adenocarcinoma of the prostate is the most commonly diagnosed cancer among men in industrialized countries, when basal cell cancer is excluded (Parkin, et al., 1997). In Finland 2 839 men were diagnosed with prostate cancer in 1997. The age standardized incidence rate was 72 new cases per 100 000 inhabitants (Finnish Cancer Registry, 2000), and it has been steadily increasing during the past 30 years: on average the yearly numbers were 28, 36, and 48 cases per 100 000 inhabitants in 1970–1974, 1980–1984, and 1990–1994, respectively (Finnish Cancer Registry, 2000). Mean age at diagnosis was 73 years in 1985–1994, and prostate cancer is rarely diagnosed in men younger than 45 years of age (Finnish Cancer Registry, 2000). The incidence of prostate cancer in 1990 was highest in Northern America, Australia, New Zealand, the Carribean, and Western Europe (Table 1) (Ferlay, et al., 1998, Parkin, et al., 1999). The incidence is higher among African-American than among white men (Hankey, et al., 1999). The lowest incidence rates in 1990 were seen in Asia and Northern Africa (Ferlay, et al., 1998, Hsing, et al., 2000). When PSA screening was introduced the incidence in the United States initially increased rapidly during 1988–1992 because a pool of men with latent prostate cancer was diagnosed. Since 1993 the incidence has been decreasing but it remains higher than before the PSA era (Fremgen, et al., 1999, Hankey, et al., 1999). In Fin-

land the incidence was still increasing in 1997 (Finnish Cancer Registry, 2000).

Autopsy studies have shown that about half of 50-year old men and 70–90% of men older than 80 years have latent prostate cancer, discovered only on postmortem examination (Franks, 1954, Sakr, et al., 1993, Sakr, et al., 1994). However, the life time risk of being diagnosed with prostate cancer is 9–11% (Scardino, 1989, Stamey, et al., 1993, Merrill, et al., 1997).

Table 1. Estimated age standardized incidence and mortality rates of prostate cancer in 1990 in various regions of the world (Ferlay 1998 and Parkin 1999).

Region	Incidence rate*	Mortality rate*
Eastern Africa	16.8	9.3
Middle Africa	29.6	17.8
Northern Africa	5.1	3.1
Southern Africa	31.0	18.7
Western Africa	23.9	14.4
Carribean	42.4	22.1
Central America	24.8	13.2
South America	26.8	15.9
Northern America	92.4	18.5
Eastern Asia	2.4	1.3
South-Eastern Asia	5.9	3.5
South Central Asia	4.5	2.8
Western Asia	7.1	4.2
Eastern Europe	14.1	8.4
Northern Europe	34.7	18.6
Southern Europe	16.9	13.2
Western Europe	39.6	19.7
Australia/New Zealand	49.7	18.2
World	19.8	8.3
More developed regions	40.1	14.3
Less developed regions	7.6	4.5

*Age standardized rate (World population) per 100 000 inhabitants

4.1.2 Mortality

The age standardized mortality rate of prostate cancer in 1990 was 18–19 deaths per 100 000 inhabitants in 1990 in Northern Europe and Northern America, whereas it was only 1.3 in Eastern Asia (Table 1) (Ferlay, et al., 1998). According to the SEER (Surveillance, Epidemiology, and End Results) database, the prostate cancer mortality rates in the United States increased 3% in 1987–1991 and decreased 5% in 1992–1997 (Ries, et al., 2000). The mortality rates have correlated with the rising and falling incidence rates caused by screening with PSA (Hankey, et al., 1999). It has been suggested that this correlation is due to attribution bias, i.e., recently diagnosed patients who die of other causes are mislabeled as dying of prostate cancer (Feuer, et al., 1999).

4.1.3 Prognosis

In Finland the survival rates have been improving and the 5-year relative survival rate was 64% in 1985–1994, as compared to 57% in 1975–1984 (Dickman, et al., 1999). In 1990 the estimated 5-year survival in the whole world was 58%. In Northern America the average 5-year survival was the highest (79%) and in Southern Europe the lowest (22%) (Parkin, et al., 1999). The survival rates of prostate cancer are mainly affected by the stage of the disease at diagnosis (Table 2) (Schröder, et al., 1992, Vaughan, et al., 1998), and the stage distribution is affected by how early and in what way the prostate cancer is diagnosed. The high 5-year survival rate in the USA is associated with stage migration, i.e., prostate cancer is increasingly detected at early stages due to screening with PSA (Smith, et al., 1997). In Finland the 5-year survival rate in 1985–1994 of patients with localized prostate cancer, regional metastases, and distant metastases was 84%, 65%, and 25%, respectively. The 10-year survival rate for patients with localized prostate cancer

Table 2. The TNM system for staging of prostate cancer (Schröder, et al., 1992, Vaughan, et al., 1998).

TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
T1	Clinically unapparent tumor–not palpable or visible by imaging
T1a	Tumor found incidentally in tissue removed at transurethral resection: 5% or less of tissue is cancerous
T1b	Tumor found incidentally at transurethral resection: More than 5% of tissue is cancerous
T1c	Tumor found by prostate biopsy because of PSA elevation
T2	Palpable tumor confined within the prostate
T2a	Tumor involves half of a lobe or less
T2b	Tumor involves more than half of a lobe
T2c	Tumor involves both lobes
T3	Palpable tumor extending through prostate capsule and/or involving seminal vesicle(s)
T3a	Unilateral extracapsular extension
T3b	Bilateral extracapsular extension
T3c	Tumor invades seminal vesicle(s)
T4	Tumor is fixed or invades adjacent structures other than seminal vesicles
T4a	Tumor invades bladder neck and/or external sphincter and/or rectum
T4b	Tumor invades levator muscles and/or is fixed to pelvic wall
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastases
N1	Metastasis in a single regional lymph node, < 2 cm in greatest dimension
N2	Metastasis in a single regional lymph node, $\geq$ 2 cm but not $\geq$ 5 cm in greatest dimension, or multiple regional lymph nodes, none $\geq$ 5 cm in greatest dimension
N3	Metastasis in a regional lymph node $\geq$ 5 cm in greatest dimension
MX	Presence of distant metastases cannot be assessed
M0	No distant metastases
M1	Distant metastases
M1a	Involvement of nonregional lymph nodes
M1b	Involvement of bones
M1c	Involvement of other distant sites

was 64%, while it was 9% for those with distant disease and 45% for all cases pooled together (Dickman, et al., 1999). In northern Sweden the 10-year survival rate of patients diagnosed with prostate cancer in 1971–1987 was 45% (Grönberg, et al., 1994), and it tended to be lower (39%) for men younger than 55 years but the difference was not significant.

4.2 Risk factors for prostate cancer

Obesity and a diet rich in fat have been shown to increase prostate cancer risk in some studies (Whittemore, et al., 1995, Giles and Ireland, 1997, Lee, et al., 1998), but a prospective study in Norway showed no correlation between fat intake and prostate cancer risk (Veierod, et al., 1997). The possible correlation may be due to increased levels of testosterone (Shaneyfelt, et al., 2000), or free testosterone in serum (Demark-Wahnefried, et al., 1997), but other studies have shown that prostate cancer risk is not affected by serum testosterone or estrogen (Vatten, et al., 1997, Heikkilä, et al., 1999). Tobacco smoking increases both the risk of being diagnosed with and the risk of dying of prostate cancer (Hsing, et al., 1990, Coughlin, et al., 1996). However, other studies indicate that there is no causal link between tobacco smoking and occurrence of prostate cancer (Adami, et al., 1996, Andersson, et al., 1996). Alcohol has not been shown to play a role in development of prostate cancer (Adami, et al., 1992, Andersson, et al., 1996). Supplementation with ß-carotene increases morbidity and mortality of prostate cancer, whereas alpha-tocopherol and Vitamin E have the opposite effect (Heinonen, et al., 1998).

4.3 Diagnosis of prostate cancer

4.3.1 Symptoms and differential diagnosis

Prostate cancer can be suspected on the basis of clinical symptoms such as reduced urinary flow, urinary retention, hematuria, urinary tract infection, blood in semen, or back pain due to bone metastases (Lukkarinen, et al., 1999). However, these symptoms are neither very specific nor sensitive for prostate cancer, and especially among men with local prostate cancer symptoms are rare. Furthermore, urinary tract symptoms are mostly caused by BPH, which is the most common prostatic disease in men. The incidence of BPH increases with age (Berry, et al., 1984), and about 80% of men older than 60 years have histological BPH while 40% have symptoms (Garraway, et al., 1991).

4.3.2 Digital rectal examination and transrectal ultrasound

By digital rectal examination (DRE) the size and shape of the prostate can be estimated. Indurations, irregularities and nodes are associated with prostate cancer but are not specific. Small tumors are mostly impossible to detect by DRE. The positive predictive value of DRE is low (4–35%) in men with serum concentrations of prostate-specific antigen (PSA) below 4 µg/L because of the low prevalence of prostate cancer in this group of men (Schröder, et al., 1998, Schröder, et al., 2000). However, among men with a PSA of 4 to 10 µg/L detection of organ confined prostate cancer can be improved by using DRE together with serum PSA (Catalona, et al., 1994a, Bangma, et al., 1995a). By transrectal ultrasound (TRUS) it is possible to get a much more precise estimate of prostate volume (Watanabe, et al., 1975, Terris and Stamey, 1991, Rietbergen, et al., 1998), and to visualize the various zones and the echogenic structure of the prostate (Babaian, et al., 1992a). Prostate cancer is usually seen as a hypoechoic area, and this has been used in some studies to predict the outcome of prostate biopsies (Watanabe, et al., 1980, Kranse, et al., 1999). Both DRE and TRUS are investigator-dependent and require much practice to master.

4.3.3 Prostate-specific antigen

PSA is a serine protease with 237 amino acids (Lilja, 1985, Watt, et al., 1986, Lundwall and Lilja, 1987, Schaller, et al., 1987), which was first isolated from prostate tissue and characterized in 1979

(Wang, et al., 1979). It is synthesized in prostatic epithelial cells as a preprotein (preproPSA) with 261 amino acids and converted to the zymogen form (proPSA) comprising 244 amino acids during synthesis (Lundwall and Lilja, 1987). ProPSA is apparently cleaved (and thus activated) when secreted into semen, because no proPSA is found in seminal fluid (Watt, et al., 1986, Schaller, et al., 1987). In semen PSA may be further degraded to 'nicked' forms, in which the peptide chain is cut or nicked at 1 to 3 points (Watt, et al., 1986, Christensson, et al., 1990, Zhang, et al., 1995). In seminal fluid PSA exists in five isoforms A–E, of which A and B are intact and enzymatically active (Zhang, et al., 1995). The physiological role of PSA is to digest the gel-forming proteins causing liquefaction of the semen (Lilja, 1985, Lilja, et al., 1987). The concentration of PSA in semen is 0.5–2 mg/L which is about one million-fold the normal concentration in serum. PSA is organ-specific and expressed both in normal and malignant prostatic epithelial cells. Low expression has been found in other tissues (Kamoshida and Tsutsumi, 1990, Diamandis and Yu, 1997). Female breast tissue has been shown to be the most notable source of nonprostatic PSA, but the concentration of PSA in seminal plasma is 1 000-fold that in nipple aspirate and 10 000-fold that in breast milk (Diamandis and Yu, 1997). The serum concentration of PSA is elevated in men with prostate cancer although the PSA expression in malignant prostatic epithelial cells has been shown to be somewhat lower than in normal cells (Abrahamsson, et al., 1988). To reach circulation from benign prostatic tissue PSA has to diffuse through the ductal walls or from the epithelial cells into extracellular fluid. Thus only a very small proportion of the PSA produced reaches serum. In prostate cancer the ductal structure of the prostate is deranged and it has been suggested that PSA can be actively secreted into extracellular fluid and the cap-

illaries of the tumor (Stenman, et al., 1999). Because of this the contribution of prostate cancer tissue to the serum concentration of PSA is 10-fold that of BPH tissue and 30-fold that of normal prostatic tissue (Stamey, et al., 1987).

Elevation of the serum PSA concentration is not specific to prostate cancer: elevated levels may also be caused by BPH and other benign prostatic diseases such as prostatitis (Brawer, 1999). In fact, about 65–75% of the men with an elevated serum PSA above the generally used cutoff value 4 µg/L do not have prostate cancer in biopsy (Table 3) (Catalona, et al., 1991, Brawer, et al., 1992, Labrie, et al., 1992, Catalona, et al., 1993, Labrie, et al., 1993, Catalona, et al., 1994a, Schröder, et al., 1998, Määttänen, et al., 1999). The number of false positive PSA results can be reduced by using higher cutoff values, but then the sensitivity will decrease as a considerable number of cancer cases are missed while an increasing proportion of the cases found are no more at a curable stage.

4.3.4 Histological diagnosis

When prostate cancer is suspected the diagnosis is confirmed by histopathological examination of tissue samples obtained by needle biopsies. Routinely, 6–8 needle biopsies are taken under TRUS guidance. Additional biopsies are often taken if suspicious areas are identified by TRUS or DRE. The sensitivity of first round sextant prostate biopsies for prostate cancer is not perfect and 10–30% of repeat biopsies taken from men with one previous negative set of sextant biopsies will be positive (Fleshner, et al., 1997, Borboroglu, et al., 2000, Djavan, et al., 2000). WHO grade and Gleason score (Table 4) describe the degree of differentiation of the tumor (Mostofi, 1975, Gleason, 1992). Poorly differentiated tumors are associated with more aggressive growth and larger risk of extraprostatic spread (Albertsen, et al., 1998).

Table 3. Proportion of men with prostate cancer on biopsy according to level of serum PSA in various screening studies.

Author	Year	PSA 4–10 µg/L PCa/Bx (%)	PSA ≥ 10 µg/L PCa/Bx (%)	PSA ≥ 4 µg/L PCa/Bx (%)
Catalona, et al.	1991	19/85 (22%)	18/27 (67%)	37/112 (33%)
Brawer, et al.	1992	23/87 (26%)	9/18 (50%)	32/105 (30%)
Labrie, et al.	1992	23/89 (26%)	18/35 (51%)	41/124 (33%)
Labrie, et al.	1993	101/716 (14%)	95/240 (40%)	196/956 (21%)
Catalona, et al.	1993	174/652 (27%)	122/208 (59%)	296/860 (34%)
Catalona, et al.	1994	143/548 (26%)	73/138 (53%)	216/686 (31%)
Schröder, et al.	1998	238/988 (24%)	113/196 (58%)	351/1184 (30%)
Määttänen, et al.	1999	63/327 (19%)	43/74 (58%)	106/401 (26%)
All		784/3 492 (22%)	491/936 (52%)	1 275/4 428 (29%)

Key: PCa = Number of prostate cancer cases; Bx = Number of biopsied men

Table 4. The Gleason grading system of prostate cancer.

Grade	Description
1	Simple round glands, close-packed in rounded masses with well-defined edges
2	Simple rounded glands, loosely packed in vague, rounded masses with loosely defined edges
3A	Medium sized single glands of irregular shape and irregular spacing with ill-defined infiltrating edges
3B	Very similar to 3A, but small to very small glands, which must not form significant chains or cords
3C	Papillary and cribriform epithelium in smooth, rounded cylinders and masses; no necrosis
4A	Small, medium, or large glands fused into cords, chains, or ragged, infiltrating masses
4B	Very similar to 4A, but with many large clear cells, sometimes resembling "hypernephroma"
5A	Papillary and cribriform epithelium in smooth, rounded masses, more solid than 3C and with central necrosis
5B	Anaplastic adenocarcinoma in ragged sheets

Reference (Gleason, 1992)

4.4 Treatment of prostate cancer and complications of the treatment

4.4.1 Local prostate cancer

If the prostate cancer is localized to the prostate gland it can be treated by surgery (Catalona, et al., 1999), radiotherapy (Shipley, et al., 1999), brachytherapy (Blasko, et al., 1996), and sometimes only actively followed (watchful waiting) (Johansson, et al.,

1992, Catalona, 1994b). The aim of radical prostatectomy is to remove the cancer tissue entirely, which is mostly achievable when the tumor is confined to the prostate (stage T1–T2N0M0). Due to the risk of complications, radical prostatectomy is usually performed only when the expected life time of the patient is at least ten years. An alternative to surgery is radical radiotherapy, which may be curative also when the cancer has spread locally outside the prostatic capsule (T1–T3N0M0) (Catalona, 1994b, Lukkarinen, et al., 1999). Radiotherapy can also be given interstitially (brachytherapy) by implanting radioactive beads in the prostate (Blasko, et al., 1996). For patients older than 70 years with a clinically localized (T1–T2N0M0) and well-differentiated (Gleason score 2–4) prostate cancer watchful waiting, i.e., follow-up by clinical and laboratory methods every sixth month is a viable alternative. Randomized studies comparing various treatment modes for local prostate cancer are still lacking. A non-randomized study in one hospital showed that the 5-year probability of freedom from PSA failure (PSA level increasing after treatment) was comparable after radical prostatectomy, radiotherapy and brachytherapy (D'Amico, et al., 1998). However, brachytherapy was associated with a lower probability of freedom from

PSA failure among high-risk patients (with a PSA above 10 µg/l and a Gleason score of 7 or higher).

After radical prostatectomy the incidence of impotence is 30–60% depending on age, tumor stage, and surgical technique (Catalona, 1994b). Thus, the risk may be reduced in selected patients by using a nerve-sparing technique (Quinlan, et al., 1991). Incontinence occurs in 5–15% of the patients (Catalona, 1994b, Walsh, et al., 1994). Radical radiotherapy also causes complications: impotence (40–60%), chronic cystitis (8%), urethral stricture (4%), chronic proctitis (2%), and incontinence (<1%) (Catalona, 1994b). Brachytherapy has been associated with a lower frequency of impotence, about 20% (Blasko, et al., 1996).

4.4.2 Advanced prostate cancer

Endocrine therapy is the main treatment of advanced metastatic prostate cancer (Catalona, 1994b, Lukkarinen, et al., 1999). Well differentiated prostate cancer cells are dependent on androgens and the aim of endocrine manipulations is to deprive the tumor cells of testosterone. This can be achieved by orchiectomy, administration of estrogens, antiandrogens or synthetic agonists of gonadotropin-releasing hormone (GnRH) derivates. Estrogens (polyestradiole phosphate, stilbestrol) suppress the secretion of pituitary luteinizing hormone (LH), which in turn leads to reduced testicular output of testosterone. Synthetic GnRH analogs act similarly. After an initial transient increase in LH secretion, long-term administration of GnRH agonists suppress LH release, achieving in effect a chemical castration. Antiandrogens (cyproterone acetate, bicalutamide, flutamide, nilutamide) block the effect of androgens on prostate cancer cells, but are not recommended as the only treatment for prostate cancer (Lukkarinen, et al., 1999). Antiandrogens are used in total androgen blockade treatment as an adjuvant to surgical or chemical cas-

tration, but in randomized trials total androgene blockade has not provided longer survival than conventional castration (Prostate Cancer Trialists' Collaborative Group, 1995, Eisenberger, et al., 1998).

Endocrine treatment based on androgen deprivation causes impotence, gynecomastia, change of the male habitus, and reduced muscle mass (Lukkarinen, et al., 1999). Estrogen treatment is associated with cardiovascular side effects, but these can be reduced by administrating estrogen parenterally (Hedlund and Henriksson, 2000).

4.5 Screening for prostate cancer with serum PSA

In most cases serum PSA becomes elevated 5–10 years before clinical diagnosis of prostate cancer (Carter, et al., 1992, Stenman, et al., 1994, Gann, et al., 1995). Thus, prostate cancer can be detected long before clinical presentation by screening with serum PSA, and most screen-detected prostate cancers are confined to the prostate and can be removed by radical prostatectomy (Mettlin, et al., 1997). Population screening by serum PSA has been recommended in order to reduce mortality and morbidity from prostate cancer (Smith, et al., 2000), but screening has not yet been shown to do this (Adami, et al., 1994, Collins and Barry, 1996). Prospective population-based studies have been initialized (Auvinen, et al., 1996), and these will hopefully reveal whether prostate cancer screening is beneficial.

4.5.1 Validity of the serum PSA test

In ongoing randomized population-based screening studies serum PSA has been found to be elevated above 4 µg/L in 8.5–12.5% of the screened population (Schröder, et al., 1998, Määttänen, et al., 1999). In 21–34% of men with a serum PSA >4 µg/L cancer was found on prostate bi-

opsy (Table 3) (Catalona, et al., 1991, Brawer, et al., 1992, Labrie, et al., 1992, Catalona, et al., 1993, Labrie, et al., 1993, Catalona, et al., 1994a, Schröder, et al., 1998, Määttänen, et al., 1999). Thus the frequency of false positive results is 66–79%. This is a drawback of the PSA test because false positive results cause considerable emotional stress for the patient and expenses for the society (Brawer, 1999, Smith, et al., 2000). However, some of the initially negative sextant biopsies are false negatives and at rebiopsy more cancer cases will be detected (Fleshner, et al., 1997, Borboroglu, et al., 2000).

Schröder et al. (Schröder, et al., 1998), took biopsies from part of the patients with PSA values below 4 µg/L and based on the detection rates in the low strata of serum PSA concentrations 7.9% (730 of 9211) of the men with a serum PSA below 4 µg/L were estimated to would have had a prostate cancer detected by biopsy. On the basis of this study it can be calculated that if all men would be biopsied regardless of the PSA concentration 35% of the cases with biopsy detected prostate cancer would have a serum PSA of 4 µg/L or higher, i.e., the sensitivity of the PSA test would be 35%. The specificity would correspondingly be 90%. This is in line with the findings in two serum bank studies that showed 91–92% sensitivity and 46–51% specificity of detecting prostate cancer within 6–10 years from serum PSA sampling (Stenman, et al., 1994, Gann, et al., 1995). In prostate cancer screening a higher sensitivity of the PSA test can be achieved by repeated screening (Carter, et al., 1997a).

The range of serum PSA concentrations of 4–10 µg/L has been called the diagnostic grey zone because the prostate cancer is detected in only about 20–25% of the men with a serum PSA in this range. There is a need to identify low-risk patients in this range who could be spared prostate biopsy. Among patients with a serum PSA of 10–20 µg/L in screening, about half have prostate cancer at biopsy, and of the cancers detected less than half are pathologically localized to the prostate and thus radically treatable (Catalona, et al., 1993).

4.5.2 Clinical relevance of detected cancers

Autopsy studies have shown that more than 50% of men older than 50 years have histological evidence of prostate cancer (Franks, 1954, Sakr, et al., 1994). Because only 9–11% are diagnosed with prostate cancer 80% of these cancers will not develop into clinical disease. In randomized population-based screening studies prostate cancer has been detected in 2.1–3.3% of the screened population when taking prostate biopsies from men with a serum PSA concentration above 4 µg/L (Schröder, et al., 1998, Määttänen, et al., 1999). Thus less than 10% of the latent prostate cancers are detected by the first round of screening. Screening appears to detect clinically relevant tumors as more than 95% of the screen-detected cancers are considered clinically significant in terms of tumor size and differentiation (Catalona, et al., 1993). Epstein et al. found that 16% of the patients with T1c prostate cancer had potentially biologically insignificant tumors (Epstein, et al., 1994). Hugosson et al. recently showed that the cumulative risk of having clinical prostate cancer is 33% for men with a PSA of 3 µg/L or higher (Hugosson, et al., 2000). However, the true clinical relevance of the screen-detected cancers, in terms of mortality and morbidity, remains to be shown in prospective studies.

4.6 Methods used to improve the performance of the serum PSA test

A method that improves the accuracy of the serum PSA test should either reduce the number of false positive or false negative PSA results, or both. Several concepts have been suggested.

4.6.1 Age-specific reference ranges

Concentrations of serum PSA correlate positively with prostate volume and age (Babaian, et al., 1990, Babaian, et al., 1992b). An increased concentration of serum PSA is mostly caused by BPH, the prevalence of which rapidly increases after age 50. Age-specific reference ranges of PSA have been suggested to compensate for the increase in PSA attributable to age (Oesterling, et al., 1993, Oesterling, et al., 1995), and to reduce the number of false negative PSA results in young men and false positive PSA results in older men. The upper reference limit calculated on the basis of the 95th percentile of serum PSA among healthy men has been determined to be 2.5, 3.5, 4.5, and 6.5 µg/L for men aged 40–49, 50–59, 60–69, and 70–79, respectively (Oesterling, et al., 1995). Use of these reference limits lowers the detection rate of prostate cancer in older age groups in which the prevalence of the disease is highest. Age specific reference ranges have been criticized for reducing the detection rate and decreasing the expected survival time of the detected cancer cases (Etzioni, et al., 1996). Therefore age-specific reference ranges giving 95% sensitivity of cancer detection have been proposed (Morgan, et al., 1996). The proportion of free PSA is not dependent on age and a cutoff of 15% has been suggested for all age groups (Oesterling, et al., 1995).

4.6.2 Increase in serum PSA with time (PSA velocity)

Men who are developing prostate cancer have a faster increase of the serum PSA concentration than other men. An increase of serum PSA larger than 0.75 µg/L per year is associated with a significantly increased risk of developing prostate cancer (Carter, et al., 1992, Smith and Catalona, 1994). This method, which is also called PSA velocity, is mainly intended to be used in men with PSA values below 4 µg/L and can

therefore reduce the number of false negative PSA results. However, this method is laborious and slow requiring repeated PSA measurements during several years. Another problem is the considerable day-to-day variation of serum PSA (Ornstein, et al., 1997). Furthermore, the proportion of free PSA has been shown to be a more accurate diagnostic variable than PSA velocity among men with a serum PSA of 2.5–4.0 µg/L (Djavan, et al., 1999a).

4.6.3 PSA in relation to prostate volume (PSA density)

At a fixed serum PSA level the average prostate volume has been shown to be smaller in men with prostate cancer than in those without (Bangma, et al., 1995b, Standaert, et al., 1997, Kranse, et al., 1999). This is due to the fact that prostate cancer tissue (per gram tissue) releases about ten times as much PSA to the circulation as BPH tissue (Stamey, et al., 1987). Veneziano et al. were the first to show that the accuracy of serum PSA can be improved by dividing the PSA value with the prostate volume (Veneziano, et al., 1990). This finding has been confirmed in later studies and the concept is called PSA density (Benson, et al., 1992, Bangma, et al., 1997, Rietbergen, et al., 1998). Because the volume of the transition zone (TZ) is preferentially enlarged in BPH, serum PSA divided by TZ volume has been considered more accurate than PSA density (Kalish, et al., 1994, Zlotta, et al., 1997, Djavan, et al., 1998, Djavan, et al., 1999a). Some investigators did not find any benefit of PSA density or TZ density in comparison with serum PSA alone (Brawer, et al., 1993, Lin, et al., 1998). The yield of a standardized number of prostate biopsies is smaller in large glands (Uzzo, et al., 1995, Karakiewicz, et al., 1997), and it has been speculated that this is the basis for the diagnostic value of PSA density and TZ density (Brawer, 1995). Prostate volume is an investigator-

dependent variable and this may affect the diagnostic accuracy of PSA density.

4.6.4 Molecular forms of PSA in serum

PSA occurs in serum as non-complexed or free PSA and in complex with various protease inhibitors (Lilja, et al., 1991, Stenman, et al., 1991). When added to serum, intact PSA rapidly forms complexes with α_2-macroglobulin (A2M) and to a smaller extent with α_1-antichymotrypsin (ACT) (Christensson, et al., 1990), and α_1-protease inhibitor (API) (Leinonen, et al., 1996). Complexes of proteases with A2M have a rapid half time (2–5 minutes) in circulation. In rats the half time of PSA-A2M has been shown to be about 7 minutes (Birkenmeier, et al., 1999). The major form of PSA in serum is the complex with ACT which constitutes 50–90% of all immuno-reactive PSA. The proportion of PSA-ACT is higher and the proportion of free PSA is lower in serum from prostate cancer cases than in men with BPH (Stenman, et al., 1991, Christensson, et al., 1993, Leinonen, et al., 1993). The proportions of free PSA and PSA-ACT have repeatedly been shown to reduce the frequency of false positive PSA results by 20–30% in men with a serum PSA above 4 μg/L (Catalona, et al., 1995, Partin, et al., 1996, Reissigl, et al., 1996, Bangma, et al., 1997, Catalona, et al., 1998), and to reduce the number of false negative PSA results when serum PSA is below 4.0 μg/L (Catalona, et al., 1997, Lodding, et al., 1998, Djavan, et al., 1999a, Törnblom, et al., 1999). The proportion of free PSA is an earlier predictor of a subsequent development of prostate cancer than total PSA (Pearson, et al., 1996), and it has also been shown to predict the aggressiveness of the cancer several years before clinical diagnosis (Carter, et al., 1997b, Arcangeli, et al., 1998).

An assay measuring the complex between PSA and API has recently been developed (Zhang, et al., 1997), and about 1–10% of serum PSA has been shown to be complexed with API (Zhang, et al., 1999), but the true concentration is somewhat smaller due to a nonspecific background in the assay. The proportion of PSA-API in serum is lower in men with than in those without prostate cancer (Zhang, et al., 1999). Assays for total PSA measure PSA-ACT, PSA-API, and free PSA. Part of PSA in serum is complexed to A2M, but due to encapsulation of PSA in A2M this complex cannot be detected by conventional immunological methods. However, incubation at high pH denatures A2M, and PSA in the complex and released from it can be measured by a conventional assay for PSA (Zhang, et al., 1998). Other methods of determining PSA-A2M have also been proposed (Espana, et al., 1996, Lilja, et al., 1999). Among subjects with a serum PSA of 4–10 μg/L the ratio of PSA-A2M to total PSA is lower in prostate cancer cases (median 0.06) than in men with BPH (median 0.12) and adds diagnostic information to the proportion of free PSA (Zhang, et al., 2000).

4.6.5 Human kallikrein 2

Human kallikrein 2 (hK2) belongs to the human glandular kallikrein protein family and shows 80% homology with PSA both at the protein and mRNA levels (Schedlich, et al., 1987). Many antibodies detecting total PSA have been shown also to detect hK2 (Lövgren, et al., 1995). Several assays for specific determination of hK2 in serum have been developed (Piironen, et al., 1996, Finlay, et al., 1998, Black, et al., 1999, Becker, et al., 2000a). The serum concentrations of hK2 correlate with total and free PSA (Piironen, et al., 1996, Recker, et al., 1998, Magklara, et al., 1999). Both the serum level of hK2 and the ratio between hK2 and the proportion of free PSA have been shown to correlate positively with prostate cancer risk (Nam, et al., 2000), and to increase the diagnostic accuracy for prostate cancer at intermediate levels of serum

PSA (Magklara, et al., 1999, Partin, et al., 1999, Becker, et al., 2000b, Becker, et al., 2000c). It is noteworthy that the ratio between hK2 and the proportion of free PSA has been shown to add diagnostic information to the proportion of free PSA at serum PSA concentrations of 2.0–4.5 µg/L (Magklara, et al., 1999, Partin, et al., 1999). Both hK2 and the ratio between hK2 and the proportion of free PSA can be used to predict organ-confined disease and the differentiation of the tumor independently of total and free PSA (Haese, et al., 2000, Recker, et al., 2000).

4.6.6 *Insulin-like growth factor-I and its binding proteins*

Insulin-like growth factor-I (IGF-I) is a mitogenic peptide that mediates the growth promoting effect of growth hormone (Daughaday and Rotwein, 1989). Elevated serum concentrations of IGF-I have been associated with both prostate cancer (Mantzoros, et al., 1997, Chan, et al., 1998, Wolk, et al., 1998), and BPH (Cohen, 1998). In serum the major part of IGF-I is bound to one of its binding proteins, IGFBP-3 (Martin and Baxter, 1992). The serum concentrations of both IGF-I and IGFBP-3 are mainly regulated by growth hormone but also affected by nutritional status, age, pregnancy and chronic disease (Martin and Baxter, 1992). PSA has been suggested to cleave IGFBP-3 (Cohen, et al., 1992, Okabe, et al., 1999), leading to release of IGF-I. It has been speculated that PSA causes release of IGF-I locally in the prostate (Cohen, et al., 1994).

Chan et al. demonstrated in a serum bank study with 152 prostate cancer cases and 152 matched controls that men in the highest quartile of serum IGF-I levels had a relative risk of prostate cancer of 2.4 in comparison with men in the lowest quartile (Chan, et al., 1998). With adjustment for the serum concentration of IGFBP-3 the relative risk was 4.3. Two other case-control studies have shown a positive correlation between serum IGF-I concentration and prostate cancer risk (Mantzoros, et al., 1997, Wolk, et al., 1998). In one of these studies adjustment for the IGFBP-3 concentration was performed and this increased the odds ratio of IGF-I for prostate cancer (Wolk, et al., 1998). Other studies have not shown higher serum IGF-I concentrations in men with than in those without prostate cancer (Cohen, et al., 1993, Kanety, et al., 1993, Ho and Baxter, 1997). A recent study indicated that IGF-I is not a useful marker for prostate cancer (Cutting, et al., 1999). whereas another study indicated diagnostic usefulness of IGF-I when it is divided by the concentration of serum PSA (Djavan, et al., 1999b).

4.6.7 *Statistical and mathematical methods combining diagnostic information of multiple variables*

Logistic regression is a statistical method especially suited for prediction of binary outcomes (Hosmer and Lemeshow, 1989). The linear predictor (x) given by the logistic regression equation is fitted to the range 0–1 by a transformation $[(e^x/(1+e^x)]$ and this is an estimate of the probability of the outcome. By logistic regression the diagnostic value of individual variables can be estimated, and variables with simultaneous importance can be combined into a diagnostic algorithm. Logistic regression has been used to construct diagnostic algorithms for diagnosis of prostate cancer in both clinical (Marley, et al., 1996, Optenberg, et al., 1997, Carlson, et al., 1998) and screening settings (Standaert, et al., 1997, Gomari, et al., 1998, Kranse, et al., 1999, Virtanen, et al., 1999). Serum PSA, the proportion of free PSA, DRE results, TRUS findings, prostate volume, age, race and family history of prostate cancer have been included in the equations.

Artificial neural networks (ANNs) are mathematical and statistical models that can

be optimized to recognize relationships within data sets. An ANN can be trained to diagnose prostate cancer (Gomari, et al., 1998, Wei, et al., 1998, Virtanen, et al., 1999). The most commonly used ANN in clinical medicine is the multilayer perceptron (MLP) (Wei, et al., 1998). A larger number of parameters can more easily be included in MLP than in logistic regression. This brings flexibility to the optimization process and ANNs can detect more complex relationships within the data set than logistic regression. In fact, as with logistic regression ANNs can also be made to fit the data set perfectly. This is called overtraining or overfitting, and it will ruin the general applicability of the the diagnostic model. To avoid overtraining the training process can be stopped when the fit of the model starts to decrease in an external data set (early stopping technique) (Bishop, 1995). Another approach is to use Bayesian regularization that in addition to the sum of squares of errors also minimizes the sum of squares and the number of the model parameters (MacKay, 1992, Foresee and Hagan, 1997). This is done after the variables have been normalized (so that their standard deviation is one) and orthogonalized.

Snow et al. were the first to develop a neural network for prediction of prostate biopsy outcome (Snow, et al., 1994). This network was based on serum PSA, age, DRE, and TRUS in men with a serum PSA above 4 μg/L, and it showed 88% specificity at 84% sensitivity. These results may not be reproducible because the test set of patients was used for early stopping of the network training (Wei, et al., 1998). Gomari et al. compared the capacity of logistic regression and three artificial neural network models: learning vector quantization (LVQ), MLP, and neurofuzzy network to predict outcome of prostate biopsy in screened men with a serum PSA of 3–10 μg/L (Gomari, et al., 1998). These diagnostic models were based on various combinations of age, total and free PSA, DRE, TRUS, first-degree family history of prostate cancer, and symptoms of the disease. The LVQ model was more accurate than the proportion of free PSA, the logistic regression and the MLP models, which did not differ significantly from each other. Virtanen et al. developed an MLP model based on total and free PSA in serum, DRE, TRUS, and family history of prostate cancer, but the model was less accurate than a corresponding logistic regression model (Virtanen, et al., 1999).

5 Aims of the study

The aim of the present investigation was:

1) to develop methods for reducing the number of false positive serum PSA results in men screened for prostate cancer (II–IV)

2) to compare and assess the validity of various diagnostic methods for prostate cancer among men with elevated serum PSA (I–IV)

3) to identify variables that independently predict the outcome of prostate biopsies and to use these variables to construct diagnostic models (III, IV).

6 MATERIALS AND METHODS

6.1 Subjects (I–IV)

The subjects were identified within the Finnish prostate cancer screening study (Määttänen, et al., 1999), which is a part of the European randomized study on prostate cancer (Schröder and Bangma, 1997). The subjects in the studies I–IV were screened in 1996 and 1997. They were aged 55–67 years, and had a serum PSA concentration of 4 µg/L or higher (Table 5). Because men with a serum PSA < 4 µg/L did not undergo prostate biopsy systematically, only those with a serum PSA > 4 µg/L were included. Of these, 6% lacked biopsy results for various reasons. In the present investigation all men without prostate cancer were used as a control group, and among these the most common biopsy finding was normal histology (56%), followed by BPH (36%), prostatitis (4%), prostatic intraepithelial neoplasia (2%), and other diagnoses (2%). Of the prostate cancer patients 34%, 47%, 11%, and 7% had a Gleason score of 2–4, 5–6, 7, and 8–10, respectively. The frequency of tumors with a low Gleason score is higher than observed in another screening study (Hoedemaeker, et al., 2000). The distribution of WHO grade was 42% grade I, 50% grade II, and 8% grade III. The prostate

cancer was clinically localized to the prostate gland (T0–T1M0) in 83%, locally advanced (T3–T4M0) in 12%, and metastasized (T1–T4M1) in 5% of the cases.

6.2 Serum samples and laboratory methods (I–IV)

Sera were kept frozen at –80ºC and were thawed once for analysis of total and free PSA, immediately refrozen, and rethawed for the analysis of IGF-I, IGFBP-3, and PSA-API. The samples were analyzed in random order blinded with regard to case-control status.

The concentrations of total and free PSA were measured simultaneously by a dual label time-resolved immunofluorometric assay (Prostatus, EG&G-Wallac) (Mitrunen, et al., 1995). Total PSA was also determined with the Beckman-Hybritech Tandem-E method which correlates strongly with the Prostatus method (Blijenberg, et al., 1997). In this study the results of the Prostatus method were used.

Total serum IGF-I was measured by a sandwich-type immunoassay (Active IGF-I ELISA, DSL-10-5600, Diagnostic Systems Laboratories, Inc., Webster, TX). The assay uses acid-ethanol extraction to dissociate IGF-I from its binding proteins. Serum IGFBP-3 was measured by an immunofluorometric assay (IFMA) using monoclonal antibodies (1B6/5C11) against recombinant IGFBP-3 (Koistinen, et al., 1994). The assay detects only intact IGFBP-3 and shows no cross-reactions with the other human IGFBPs or IGFs. IGF-I

Table 5. Number of subjects and their PSA concentrations in the original publications.

Study	I	II	III	IV
Men with prostate cancer (n)	179	78	200	148
Men without prostate cancer (n)	486	226	558	508
All subjects (n)	665	304	758	656
Range of serum PSA (µg/L)	≥ 4	≥ 4	4–20	4–10

and IGF-II do not interfere with the assay (Koistinen, et al., 1994).

The serum concentration of PSA-API was measured with an immunofluorometric assay with a monoclonal antibody (H117) to total PSA on the solid phase and a polyclonal antibody to API as the detector antibody (Zhang, et al., 1999). For each sample the nonspecific background signal (produced by nonspecific binding of the huge amount of API to the solid phase) was measured with an assay using an unrelated solid-phase antibody but the same detector antibody. By subtracting the signal caused by the nonspecific binding of API the detection limit of the assay could be improved. The biological detection limit of the assay was estimated as the mean value plus 2 SD of PSA-API measured in sera from 42 females. Patients with a serum PSA-API value below this were given a value of half the detection limit (0.025 µg/L).

6.3 DRE, prostate volume and prostate biopsies (I–IV)

Clinical investigation of the patients was performed by several urologists in four medical centers. DRE was defined as positive if any abnormality was palpated. Prostate volume was estimated by TRUS according to the formula $(\pi/6)$ x (transverse diameter x anteroposterior diameter x cephalocaudal diameter) (Terris and Stamey, 1991). Sextant biopsies of the prostate were taken under TRUS guidance, and additional biopsies were obtained from suspicious lesions identified by TRUS or DRE. Men without cancer in the first biopsy were rebiopsied within six months if the concentration of serum PSA was higher than 10 µg/L or if they had high grade prostatic intraepithelial neoplasia.

6.4 Statistical methods

6.4.1 General (I–IV)

The Mann-Whitney U test was used to compare the distribution of age, serum total PSA, the proportion of free PSA, IGF-I, IGFBP-3, PSA-API, and prostate volume between cases and controls. The difference in distributions of binary variables (DRE findings, and family history of prostate cancer) between cases and controls were assessed by the chi square test. Correlations between continuous variables were estimated by partial correlation (I), and by calculating Pearson's correlation coefficient (II). When necessary, the variables were logarithmically transformed to achieve normal distribution before analyses of correlations. The difference in serum PSA-API levels in sera from 42 females before and after background extraction was tested by Wilcoxon matched-pairs signed-ranks test (II). To evaluate the diagnostic validity of the variables, clinical sensitivity, specificity (I-IV), positive predictive value, and negative predictive value were calculated (IV) (Table 6). Specificities at various levels of sensitivity were compared by the McNemar test (II, III). To evaluate the diagnostic accuracy of various

Table 6. Explanations for various concepts describing diagnostic validity.

Concept	Definition
Specificity	Proportion of subjects without disease who have a negative test
Sensitivity	Proportion of subjects with disease who have a positive test
Negative predicitve value	Proportion of subjects with a negative test who do not have the disease
Positive predictive value	Proportion of subjects with a positive test who have the disease
Accuracy	Proportion of subjects with a correct test result

diagnostic tests, receiver-operating charac-teristic (ROC) curves were constructed and the area under the curve (AUC) was calcu-lated according to the method of Hanley and McNeil (I, II) (Hanley and McNeil, 1982). The AUC represents the probabil-ity of a randomly selected case patient to have a higher value than a randomly selected control patient. If the AUC is 0.5 the diag-nostic test is of no value and if AUC is 1 the test is perfect.

6.4.2 Logistic regression (I–IV)

Multiple logistic regression analysis was used to identify and quantify the diagnostic value of variables that simultaneously affect the probability of having prostate cancer. The binary response variable in the logistic regression analyses was presence or absence of prostate cancer in prostate biopsy (I–IV). Explanatory variables were the concentra-tion of total PSA in serum (I-IV), age (I, III, IV), serum IGF-I (I), serum IGFBP-3 (I), prostate volume (I, III, IV), the proportion of free PSA (II–IV), the concentration of PSA-API (II), DRE findings (III–IV), and a family history of prostate cancer (father or brother having prostate cancer) (III–IV). Various transformations (logarithmic, qua-dratic, square root, categorical, and dichoto-mous) of continuous explanatory variables were tested in order to achieve a better fit of the model as assessed by the Hosmer-Lemeshow test and the deviance of the model (Hosmer and Lemeshow, 1989). No first or higher order interactions were found be-tween any of the explanatory variables. Prob-abilities of having prostate cancer in biop-sies were calculated on the basis of the lo-gistic regression models (II–IV). 95% con-fidence intervals (CI) of these probabilities were calculated with SAS 6.12 for Windows. Other analyses were performed with SPSS 6.0 for the Macintosh. Odds ratios of pros-tate cancer for various variables were ob-tained by calculating the exponents of the regression coefficients of the variables.

6.4.3. MLP with Bayesian regularization (IV)

The neural network model used (IV) was an MLP network composed of one input layer with four preprocessed variables (total PSA, the proportion of free PSA, prostate volume and DRE), one hidden layer with two neu-rons, and one output layer with one neuron giving the output value that is a measure of the probability of cancer (Figure 1). The four input variables were normalized so that their standard deviations were 1 and orthogonal-ized by principal component analysis (Bishop, 1995), which has the capability to reduce the number of input variables but did not do so in this study. The network model contained 13 parameters (weights) to be optimized. The activation function used in the hidden layer and the output layer was the hyperbolic tangent sigmoid function $[a=\tanh(s)=(e^{s}-e^{-s})/(e^{s}+e^{-s})]$ that generates output values (a) between -1 and 1; s is the weighted sum of preceding neuron outputs.
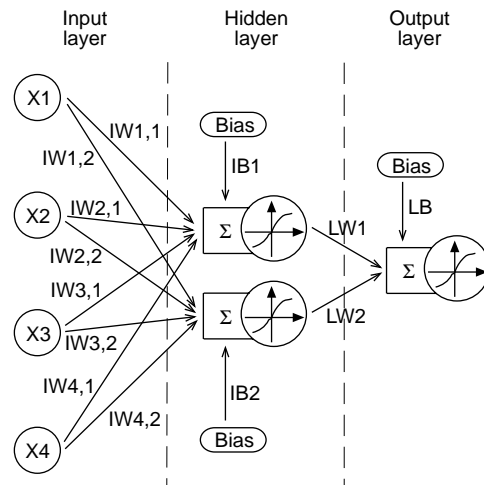

Figure 1. Schematic picture of the MLP model used. X1–X4 represent the input variables (total PSA, the proportion of free PSA, DRE and prostate volume). IW stands for input weight, IB for input bias, LW for layer weight, and LB for layer bias.

The formula for the whole network in Figure 1 can be written mathematically as following:

$$a_1 = \tanh(IW1,1*X1 + IW2,1*X2 + IW3,1*X3 + IW4,1*X4 + IB1)$$
$$a_2 = \tanh(IW1,2*X1 + IW2,2*X2 + IW3,2*X3 + IW4,2*X4 + IB1)$$
$$a_{out} = \tanh(LW1*a_1 + LW2*a_2 + LB)$$

where a_1 and a_2 are the outputs of the hidden neurons and a_{out} is the output of the network. After the model building, the output value was linearly transformed into the range 0–1. The network was trained using Levenberg-Marquardt optimization combined with Bayesian optimization of the regularization parameters (Foresee and Hagan, 1997). The aim of regularization is to avoid overtraining of the model by minimizing the sum of squares of errors and the sum of squares of model parameters. The Bayesian technique controls the regularization so that these two minimizing processes occur in a balanced manner (MacKay, 1992, Foresee and Hagan, 1997).

Suitable initial weights for the MLP model were estimated by randomly dividing the 655 (656–1) patients in each training set into two groups. The model was initially fitted on 75% and tested on 25% of the patients in the training set. The random-ization, fitting and testing sessions were repeated 5 times and the weights producing the smallest sum of squared errors on the initial test set were selected as initial weights for the final training of the MLP. The MLP models were optimized with the MATLAB Neural Network Toolbox, version 3.

6.4.4. *Validation of diagnostic models (II, IV)*

The generalizability of the diagnostic models were assessed by cross validation (II) and leave-one-out validation (IV) (Moody and Utans, 1995). The patients were divided into a training set (with all minus n patients) and a test set with n patients. The training and testing sessions were repeated till every patient had been a part of the test set once. In the cross validation in study (II) the patients were randomly divided into four groups and consequently trained on 75% and tested on 25% of the patients. This was repeated four times. In leave-one-out validation n is one, and as the study (IV) comprised 656 patients there were 656 rounds of training and testing. To obtain a desired sensitivity level among the tested subjects, a cut-off of the output value was determined on the basis of the respective sensitivity level in the training set.

7 RESULTS

7.1 IGF-I and IGFBP-3 in serum of patients with elevated serum PSA (I)

7.1.1 Correlations between variables and differences between diagnostic groups.

The serum concentration of IGF-I correlated positively with serum IGFBP-3 and prostate volume but was not associated with the serum PSA level (Table 7). Serum IGF-I and IGFBP-3 correlated negatively with the age of the patients. There was no significant difference in mean age (62 vs. 63 years; p=0.122), serum IGF-I (183 vs. 194

μg/L; p=0.094) or IGFBP-3 (4 600 vs. 4 500 μg/L; p=0.948) between prostate cancer cases (n=179) or men without prostate cancer (n=486). The median concentration of serum PSA (8.3 vs. 5.7 μg/L; p<0.001) was higher, whereas prostate volume was lower (28 vs. 38 mL; p<0.001) among cases than controls.

7.1.2 Odds ratios of serum IGF-I and IGFBP-3 for prostate cancer

Men with a high serum IGF-I concentration (highest quartile of IGF-I concentrations) had a smaller risk of having prostate cancer on biopsy than those with a low serum IGF-I concentration (lowest quartile) when adjusting for the confounding effect of serum IGFBP-3, serum PSA, and age (OR 0.50; 95% CI 0.26–0.97) (Table 8). When also adjusting for prostate volume the association between serum IGF-I level and prostate cancer risk was weaker and no longer statistically significant.

Table 7. Correlations (r) between serum IGF-I, IGFBP-3, age and prostate volume among subjects with a serum PSA of 4 μg/L or higher.

	IGF-I		IGFBP-3	
	r	P value	r	P value
Age	-0.16	< 0.001	-0.20	< 0.001
IGF-I			0.60	< 0.001
IGFBP-3	0.60	<0.001		
Total PSA	0.00	0.983	0.00	0.954
Prostate volume	0.09	0.021	0.03	0.456

Table 8. Odds ratio of prostate cancer in relation to quartiles of serum IGF-I concentration.

IGF-I quartile	Range (μg/L)	OR[1]	(95% CI)	OR[2]	(95% CI)	OR[3]	(95% CI)
1	< 134	1.00	reference	1.00	reference	1.00	reference
2	134–186	0.76	(0.45–1.26)	0.71	(0.42–1.20)	0.75	(0.42–1.35)
3	187–242	0.62	(0.36–1.07)	0.56	(0.32–1.00)	0.60	(0.32–1.10)
4	> 242	0.60	(0.34–1.04)	0.50	(0.26–0.97)*	0.57	(0.28–1.16)
IGF-I continuous[4]		0.63	(0.41–0.96)	0.51	(0.30-0.86)	0.61	(0.34-1.09)

[1]Adjusted for age and serum PSA
[2]Adjusted for age, serum PSA, and serum IGFBP-3
[3]Adjusted for age, serum PSA, serum IGFBP-3, and prostate volume
[4]Per 160 μg/L increment of serum IGF-I concentration
*p<0.05 for difference between first and fourth quartile

7.1.3 Serum IGF-I and IGFBP-3 as diagnostic tests for prostate cancer

As evidenced by ROC analysis the number of false positive PSA results could not be reduced by using serum IGF-I or IGFBP-3 as diagnostic tests. The areas under the ROC curve, 0.55 for IGF-I and 0.50 for IGFBP-3, did not differ statistically significantly from 0.50, which is the AUC of a test without diagnostic value (Figure 2).
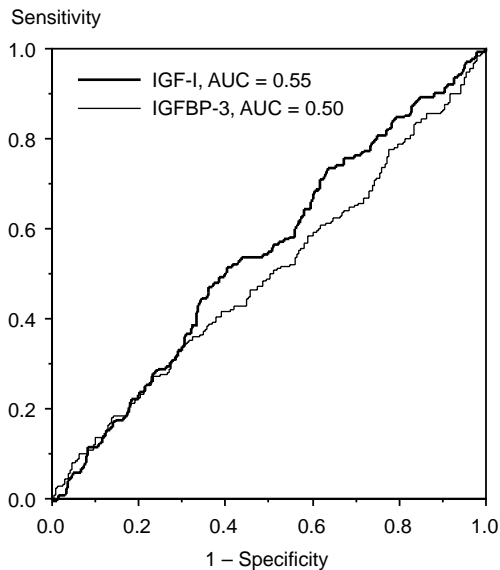
Figure 2. ROC analysis of serum IGF-I and IGFBP-3.

7.2 PSA-API in serum of patients with elevated serum PSA (II)

7.2.1 Subtraction of the nonspecific background in the PSA-API assay

In 42 female sera without PSA immunoreactivity, the PSA-API concentration was measured both with a PSA antibody and an unrelated antibody (to human chorionic gonadotropin) on the solid phase. The apparent mean concentration of PSA-API in female sera measured by the two methods did not differ significantly from each other (0.09 vs. 0.08 µg/L; p=0.17). Thus, the nonspecific background could be eliminated by subtraction. The biological detection limit of the assay with background subtraction was 0.05 µg/L, as compared to 0.21 µg/L without extraction.

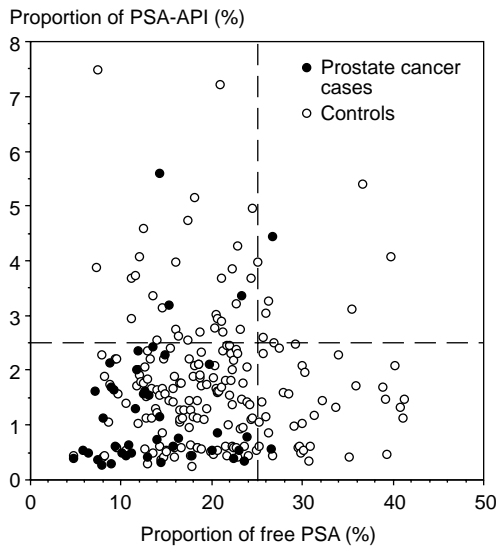*7.2.2 PSA-API and free PSA in serum from men with a serum PSA of 4–10 µg/L*

In men with a serum PSA concentration of 4–10 µg/L the proportion of free PSA and PSA-API in serum were lower in men with prostate cancer than in those without (Table 9). The concentration of serum PSA did not differ between prostate cancer cases and controls. There was no correlation between the proportions of free PSA and PSA-API (r=0.03, p=0.56) among men with a serum PSA of 4 to 10 µg/L. Of the cancer cases 42 of 44 had a proportion of free PSA lower than 25%. Only 7% of the cancer

Table 9. Medians and 95% confidence intervals of the concentrations and proportions of the various forms of PSA in serum from subjects with serum PSA in the range 4–10 µg/L.

Patient group	N	Total PSA (µg/L) Median (95% CI*)	PSA-F/T (%) Median (95% CI*)	PSA-API, µg/L Median (95% CI*)	PSA-API/T (%) Median (95% CI*)
Prostate cancer	44	5.5 (4.9–6.6)	13.0 (10.8–15.3)	0.053 (0.025–0.278)	0.78 (0.53–1.63)
Benign	210	5.4 (5.1–5.6)	19.2 (18.1–20.6)	0.091 (0.025–0.503)	1.61 (1.46–1.76)
P value		0.28	< 0.001	0.012	0.002

*95% confidence intervals for the median
Key: PSA-F/T = proportion of free PSA; PSA-API/T = proportion of PSA-API

Proportion of PSA-API (%)

Figure 3. The proportion of PSA-API as a function of the proportion of free PSA in serum from men with a serum PSA of 4–10 µg/L. The broken lines denote a cutoff of 2.5% for the proportion of PSA-API and 25% for the proportion of free PSA.

cases, but 20% of the controls with a proportion of free PSA lower than 25% had a proportion of PSA-API higher than 2.5% (Figure 3). Logistic regression analysis showed that the proportion of free PSA and the concentration of PSA-API were simultaneously significant diagnostic variables (p<0.001 and p=0.009, respectively). The diagnostic information of these two variables was combined by using logistic regression to calculate the probability of prostate cancer. At high sensitivity levels this probabil-

ity had a higher specificity than the proportion of free PSA (Table 10). In ROC analysis the AUCs of the proportion of free PSA (0.73) and the probability of prostate cancer estimated by logistic regression (0.75) were not significantly different (p=0.58).

7.3 Estimation of probability of detecting prostate cancer on biopsy (III)

7.3.1 Selection of important diagnostic variables

Men with a serum PSA of 4 to 20 were included in the study (III) in order to enable identification of patients with a low probability of prostate cancer although their serum PSA was 10–20 µg/L. There was no significant difference in age distribution (62 vs. 63 yrs; p=0.118) or frequency of a family history of prostate cancer (5.7% vs. 7.9%; p=0.371) between the prostate cancer cases (n=200) and controls (n=558). The median serum concentration of total PSA (6.8 vs. 5.5 µg/L; p<0.001) and frequency of positive DRE findings (37% vs. 12 %; p<0.001) were higher, whereas the proportion of free PSA 14% vs. 20%; p<0.001) and prostate volume (33 mL vs. 42 mL; p<0.001) were lower among cases than controls. When variables were selected into the logistic regression equation by forward and backward stepwise methods, serum PSA, the proportion of free PSA, prostate volume and DRE findings were retained in the

Table 10. Clinical sensitivities and specificities of the concentration of total PSA in serum, the proportions of PSA-API and free PSA, and LR (probability of prostate cancer calculated by logistic regression to combine the diagnostic information of PSA-API and the proportion of free PSA) in prostate cancer patients (n = 44) and controls (n = 210) with serum PSA concentrations of 4–10 µg/L.

Sensitivity (%)	Specificity (%)				Significances (p values) of differences in specificities of:		
	Total PSA	PSA-API/T	PSA-F/T	LR* of PSA-API and PSA-F/T	PSA-API/T vs. total PSA	PSA-F/T vs. total PSA	LR vs. PSA-F/T
95	5	10	26	41	0.082	<0.001	<0.001
90	6	21	28	42	<0.001	<0.001	<0.001
85	22	27	33	52	0.337	0.038	<0.001

Key: PSA-F/T = proportion of free PSA; PSA-API/T = proportion of PSA-API.

Table 11. Logistic regression equation used to calculate the probabilities in table 12.

Variable	Coefficient (ß)	Standard error	Deviance increase*	P Value
ln(PSA-T, µg/L)	0.80	0.247	10.5	0.001
PSA-F/T, 10 percent units	-0.96	0.163	41.6	<0.001
DRE	1.03	0.217	22.2	<0.001
Prostate volume ≥ 37 mL	-0.63	0.205	9.6	0.002
Intercept	-0.86	0.563		

Deviance = 719.8
Hosmer-Lemeshow test, p = 0.94
*The impact of the variables is reflected by the deviance increase if the variable is excluded from the model. Larger numbers denote a stronger impact of the variable.

model (Table 11). As evidenced by the largest deviance increase if excluded from the model, the proportion of free PSA was the most important diagnostic variable. Prostate volume was used as a binary variable for purposes of presentation, but even if used as a continuous variable its deviance increase was the smallest.

7.3.2 Probability of cancer detection on biopsy

Of the subjects with a serum PSA of 4.0–4.5 µg/L 18% had prostate cancer, indicating that the overall probability of prostate

Table 12. Probability of detecting prostate cancer at biopsy for a negative and positive DRE, a low or high prostate volume, and for various concentrations of total PSA and proportions of free PSA. The probabilities are based on the equation in Table 11.

F/T (%)	Probability					
	DRE −, Volume ≥37 mL			DRE +, Volume ≥37 mL		
5	0.30	0.47	0.60	0.54	0.71	0.81
15	0.14	0.25	0.37	0.31	0.48	0.62
25	0.06	0.11	0.18	0.15	0.27	0.39
35	0.02	0.05	0.08	0.06	0.12	0.19
	4	10	20	4	10	20
	PSA-T (µg/L)					

F/T (%)	Probability					
	DRE −, Volume <37 mL			DRE +, Volume <37 mL		
5	0.44	0.62	0.74	0.69	0.82	0.89
15	0.23	0.39	0.52	0.46	0.64	0.76
25	0.10	0.20	0.30	0.25	0.41	0.54
35	0.04	0.09	0.14	0.11	0.21	0.31
	4	10	20	4	10	20
	PSA-T (µg/L)					

Key: F/T = proportion of free PSA

cancer when serum PSA is 4 µg/L is about 0.18. The probability, as calculated by logistic regression analysis, was lower in cases with a high proportion of free PSA, a large prostate volume (> 37 mL), and when the DRE findings were negative (Table 12). The probability of prostate cancer increased with increasing serum PSA concentration, but a two-fold increase in serum PSA (from 4 to 10 µg/L or from 10 to 20 µg/L) increased the probability less than a decrease of 10 percent units in the proportion of free PSA. The probability of having prostate cancer of subjects with a prostate volume below 37 mL was 1.1 to 2-fold that of subjects with a larger prostate volume. A positive DRE finding increased the probability of detecting prostate cancer 1.2–3-fold. The mean probability (calculated before biopsy) of detecting prostate cancer was 0.41 (95% CI 0.38–0.44) for prostate cancer cases and 0.21 (95% CI 0.20–0.22) for controls (Figure 4).

7.4 Predicting outcome of prostate biopsy by using an MLP network (IV)

7.4.1 Diagnostic variables among subjects with a serum PSA of 4–10 µg/L

Among men with a concentration of serum PSA of 4–10 µg/L there was a significant difference in serum PSA level (5.7 vs. 5.3; p=0.036), proportion of free PSA (15% vs. 21%; p<0.001), prostate volume (32 mL vs. 42 mL; p<0.001) and frequency of posi-

Subjects with prostate cancer (n)

Subjects without prostate cancer (n)
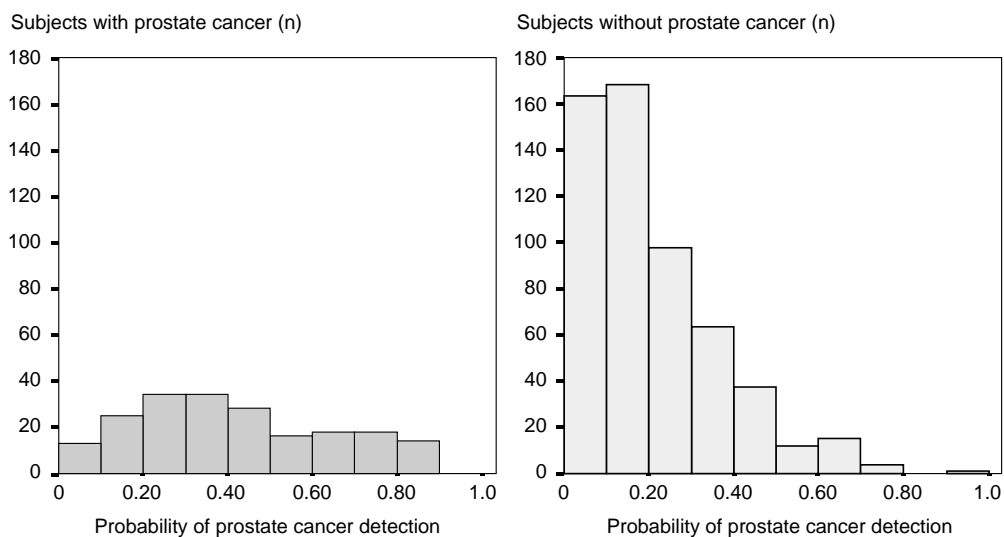
Figure 4. Distribution of the calculated probability of prostate cancer detection at biopsy among men with (n=200) and without prostate cancer (n=558).

tive DRE findings (28% vs. 11%; p<0.001) between prostate cancer cases (n=148) and controls (n=508), whereas there was no difference in age distribution (62 vs. 63 yrs; p=0.168) or frequency of a family history of prostate cancer (5.9% vs. 5.9%; p=0.937). All four variables that were significant in univariate analysis were found to be simultaneously significant diagnostic variables in a logistic regression model. These variables were also used to construct a neural network model (MLP).

7.4.2 Accuracy of the diagnostic models and the proportion of free PSA

At sensitivity levels of 90–100% the MLP model was more accurate than the logistic regression model, and both models were more accurate than the proportion of free

Table 13. Diagnostic performance of the proportion of free PSA, the LR and MLP models in 148 prostate cancer cases, and 508 controls with a serum PSA concentration of 4–10 ng/mL.

Target sensitivity	Actual sensitivity			Specificity			Accuracy			P1	P2	P3
	F/T	LR	MLP	F/T	LR	MLP	F/T	LR	MLP			
100	99	99	99	1	9	13	23	30	33	<0.001	<0.001	0.001
95	95	95	95	19	24	33	36	40	47	0.002	<0.001	<0.001
90	90	89	89	38	41	46	49	52	56	0.017	<0.001	0.001
85	85	84	82	47	52	53	51	59	59	<0.001	<0.001	0.651
80	80	78	79	50	59	57	56	63	62	<0.001	<0.001	0.201

Key: F/T = proportion of free PSA; LR = logistic regression model; P1 = P Value (McNemar test) for difference in accuracy between LR and F/T; P2 = difference between MLP and F/T; P3 = difference between MLP and LR. Target sensitivity is the sensitivity aimed at in the training session; the actual sensitivity is the sensitivity obtained in the testing session. To make the various algorithms comparable the proportion of free PSA has been treated in the same way (with target and actual sensitivity) as the diagnostic models based on logistic regression and MLP.

PSA (Table 13). If missing 5% of the cancer cases was accepted (95% sensitivity) the MLP model reduced 33%, the logistic regression model 24%, and the proportion of free PSA 19% of the false positive PSA results. At lower sensitivity levels (80–85%) the MLP and logistic regression models performed equally, but both had a higher specificity (reduced more false positive PSA results) than the proportion of free PSA.

8 DISCUSSION

The goal of prostate cancer screening is to reduce mortality and morbidity in prostate cancer. However, screening with serum PSA has not yet been proved to achieve this goal in randomized trials.

8.1 PSA and prerequisites for screening

Principles for early disease detection have been suggested by Wilson and Jungner (Wilson and Jungner, 1968). 1) The disease should be an important health problem. 2) There should be an accepted treatment available. 3) The natural history of the disease should be adequately understood and the disease should have a preclinical phase. 4) There should be a valid test available that is acceptable to the population and results in earlier detection of the disease. 5) The cost of screening and treatment should be economically possible and the screening should be a continuous process.

PSA-based screening fulfills most, but not all, of the above mentioned prerequisites for a successful screening program. First, prostate cancer is a major health problem. It is the most common cancer among Finnish men with an incidence of 72 new cases/100 000 person-years in 1997, and it is the second most common cause of cancer death among men. Second, treatment of prostate cancer by radical prostatectomy and radiation therapy is effective if the disease is localized to the prostate gland. However, radical treatment causes complications. Third, the natural history of prostate cancer is fairly well known. Prostate cancer is a slowly growing disease, and therefore screening and treatment of men with a short life expectancy is not likely to reduce prostate cancer specific mortality (Albertsen, et al., 1998, Carter, et al., 1999). Most prostate cancers have a long latent phase and do not develop into clinical disease during the patient's life time. Thus it is inevitable that prostate cancer screening will lead to detection and treatment of some prostate cancers that would not otherwise have been diagnosed during the patient's life time. Fourth, the PSA test is available and acceptable to the population. PSA-based screening has been estimated to result in a lead time for detection of prostate cancer of about 5 to 10 years (Stenman, et al., 1994, Gann, et al., 1995). Most prostate cancers detected on the basis of an elevated PSA are clinically relevant, organ-confined and potentially curable (Catalona, et al., 1993). However, the majority of men with a positive PSA test do not have prostate cancer at biopsy, and this has been considered to be the principal drawback of PSA testing (Smith, et al., 2000).

The screening should also improve the outcome of the disease, and do more good than harm. A screening study in Quebec reported a reduction in prostate cancer mortality of 69% (Labrie, et al., 1999). However, the study participants were not properly randomized into screening and control arms, and the study has been criticized for being heavily biased in several ways (Alexander and Prescott, 1999, Boer and Schröder, 1999). The true effect of prostate cancer screening will hopefully be clarified

by ongoing randomized prostate cancer screening trials (Auvinen, et al., 1996).

8.2 Existing methods for reducing the number of false positive PSA results

The aim of the present investigation was to develop methods by which the number of false positive serum PSA results can be reduced. This is important because more than two thirds of the subjects with an elevated PSA do not have prostate cancer on biopsy. Needle biopsy is complicated by infection or bleeding in 0.1 to 4%, and by discomfort and anxiety in 58 to 68% of the patients (Cooner, et al., 1990, Aus, et al., 1993, Desmond, et al., 1993). Waiting for the biopsy result also causes anxiety. Increasing the cutoff value of serum PSA would lead to a reduction of the number of false positive PSA results, but this would not serve the goal of the screening as the number of curable cancers detected would decrease.

The proportions of free PSA and PSA-ACT can be used to reduce the number of false positive PSA results by 20 to 30% in the serum PSA range 4 to 10 μg/L with little or no decrease in the number of detected cancer cases (Reissigl, et al., 1996, Catalona, et al., 1998). Among men with an elevated concentration of serum PSA, prostate volume has been shown to be smaller among those with than those without prostate cancer. This finding has formed the basis for PSA density. PSA density has been claimed to reduce as many false positive PSA results as the proportion of free PSA (Bangma, et al., 1997), but it is available only when TRUS has been performed. Furthermore, many urologists prefer to always take biopsies when performing TRUS. In this case TRUS findings and prostate volume are not utilized when deciding whether to perform a biopsy. It is not clear whether prostate volume is useful when combined with the proportion of free PSA because a large prostate volume may increase the percentage of free PSA. The present investigation showed a positive correlation between the proportion of free PSA and prostate volume, but logistic regression analysis indicated that they still are simultaneously important as diagnostic variables (III, IV). Thus, prostate volume can be used to improve the detection of prostate cancer even when free and total PSA are available.

Before the PSA era DRE was the main screening tool for prostate cancer. DRE is a less sensitive test than PSA, but among men with a serum PSA of 4 to 10 μg/L a clearly larger proportion of the men with prostate cancer has a positive DRE finding (28%) than men without prostate cancer (11%) (IV). A problem with DRE is the large inter-observer variation (Schröder, et al., 1998). However, although several urologists performed the DRE:s in the present investigation, the DRE result was an independently important diagnostic variable, which the finding of a hypo-echoic area by TRUS was not. The validity of our diagnostic algorithms could possibly be improved if DRE would be performed in a systematic manner by persons specifically trained to achieve inter-observer agreement (Varenhorst, et al., 1993, Schröder, et al., 1998).

A family history of prostate cancer has been shown to be an important risk factor for prostate cancer (Walsh and Partin, 1997). However, in the present investigation (III) the frequency of subjects with a first degree familial prostate cancer (brother or father with prostate cancer) was similar (6 vs. 8%) in men with and without prostate cancer. This finding is surprising especially as a recent study on 44 788 pairs of twins in Sweden, Denmark, and Finland showed that heritability has a substantial effect on prostate cancer risk: as much as 42% of the prostate cancer risk may be explained by heritable factors (Lichtenstein, et al., 2000). Our finding could possibly be explained by the fact that the subjects were selected on the basis of an elevated serum PSA. However, an accumulation of a fam-

ily history of prostate cancer among prostate cancer cases has been observed in screen positive subjects in other countries (Virtanen, et al., 1999).

The prevalence of prostate cancer increases strongly with age. Age-specific cutoff values for PSA have been proposed (Oesterling, et al., 1993), but there are strong arguments for the use of a constant PSA cutoff for all age groups (Etzioni, et al., 1996, Carter, 2000), and instead decreasing the intensity of the screening among older men by prolonging the test intervals (Carter, et al., 1999). Because age is a risk factor for prostate cancer, age has been used to assist in the biopsy decision in some studies (Chen, et al., 1996, Optenberg, et al., 1997). In our study and some other ones comprising a limited age range the correlation between age and prostate cancer risk was not statistically significant (Kranse, et al., 1999, Virtanen, et al., 1999).

8.3 Serum IGF-I

Because serum IGF-I has been suggested to be associated with an increased risk of prostate cancer (Mantzoros, et al., 1997, Chan, et al., 1998, Wolk, et al., 1998), we decided to determine whether serum IGF-I can be used to reduce false positives PSA results. In our study population that included men with a serum PSA of 4 µg/L or higher, serum IGF-I was slightly but not significantly lower among men with prostate cancer than in those without. This was possibly partly explained by a weak positive correlation between serum IGF-I concentration and prostate volume, which was larger in controls than in cases. Recently, a strong association between high IGF-I levels, BPH and enlarged prostate volume was observed in acromegaly patients with elevated serum concentrations of growth hormone and IGF-I, whereas successfully treated patients had normal prostate volume and growth hormone deficient cases had reduced prostate volume (Colao, et al., 1999). These find-

ings indicate that a high serum IGF-I may cause BPH. A causal link between serum IGF-I and prostate volume could explain the positive correlation between serum PSA and prostate cancer in earlier studies (Cohen, 1998). Before the PSA era prostate cancer was often detected because of urinary symptoms, which mainly are caused by BPH and an enlarged prostate. It has even been suggested that the positive correlation between serum IGF-I and prostate cancer risk seen in some studies may be due to a bias caused by prostate volume (Cohen, 1998). In earlier studies on the correlation between serum IGF-I and prostate cancer risk, prostate volume has not been measured and the controls have not been selected on the basis of an elevated PSA value. We could study the effect of prostate volume and when statistically adjusting for prostate volume the negative correlation between serum IGF-I and prostate cancer risk became weaker. The conclusion of our study is that serum IGF-I not is a useful marker for reducing the number of false positive PSA results in a screening setting (I). Furthermore, our findings support the notion that serum IGF-I is associated with prostate volume rather than with prostate cancer (Colao, et al., 1999).

8.4 Serum PSA-API

The proportion of PSA-ACT is larger (Stenman, et al., 1991), whereas the proportions of free PSA and PSA-API are smaller in men with prostate cancer than in those without (Zhang, et al., 1999). Thus PSA-API is a potentially useful marker for prostate cancer. However, the original assay for PSA-API was hampered by a nonspecific background, which limited its sensitivity. The background signal is caused by the large excess of serum API that binds nonspecifically to the solid phase as such or possibly in complex with other proteases. A complex between ACT and cathepsin G is thought to cause the nonspecific background in assays for PSA-ACT (Heidtmann

and Havemann, 1993, Pettersson, et al., 1995). The PSA-API assay used in the present investigation was improved by subtracting the nonspecific background caused by binding of API to the solid phase. Thus the detection limit could be lowered and PSA-API could be determined in most of the subjects with a serum PSA of 4 to 10 μg/L. The new assay is a bit more laborious than a conventional PSA assay, requiring the performance of one assay for PSA-API and one for the background signal. However, both assays can easily be performed on an automatic analyzer.

Most cancer cases (95%) with a serum PSA of 4–10 μg/L had a proportion of free PSA below 25%, but so had most controls (78%) too. A high proportion of PSA-API indicated a low probability of prostate cancer although the proportion of free PSA was lower than 25%. Seven percent of the cancer cases with a proportion of free PSA below 25% had a proportion of PSA-API higher than 2.5% as compared to 20% of the controls. We could therefore combine the diagnostic information of PSA-API and the proportion of free PSA using logistic regression analysis to calculate the probability of prostate cancer, and at high sensitivity levels this combination could reduce more false positive PSA results than the proportion of free PSA alone.

8.5 Optimal use of available variables to reduce false positive PSA results

Logistic regression is a statistical technique that can be used to identify among multiple variables those which provide independent diagnostic information. Logistic regression can also be used to construct diagnostic algorithms for estimating the probability of a disease. This probability optimally combines the diagnostic information of the variables in the logistic regression equation. In some types of data sets even more optimal combinations can be achieved by neural networks that are more complex

mathematical models than logistic regression. Diagnostic algorithms based on logistic regression and neural networks facilitate the interpretation and utilization of multiple diagnostic variables.

In the present investigation we showed by logistic regression analysis that the variables that contribute simultaneously to the diagnostic information among men with a serum PSA of 4 to 20 μg/L were total PSA, the proportion of free PSA, prostate volume, and DRE findings, whereas age and a family history of prostate cancer did not provide diagnostic information. The independent variables were used to establish algorithms for calculation of the probability of detecting prostate cancer at biopsy. These algorithms showed that the probability varied greatly at a fixed level of serum PSA. The overall probability of prostate cancer for a man with a serum PSA of 4 μg/L was about 0.18, but it varied between 0.02 and 0.69. This was mainly explained by variation in the proportion of free PSA but also by prostate volume and DRE findings. This finding demonstrates that combinations of many variables can reduce the frequency of false positive PSA results more efficiently than the proportion of free PSA. Among men with a serum PSA of 4–10 μg/L a neural network model, MLP, was shown to reduce false positive PSA results more effectively than logistic regression. Using cutoffs so that 95% of the cancer cases could be detected 33%, 24%, and 19% of the false positive PSA results could be eliminated, respectively, with the MLP model, logistic regression model, and the proportion of free PSA.

The diagnostic models used in the present investigation indicate that prostate volume can predict negative biopsies simultaneously with the proportion of free PSA. Predictable negative biopsies should be avoided because they cause unnecessary expenses and anxiety. Prostate volume cannot form the basis for a biopsy decision if most urologists always perform prostate biopsies when they

measure prostate volume by TRUS. The diagnostic algorithms developed in this study can be made available on the internet or in a pocket calculator or incorporated in the software of the TRUS device. Thus, the probability of detecting prostate cancer can be obtained immediately when prostate volume has been measured and the biopsy decision can be made bed-side. Furthermore, in screening programs paramedical personnel can be trained to perform TRUS and DRE (Schröder, et al., 1998).

8.6 Future prospects of early detection of prostate cancer

Most screening programs use serum PSA with a cutoff of 4 μg/L as a primary screening test to select men who should have a prostate biopsy because of an increased risk of prostate cancer. In some screening studies DRE or the proportion of free PSA has been introduced as a second screening test to identify subjects with increased prostate cancer risk among men with a serum PSA of 2.6 to 4 μg/L (Catalona, et al., 1997, Määttänen, et al., 1999). The primary test by which the whole population is screened should be reasonably inexpensive for the society and highly acceptable for the screened subjects. This is true for PSA but not for DRE or TRUS. Additional serum markers can easily be determined from the initial serum sample without bothering the patient and thus serum markers are well suited to increase the diagnostic accuracy in the large group of men with a serum PSA below 4 μg/L. The prevalence of prostate cancer among men with a serum PSA below 2 μg/L is very low (Schröder, et al., 1998), and use of additional tests to identify high-risk cases among these is probably not worthwhile, especially if the screening is continuous.

A possible future model for improving the accuracy of prostate cancer screening could be measuring free PSA, PSA-API, PSA-A2M and hK2 in serum from all men with a PSA above 2 μg/L. Other serum variables could also be measured if found to be useful. Men with a serum PSA below 2 μg/L could be spared further investigations but would have a new PSA test within 2–4 years. All serum markers and anamnestic variables (age and family history if found to be useful) would be used in a diagnostic algorithm to identify patients who need a clinical investigation including DRE and TRUS. The clinical findings would be used together with all other available important variables in a second diagnostic algorithm to identify patients who should have a prostate biopsy. If indicated, this could be performed immediately after the clinical investigation. This model for prostate cancer screening would lead to performing biopsy on many men with a serum PSA below 4 μg/L but avoiding it on a number of men with a serum PSA higher than 4 μg/L. The biopsy decision would be based on a prostate cancer probability calculated on the basis of all important variables available.

9 Summary and conclusions

Prostate cancer is the most common cancer among men in the industrialized world, and it is the second most common cause of cancer death in men. Serum PSA is being used for early detection of prostate cancer in population screening but its utility is hampered by a high frequency of false positive test results. The aim of the present investigation was to develop and validate methods for reducing the number of false positive PSA results. The subjects studied were men with a serum PSA of 4 µg/L or higher in the Finnish prostate cancer screening trial.

Among men with a concentration of serum PSA of 4 µg/L or higher, serum levels of IGF-I were not significantly different between subjects with cancer and those without. When adjusting for serum IGFBP-3, serum IGF-I showed a negative association with prostate cancer risk (OR 0.50, CI 0.26–0.97 for the highest vs. the lowest quartile of IGF-I concentrations). Serum IGF-I correlated positively with prostate volume, and when also adjusting for prostate volume the negative association between serum IGF-I and prostate cancer risk was no longer statistically significant. In ROC analysis the AUC of serum IGF-I was 0.55, indicating that it had no diagnostic value.

A new assay for serum PSA-API was developed. By subtraction of the nonspecific background signal the detection limit of the assay could be lowered. The diagnostic information of serum PSA-API and the proportion of free PSA were combined using logistic regression analysis. Among men with a serum PSA of 4–10 µg/L this combination gave higher specificity (41% vs. 26%) at 95% sensitivity than the proportion of free PSA (p<0.001).

The probability of detecting prostate cancer in men with a serum PSA of 4–20 µg/L was estimated by logistic regression analysis. Among those with a serum PSA of 4 µg/L the overall probability was 0.18 but depending on the proportion of free PSA, DRE findings and prostate volume the probability varied between 0.02 and 0.69. An increase in serum total PSA from 4 to 10 µg/L increased the probability less than a decrease in the proportion of free PSA of 10 percent units.

Diagnostic models were constructed to predict biopsy outcome among men with a serum PSA of 4–10 µg/L using an artificial neural network (MLP) and logistic regression. Serum PSA, the proportion of free PSA, DRE findings and prostate volume were found to be important diagnostic variables. At 95% sensitivity the MLP model reduced the frequency of false positive PSA results more effectively than the logistic regression model or the proportion of free PSA (33%, 24%, and 19%, respectively, p<0.001).

In conclusion, serum IGF-I was not a useful marker for prostate cancer among men with elevated serum PSA. Use of PSA-API in combination with the proportion of free PSA provided higher diagnostic accuracy than the proportion of free PSA alone. The probability of detecting prostate cancer calculated by logistic regression or MLP eliminated false positive PSA results more accurately than the proportion of free PSA.

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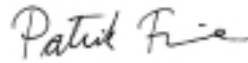
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